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Total mercury in hair as biomarker for methylmercury exposure among women in central Sweden– a 23 year long temporal trend study☆

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ABSTRACT

Exposure to methylmercury (MeHg) through fish is a global public health problem. Exposure monitoring is essential for health risk assessment, especially in pregnant women and children due to the documented neurotoxicity. Herein, we evaluate a time series of MeHg exposure via fish in primiparous Swedish women, covering a time period of 23 years (1996–2019). The 655 included mothers were part of the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas) conducted by the Swedish Food Agency (SFA). MeHg exposure was assessed via measurements of total mercury (Hg) in hair using either cold vapor atomic fluorescence spectrophotometry or inductively coupled plasma mass spectrometry, showing very good linear agreement ($R^2 = 0.97$). Maternal characteristics and fish consumption were obtained via questionnaires. The median concentration of total Hg in hair was 0.38 mg/kg (range 0.17 −1.5) in 1996 and 0.25 mg/kg (range 0.03−1.1) in 2019. On average the women consumed 11 ± 8.2 meals of fish per month, and fish consumption was positively correlated with total Hg in hair (Spearman correlation: 0.39; $p < 0.001$). In multiple regression analyses, the geometric mean annual decrease of total Hg in hair was −2.5% (95% CI: -3.2, −1.8%). Total fish consumption increased up to 2011 (B: 0.32 times/month per year; 95% CI 0.17, 0.46) after which it started to decline (B: -0.66 times/month per year; 95% CI -0.92, −0.40). Moreover, both total Hg in hair and fish consumption was positively associated with maternal age and education, and inversely associated with pre-pregnancy BMI. In conclusion, the exposure to MeHg via fish appears to be slowly declining among Swedish pregnant women.

1. Introduction

Fish is considered to be a part of a healthy diet, although the simultaneous exposure to environmental contaminants present in fish raises concern for human health (Mozaffarian and Rimm, 2006). Exposure to methylmercury (MeHg) occurs almost exclusively via consumption of fish and seafood (EFSA, 2012). However, the MeHg concentrations have been shown to vary widely between different species of fish, and the highest concentrations have been found in predatory fish (EFSA, 2012). The ingested MeHg is efficiently absorbed in the gastrointestinal tract (Aberg et al., 1969), after which it is distributed to all tissues in the body, readily passing the blood-brain and placenta barrier (Berlin and Ullberg, 1963; Reynolds and Pitkin, 1975). The target organ for MeHg toxicity is the brain, which is especially susceptible during development (EFSA, 2012; NRC, 2000). Based on the established neurotoxicity of MeHg, several authorities, including the Swedish Food Agency (SFA), have established recommendations to pregnant and breastfeeding women concerning restricted consumption of certain fish species. Still, it is highly important to conduct biomonitoring of exposure to MeHg in susceptible groups such as pregnant women.

Exposure to MeHg in fish consumers can be assessed by the MeHg or the total Hg concentration (as a proxy) in whole blood or in erythrocytes, with the assumption that the inorganic Hg exposure is much lower than that of MeHg (Hallgren et al., 2001;

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Skerfving, 1988). Exposure to MeHg can also be assessed by the total Hg concentration in hair as more than 90% of Hg in hair is in the form of MeHg (Berglund et al., 2005). Indeed, total Hg concentration in hair is highly correlated with the concentration of MeHg in blood, but not with inorganic Hg in blood (Berglund et al., 2005). It has been proposed that total Hg in hair reflects inorganic mercury exposure at low MeHg exposure in populations with no or low fish consumption (NRC, 2000). However, studies in non-fish consumers found no association between total Hg in hair and inorganic Hg in blood, whereas total Hg in hair was associated with MeHg in blood, despite a very low MeHg exposure (Lindberg et al., 2004). Inorganic Hg in hair was also associated with fish consumption (Berglund et al., 2005), but as fish contain very small amounts of inorganic mercury (Marrugo-Negrete et al., 2008) and the gastrointestinal absorption is low (EFSA, 2012), this minor fraction of inorganic Hg in hair probably emanates from MeHg that has been demethylated in the body and/or during sample preparation and analysis. Thus, the concentration of total Hg in hair appears to be a very good biomarker of MeHg exposure.

Despite the adoption of the Minamata Convention on mercury, designed to protect human health and the environment from Hg pollution (UNEP, 2017), it has been estimated that climate change is likely to increase the human exposure to MeHg via fish and seafood (Alava et al., 2017). The aim of the present study was to evaluate a time series of MeHg exposure via fish in primipara Swedish women during a period of 23 years (1996–2019).

2. Materials and methods

2.1. Participants

Participants were primiparous women living in Uppsala County, Sweden, included in the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas) conducted by the SFA (Glynn et al., 2007; Lignell et al., 2009). The details about recruitment and collection of data have been described elsewhere (Glynn et al., 2007; Lignell et al., 2009). In short, participants were randomly recruited among primiparous mothers who were Swedish by birth and delivered at Uppsala University Hospital. Hair samples for assessment of MeHg were collected by a midwife who visited the mothers three weeks after delivery. Data on maternal characteristics were obtained via personal interviews and questionnaires, and included age, weight before pregnancy, height, level of education, and smoking before and during pregnancy. Also, information regarding fish consumption was collected using a detailed self-administered questionnaire where type of fish consumed and consumption frequency (number of meals per month or week) were specified. The validation of this questionnaire has been described in detail elsewhere (Strom et al., 2011). The study was approved by the local ethics committee of Uppsala University, and participants gave their informed consent prior to the inclusion in the study.

In total, 658 mothers were recruited from 1996 to 2019 out of which three (one in 2008 and two in 2019) did not donate a hair sample, resulting in 655 mothers with data on MeHg exposure. Furthermore, some mothers lacked information on fish consumption ($n = 12$), age ($n = 7$), level of education ($n = 5$), body mass index ($n = 18$), and pre-pregnancy smoking ($n = 5$). Thus, the final study sample with exposure data and complete characteristics comprised 622 mothers.

2.2. Sample collection and preparation

Full length hair samples (about the thickness of a toothpick and 9 cm of length from the scalp, whenever available) were tied with a cotton thread in the back of the head, cut close to the scalp, put into a plastic bag and stored at room temperature. Hair samples collected between 1996 and 2006 were digested using alkaline solubilization as described in detail previously (Berglund et al., 2005). In short, about 20 mg hair was treated with L-cysteine, sodium hydroxide and sodium chloride and heated to 90–95 °C for 20 min. Hair samples collected between 2007 and 2019 were digested using a Milestone ultraCLAVE II microwave digestion system (EMLS, Leutkirch, Germany) (Gustin et al., 2017). Approximately 50 mg of hair was weighted into quartz or teflon tubes together with 2 mL nitric acid (65% w/w) and 3 mL deionized water and digested for 30 min (250 °C and a pressure of 160 bar). Digested samples were cooled down to a temperature below 30 °C, and thereafter diluted with deionized water to an acid concentration of 20%. Finally, 2 mL of each sample was stabilized with 2% hydrochloric acid (30% w/w, Merck, Darmstadt, Germany). In 2018, the hydrochloric acid used to stabilize Hg was exchanged to 1 ppm of a gold standard (Sigma-Aldrich, Stockholm, Sweden). The main reason for shifting the stabilizer was that when using hydrochloric acid in ICPMS analysis it decreased the sensitivity of other element ions of interest. Gold is commonly used for stabilizing Hg in ICPMS analyses (EPA method 6020B) and it mainly acts as an oxidizer keeping Hg in solution as ions.

2.3. Analytical methods

In hair samples collected from 1996 to 1999, the total Hg concentration was measured on a yearly basis using cold vapor atomic fluorescence spectrophotometry (CVAFS; Merlin PSA 10.023; P.S. Analytical Ltd., Orpington, Kent, UK) as previously described in detail (Berglund et al., 2005). The limit of detection (LOD; calculated as three times the standard deviation of the reagent blanks) was <0.03 mg/kg. From 2000 and onwards, total Hg in hair was instead measured using inductively coupled plasma mass spectrometry (ICPMS; Agilent 7500ce, 7700x and 7900, Agilent Technologies, Tokyo, Japan) (Gustin et al., 2017). Hair samples collected between 2000 and 2006 were measured together, and thereafter samples were measured on a yearly basis. We continuously monitored the Hg isotope 201 in standard mode (no gas). The LOD was <0.08 mg/kg for all runs. Total Hg concentrations below the LOD of each respective method were kept as measured because, even though they are uncertain, they usually contain more information than applying the value of LOD/2 (Helsel, 1990). Analytical quality was ascertained by inclusion of several reference materials for Hg in hair (IAEA 086 Hair, NCSZC81002b, GBW 09101) in each analytical run (Table 1), and in general there was a good agreement between obtained concentrations and target concentrations.

In connection to the switch of analytical method from CVAFS to ICPMS, 14 different hair samples were analyzed using both methods. The Hg concentrations in the hair samples showed a good agreement between the two analytical methods [Linear regression: $CVAFS = 0.99*ICPMS+0.009$ ($R^2 = 0.97$; $n = 14$)] within the range of 0.1–0.8 mg Hg/kg hair (Fig. 1a). Similarly, the shift of Hg stabilizer from hydrochloric acid to gold was evaluated by ICPMS analysis of 71 hair samples and the methods showed good agreement [Linear regression: $HCl = 1.03*Gold-0.015$ ($R^2 = 0.97$; $n = 71$)] (Fig. 1b).

2.4. Statistics

The statistical analyses were performed with Stata 15.0 (StataCorp LLC, TX, USA) and $p \leq 0.05$ was considered statistically significant. Bivariate associations between total Hg in hair and potential influencing factors [year of collection, maternal age (years), pre-pregnancy BMI (kg/m^2), level of education (upper secondary school, ≤3 y of higher education, >3 y of higher

Table 1
Results of the analytical quality control used for total mercury in hair (mg/kg).

Material	Year	n	Obtained values (mg/kg)	Reference values (mg/kg)
IAEA-086 Hair[a]	2000–2006	15	0.56	0.57 (0.534–0.612)
	2008	7	0.55	0.57 (0.534–0.612)
	2009	4	0.54	0.57 (0.534–0.612)
	2010	4	0.54	0.57 (0.534–0.612)
	2011	7	0.47	0.57 (0.534–0.612)
	2012	3	0.47	0.57 (0.534–0.612)
	2013	3	0.44	0.57 (0.534–0.612)
	2014	3	0.53	0.57 (0.534–0.612)
	2015	2	0.53	0.57 (0.534–0.612)
	2017	3	0.53	0.57 (0.534–0.612)
	2018	3	0.54	0.57 (0.534–0.612)
GBW 09101[b]	2000–2006	7	2.18	2.16 ± 0.21
NCSZC81002b[c]	2007	3	1.18	1.06 ± 0.28
	2008	7	1.21	1.06 ± 0.28
	2009	4	1.19	1.06 ± 0.28
	2010	4	1.19	1.06 ± 0.28
	2011	7	0.98	1.06 ± 0.28
	2012	3	1.02	1.06 ± 0.28
	2013	5	0.88	1.06 ± 0.28
	2014	9	1.08	1.06 ± 0.28
	2015	2	1.11	1.06 ± 0.28
	2016	3	0.99	1.06 ± 0.28
	2017	3	1.35	1.06 ± 0.28
	2018	3	1.31	1.06 ± 0.28
	2019	2	0.99	1.06 ± 0.28

[a] IAEA 086 Hair: International Atomic Energy Agency, Vienna, Austria.
[b] GBW 09101: Shanghai Institute of Nuclear Research Academia Sinica, Shanghai, China.
[c] NCSZC81002b: China National Analysis Center for Iron and Steel, Beijing, China.

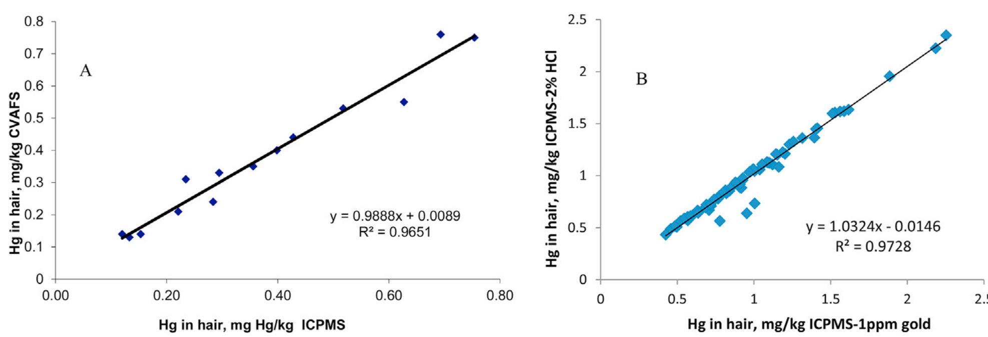

Fig. 1. A-B. Comparison of total mercury (Hg) in hair A) measured using ICPMS or CVAFS, and B) using ICPMS following Hg stabilization with gold (1 ppm) or hydrochloric acid (HCl). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

education), smoking before pregnancy (no/yes/former), total fish consumption (times/month, making no distinction concerning type of fish consumed), and awareness of dietary advice concerning fish (yes/no)] were, depending on the type of data (continuous or categorical), assessed using either Spearman's rank correlation, Mann-Whitney *U* Test, or Kruskal-Wallis. The Jonckheere-Terpstra test was used to assess the change in total Hg in hair and fish consumption over the entire time period (1996 throughout 2019). Associations of total Hg in hair and fish consumption with date/year of collection were also visually examined using both scatter plots with Lowess smoothing lines and box plots.

For exploring predictors of total Hg in hair, we used multiple linear regression analysis, in which we included all predictors that were significantly associated with total hair Hg in the bivariate analyses [year of collection (continuously), maternal age (<25 y, ≥25 to <30 y, ≥30 to <35 years, and ≥40 years), level of education (upper secondary school, ≤3 y of higher education, >3 y of higher education), pre-pregnancy BMI (<25 and ≥ 25 kg/m^2), and fish consumption (no fish, <5 times/month, ≥5 to <10 times/months and ≥10 times/months)]. As total Hg in hair was not normally distributed it was log-transformed using the natural logarithm. To ease the interpretation, the unstandardized beta estimates were sometimes exponentiated and expressed as percent change in total Hg in hair. For fish consumption, we applied spline linear regression analysis as the association with year of collection appeared non-linear (a spline knot was placed at year 2011). We also included the same predictors specified above in the model, except for total fish consumption. In addition, we explored the adjusted standardized estimates (β) for each predictor in relation to total Hg in hair and fish consumption by adding the *beta* option for regression analysis in Stata, which standardizes all variables to have a mean of 0 and a standard deviation of 1. The residual distribution in each model was checked using q–q plots and residual versus fitted plots.

In sensitivity analyses, we explored the association of total Hg in hair and year of collection categorized into 5-year intervals (1996–2000, 2001–2006, 2007–2011, 2012–2016, ≥2017) using 1996–2000 as the reference category. In addition, we restricted the model of total Hg in hair and potential predictors to samples collected before 2011 when fish consumption was still increasing over time.

3. Results

The primiparous Swedish mothers included in the study from 1996 to 2019 had a mean age of 29 years (range 19–45 years; Table 2). The mean pre-pregnancy BMI was 23 kg/m^2 (range 16–44 kg/m^2). About 55% of the participants had more than three years of studies after upper secondary school, and 76% of the mothers reported that they were non-smokers. The total concentration of Hg in hair (mg/kg) and fish consumption (times/month) per year of collection is presented in Table 3. The overall median concentration of total mercury in hair from 1996 throughout 2019 was 0.34 mg/kg with a range of 0.01–1.53 mg/kg. None of the women exceeded WHO/JECFA provisional tolerable daily intake (PTDI) of 0.23 μg/kg body weight - corresponding to 2.3 mg Hg/kg in hair, or EFSAs PTDI of 0.19 μg/kg body weight – corresponding to about 1.9 mg Hg/kg hair, and less than 3% of all the women ($n = 17$ out of 655) exceeded US NRC tolerable daily intake (TDI) of 0.1 μg/kg body weight - corresponding to about 1 mg Hg/kg in hair (IOMC, 2008). On average the women consumed 11 ± 8.2 meals of fish per month, and thus, many of the women (63%) followed the dietary advice given by the SFA of eating fish two to three times per week. However, it should also be noted that about 5% of the women reported that they did not consume fish, but still total Hg was detected in their hair (median 0.12 mg/kg).

In bivariate analyses, there was a significant trend indicating that the concentration of total Hg in hair decreased over time ($p < 0.001$; Fig. 2a and Supplemental Fig. S1a), whereas fish consumption increased significantly up to 2011 ($p < 0.001$) after which the trend turned negative ($p = 0.001$; Fig. 2b and Supplemental Fig. S1b). Overall, total Hg in hair was positively correlated with total fish consumption [Spearman correlation (r_s) = 0.39; $p < 0.001$]. Moreover, total Hg in hair was positively correlated with maternal age ($r_s = 0.26$; $p < 0.001$) and level of education ($r_s = 0.21$; $p < 0.001$), and inversely correlated with pre-pregnancy BMI ($r_s = -0.19$; $p < 0.001$). Similarly, fish consumption was positively correlated with maternal age ($r_s = 0.16$; $p < 0.001$) and level of education ($r_s = 0.18$; $p < 0.001$), and weakly inversely correlated with pre-pregnancy BMI ($r_s = -0.077$; $p = 0.053$). Neither total Hg in hair nor fish consumption was correlated with the women's smoking status prior to pregnancy ($p > 0.05$, respectively).

In a linear regression model, the total Hg in hair was inversely associated with year of collection (Table 4), corresponding to a geometric mean annual decrease of total Hg in hair by −2.5% (95% CI -3.2, −1.8%). Moreover, mothers older than 30 year had on average higher total Hg in hair than mothers who were younger than 25 years, and mothers with more than 3 years of higher education had on average higher total Hg in hair than mothers who only attended upper secondary school. Mothers with a pre-pregnancy BMI equal to or above 25 kg/m^2 had on average lower total Hg in hair than mothers with a BMI below 25 kg/m^2. Finally, mothers consuming fish, independent of the amount, had on average higher total Hg in hair than those who did not consume fish. Fish consumption was the most important predictor for total hair Hg followed by year of collection, maternal age, education and pre-pregnancy BMI and the R^2 of the model was 0.33.

In a spline regression model (Table 4), there was an overall mean increase in fish consumption annually up to 2011, after which the trend turned negative with a mean annual decrease in fish consumption. There was no clear linear trend with maternal age, although there was an indication that mothers in the age range of ≥30 to ≤35 years ate on average fish more often than mothers who were younger than 25 years. Also, mothers with more than 3 years of higher education ate fish more often than mothers who only attended upper secondary school. Mothers with a pre-pregnancy BMI equal to or above 25 kg/m^2 ate on average less fish than mothers with a BMI below 25 kg/m^2. Out of the studied predictors the strongest predictor for fish consumption was year of collection followed by maternal age, pre-pregnancy BMI and education, although it is noteworthy that the R^2 of the model was very low 0.089.

In a sensitivity analysis, we explored the change in total Hg in hair over time categorizing year of collection into 5-year intervals (Supplemental Table S1), confirming decreasing concentrations over time. The geometric mean decrease of total Hg in hair was −42% (95% CI 51, 31%) when comparing the last interval with the first 5-year interval. Moreover, we restricted the model with total Hg in hair to only include samples before 2011 ($n = 342$), which resulted in a geometric mean decrease of total Hg in hair by −3.4% (95% CI: -4.8, −1.9; $p < 0.001$). Also, total Hg in hair was still positively associated with fish consumption, maternal age, and level of education, and inversely associated with pre-pregnancy BMI (data not shown).

4. Discussion

To our knowledge, this is the longest time series of MeHg exposure, assessed via total Hg in hair in primiparous Swedish women sampled between 1996 and 2019. Despite that the MeHg exposure varied between the different sampling years, there was an overall adjusted geometric mean decrease in total Hg in hair by −2.5% per year. This minor annual decrease of total Hg in hair suggests that it will take decades to see major changes in human exposure via fish, in spite of decreased Hg air emissions in Europe (UNEP, 2019). In contrast to Europe, on a global scale air emission was still increasing from 2010 to 2015. Surprisingly, the present decrease in MeHg exposure via fish appeared to be occurring in concordance with a trend of increasing fish consumption during most of the time period. Moreover, we found that MeHg exposure increased with maternal age and education, and decreased with maternal BMI, which is in accordance with findings of previous

Table 2
General characteristics of the participating primiparous Swedish women, sampled from 1996 throughout 2019 ($n = 655$).

Characteristics	n	Mean ± SD or percent	Median	5–95th percentile
Age (years)	648	29 ± 4.0	29	22–36
Pre-pregnancy BMI (kg/m^2)	637	23 ± 3.5	22	19–30
Education	650			
Upper secondary school (%)	159	24		
≤3 years higher education (%)	134	21		
>3 years higher education (%)	357	55		
Pre-pregnancy smoking	650			
Never (%)	494	76		
Former (%)	70	11		
Current (%)	86	13		

Abbreviations: SD, standard deviation; BMI, body mass index.

Table 3
Total mercury in hair (mg/kg) and total fish consumption (times per month) in primiparous Swedish women from 1996 throughout 2019.

Sampling year	n	Total mercury in hair (mg/kg)					Total fish consumption (times/month)			
		Mean ± SD	Median	25–75th percentile	Range	n	Mean ± SD	Median	25–75th percentile	Range
1996	28	0.46 ± 0.28	0.38	0.27–0.58	0.17–1.5	28	9.8 ± 6.7	9.1	4.5–13	1.5–27
1997	45	0.38 ± 0.24	0.30	0.24–0.41	0.07–1.1	45	8.2 ± 5.4	8.0	3.8–12	0–18
1998	43	0.48 ± 0.38	0.40	0.31–0.55	0.09–1.4	43	8.3 ± 5.5	6.8	4.3–11	0.25–23
1999	8	0.45 ± 0.28	0.35	0.28–0.51	0.21–1.1	8	10 ± 4.1	10	8.8–13	3.0–17
2000	17	0.40 ± 0.16	0.37	0.26–0.56	0.19–0.66	17	14 ± 16	12	6.3–15	4.0–72
2001	11	0.45 ± 0.24	0.41	0.26–0.59	0.21–0.99	11	8.5 ± 6.7	7.3	4.0–9.0	0.5–23
2002	25	0.54 ± 0.39	0.36	0.30–0.75	0.04–1.4	25	9.8 ± 4.7	11	6.3–12	0.5–23
2003	4	0.39 ± 0.11	0.38	0.31–0.47	0.25–0.52	4	6.9 ± 2.2	6.5	5.1–8.6	4.8–9.8
2004	31	0.46 ± 0.27	0.42	0.30–0.59	0.040–1.3	31	12 ± 8.3	10	6.8–17	0–39
2006	38	0.37 ± 0.23	0.32	0.19–0.49	0.12–1.1	38	13 ± 8.7	11	7.3–15	0–36
2007	29	0.35 ± 0.15	0.34	0.26–0.40	0.016–0.73	29	11 ± 7.3	12	6.8–17	0–24
2008	29	0.43 ± 0.26	0.39	0.24–0.56	0.025–0.95	30	11 ± 8.2	9.5	6.3–16	0–34
2009	30	0.40 ± 0.22	0.39	0.23–0.51	0.071–1.1	28	15 ± 9.1	14	7.9–19	2.8–50
2010	30	0.38 ± 0.26	0.29	0.21–0.50	0.020–0.91	28	14 ± 8.8	12	9.5–17	0–41
2011	29	0.36 ± 0.24	0.33	0.23–0.42	0.020–1.3	28	16 ± 14	13	9.3–16	0–65
2012	30	0.24 ± 0.14	0.23	0.13–0.37	0.020–0.53	30	9.5 ± 6.9	8.6	4.3–15	0–21
2013	30	0.37 ± 0.23	0.35	0.23–0.49	0.010–0.95	29	9.9 ± 6.9	9.8	6–16	0–22
2014	30	0.41 ± 0.24	0.38	0.27–0.61	0.010–1.1	27	16 ± 9.4	14	9–22	3.3–37
2015	30	0.42 ± 0.24	0.36	0.27–0.60	0.040–1.0	30	9.7 ± 7.2	7.9	5.8–13	0.5–39
2016	30	0.43 ± 0.21	0.40	0.29–0.60	0.070–0.96	30	12 ± 6.6	11	7.9–14	0–30
2017	30	0.34 ± 0.21	0.31	0.18–0.51	0.016–0.72	30	9.6 ± 9.0	7.1	3.2–12	0–37
2018	30	0.27 ± 0.15	0.21	0.15–0.34	0.072–0.73	30	9.1 ± 6.6	8.1	4.7–13	0–30
2019	48	0.28 ± 0.19	0.25	0.17–0.37	0.030–1.1	47	8.3 ± 5.3	7.7	4.0–11	0–21

Abbreviations: SD, standard deviation.

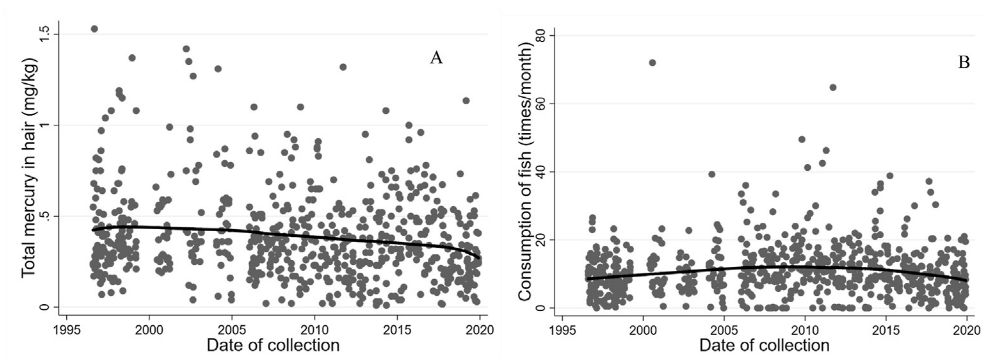

Fig. 2. A-B. Scatter plots with smoothed lowess lines indicating decreasing concentrations of total hair mercury (Jonckheere-Terpstra trend test $p < 0.001$) in Swedish primiparous women from 1996 throughout 2019, in combination with B) a trend of increasing total fish consumption up until 2011 ($p < 0.001$) after which it started to decline ($p = 0.001$).

studies (Cusack et al., 2017; Taylor and Williamson, 2017; Trdin et al., 2019).

We have not been able to identify any other studies which have used hair to biomonitor the exposure to MeHg via fish over time, enabling a direct comparison. Our results are similar to those of a literature review of epidemiological data between 1966 and 2015, finding declining trends in total Hg in whole blood among pregnant women globally and regionally (Europe, Asia and North America) (Sharma et al., 2019). On the other hand, it should be noted that Hg in whole blood is a less specific biomarker of MeHg than hair and may also reflect exposure to inorganic mercury originating from dental amalgam or other dietary sources. However, in all population groups focusing only on studies with documented dietary MeHg exposure there was also a significant decline in whole blood levels of total Hg (Sharma et al., 2019).

The decreasing trend of exposure to MeHg over time in Swedish primiparous women is most likely due to a combination of many different factors, such as changes in fish species available on the Swedish market, leading to an altered consumption pattern of the fish species most commonly consumed, and/or decreasing Hg levels in fish. Unfortunately, the detailed questions on consumption of different fish species in the questionnaire have been altered during this time period, making it difficult to determine if a change in the consumption pattern of different fish species has occurred. In the Swedish Market Basket Survey 2015 (SFA, 2017), including fish baskets representative for the fish/fish products purchased by the average Swedish consumer, the median Hg concentration in the fish baskets was 30 μg/kg, which is well below the lowest FAO/WHO guideline for fish (500 μg/kg). Lean marine fish and farmed salmon were the types of fish most frequently purchased by the Swedish consumers. In the Swedish POPUP study, in which the present women are included, a mean increase in salmon consumption of 0.7 g/day per year was reported from 1996 throughout 2006, and national surveys by SFA in 1996–97 and 2010 support an increased intake of salmon, especially farmed salmon (SFA, 2011). Globally, farmed Atlantic salmon has become an increasingly important source of seafood (Bostock et al., 2010), and farmed Atlantic salmon contains lower Hg levels than wild Atlantic salmon (Lundebye et al., 2017). In addition, levels of Hg in farmed Atlantic salmon have also been declining during the last decades

Table 4
Multiple regression models of total mercury in hair (mg/kg; natural log-transformed) and total fish consumption (times/month) with year of collection and other potential predictors in primiparous Swedish women from 1996 throughout 2019 (n = 622).

Predictors	Total mercury in hair (natural log-transformed)[a]			Total fish consumption (times/month)[b]		
	B (95% CI)	β (95% CI)	p	B (95% CI)	β (95% CI)	p
	R^2 = 0.33			R^2 = 0.089		
Year of collection (per year)	−0.025 (−0.032; −0.018)	−0.25 (−0.31; −0.18)	<0.001			
Year of collection <2011				0.32 (0.17; 0.46)	0.20 (0.11; 0.29)	<0.001
Year of collection ≥2011				−0.66 (−0.92; −0.40)	−0.22 (−0.31; 0.13)	<0.001
Maternal age (years)						
<25	Reference	Reference		Reference	Reference	
≥25 to <30	0.079 (−0.11; 0.27)	0.053 (−0.073; 0.18)	0.42	0.82 (−1.7; 3.3)	0.049 (−0.10; 0.20)	0.52
≥30 to <35	0.31 (0.12; 0.49)	0.20 (0.078; 0.32)	0.001	2.6 (−0.013; 5.2)	0.15 (−0.00077; 0.31)	0.051
≥35	0.24 (−0.038; 0.51)	0.087 (−0.014; 0.19)	0.090	1.1 (−2.2; 4.5)	0.037 (−0.074; 0.15)	0.51
Education						
Upper secondary school	Reference	Reference		Reference	Reference	
≤3 years of higher education	0.15 (−0.012; 0.031)	0.083 (−0.0067; 0.017)	0.069	−0.061 (−1.7; 1.5)	−0.0030 (−0.083; 0.073)	0.94
>3years of higher education	0.27 (0.13; 0.42)	0.18 (0.088; 0.29)	<0.001	1.5 (−0.025; 3.0)	0.089 (−0.0015; 0.18)	0.054
Pre-pregnancy BMI (kg/m^2)						
<25	Reference	Reference		Reference	Reference	
≥25	−0.18 (−0.30; −0.057)	−0.097 (−0.16; −0.031)	0.004	−2.6 (−4.0; −1.3)	−0.13 (−0.20; −0.064)	<0.001
Fish consumption (times/month)						
0	Reference	Reference				
<5	0.59 (0.18; 1.0)	0.30 (0.092; 0.51)	0.005			
≥5 to <10	1.0 (0.64; 1.4)	0.64 (0.41; 0.90)	<0.001			
≥10	1.2 (0.77; 1.5)	0.78 (0.50; 0.98)	<0.001			

Abbreviations: B, unstandardized beta; β, standardized beta; CI, confidence interval, R^2, R-squared - goodness of fit; BMI, body mass index.
[a] Predictors significantly associated with total mercury concentrations in hair in bivariate analyses (year of collection, maternal age, education, pre-pregnancy BMI, and fish consumption).
[b] Predictors significantly associated with total fish consumption in bivariate analyses [year of collection (spline knot at 2011), maternal age, education, and pre-pregnancy BMI].

(Nostbakken et al., 2015). On the other hand, recent findings from a birth cohort study in Northern Sweden, indicate that pregnant women only consume marginally more fatty fish (mean ± SD: 18 ± 14 g/day) than lean (14 ± 12 g/day) fish (Stravik et al., 2019) and that the consumption of fresh water fish is low (2.5% out of 589 participants) (Gustin et al., 2020). For lean marine fish, Atlantic fish has been shown to contain lower MeHg levels than Mediterranean fish (0.43 μg/g versus 1.5 μg/g wet weight) (Junque et al., 2018), but we have not found any data concerning temporal trends of Hg in lean marine fish. The Hg concentrations in Swedish freshwater fish have also declined, particularly in fish from waters in southwestern Sweden where Hg decreased by up to 10% per year (Akerblom et al., 2014). However, the authors noted that 52% of Swedish freshwater fish still exceeded the MeHg guidelines by FAO/WHO in fish (0.5–1.0 mg Hg/kg) (Akerblom et al., 2014). As only 3% of the women in the present study exceed a hair Hg concentration of 1 mg/kg, corresponding to the reference dose set by US EPA (2001) of 0.1 μg MeHg/kg body weight per day, their consumption of freshwater fish appears to be low.

Low intake of freshwater fish and declining MeHg exposure may be a result of dietary advisories established by the SFA, which have been reinforced during the last 50 years, recommending pregnant and lactating women to avoid consumption of fish known to contain high levels of Hg. Indeed, starting from the sampling year 2001–2002, 95% of the present women responded that they were aware of the dietary advisories concerning fish. The custom of informing pregnant women of risks associated with intake of certain fish species has been widely debated, as pregnant women should not be stressed, and that they may avoid or decrease their fish consumption instead of choosing fish naturally low in MeHg. Our results show, on the contrary, that this does not appear to be the case. More than 60% of the pregnant women reported that they consumed fish 2–3 times/week or more, which is likely due to an increased awareness of the beneficial effects of fish consumption on fetal outcomes, especially on fetal brain development (Oken et al., 2005). Interestingly, we were able to detect MeHg in mothers who reported that they did not consume fish, which may for some individuals be related to misclassification of fish consumption. However, measurable MeHg levels have previously been reported in non-fish-eating individuals (Lindberg et al., 2004), and hypothesized to be related to the use of fish meal in animal feed, resulting in exposure via intake of meat and poultry (Dabeka et al., 2003; Shah et al., 2010).

The main strengths of the present study include the 23-year long exposure monitoring period, that the exposure assessment was conducted by measurements in hair, a well-established marker of MeHg exposure (Berglund et al., 2005), and the use of a validated questionnaire for estimating the mothers fish consumption. On the other hand, this study also has several limitations which might have affected the observed declining trend in MeHg exposure. The laboratory method for measuring total Hg in hair has been exchanged once, shifting from CVAFS to ICPMS (2000) and thereafter the ICPMS method was altered in 2018, to using gold instead of hydrochloric acid for stabilization of Hg. Thus, we cannot exclude that some of the observed changes in total Hg are related to these changes. However, in relation to each change in laboratory method we conducted thorough method comparisons analyzing several hair samples in duplicates using the two different methods, showing very good agreement each time within the range of 0.1–0.8 mg Hg/kg and 0.4–2.3 mg Hg/kg (Fig. 1a and b) in hair, respectively. Secondly, misclassification of fish consumption due to measurement error is inevitable in dietary assessment studies. Indeed, an earlier validation of the applied frequency questions in 130 women indicated that individuals tend to overestimate their fish consumption, but despite this there was a good correlation between total Hg in hair and fish intake per month (Strom et al., 2011). Similarly, we found a reasonable correlation between total Hg in hair and fish consumption (Spearman correlation: 0.39; $p < 0.001$). Finally, this temporal trend study only included primiparous women from Uppsala county in central Sweden, and therefore, geographical differences within Sweden cannot be excluded. Biomonitoring of Hg in blood in other countries have indicated differences among women in coastal regions compared to those in the inland (Cusack et al., 2017). In Sweden, fish consumption is not only the main source of MeHg exposure, but also a major source of exposure to polychlorinated biphenyls (PCBs), dibenzo-p-dioxins and dibenzofurans (PCDD/Fs). In a comparison of temporal trends of PCBs and PCDD/Fs in breast milk, the POPUP trends between 2000 and 2017, using the same participants as in the present study, were similarly decreasing as in breast milk from Stockholm and Gothenburg, the two largest urban areas of Sweden (Glynn et al., 2020). This suggests that the POPUP population is representative for populations of young pregnant and nursing women in urban areas of Sweden.

5. Conclusion

The present findings indicate a trend of decreasing MeHg exposure in Swedish primiparous women despite a trend of increasing fish consumption during the majority of the biomonitoring period. In general, the MeHg exposure in Sweden was low with only a few percent of primiparous women exceeding the reference dose for neurotoxic effects. Even though the present study indicate that the MeHg exposure via fish has been decreasing during the last decades further monitoring is warranted, especially as it has been foreseen that ongoing and upcoming climate changes are likely to increase the human exposure to MeHg via fish and seafood (Alava et al., 2017). Also, emerging evidence suggest that Hg exposure below current health-based guideline values may be associated with various adverse health outcomes (Sharma et al., 2019), emphasizing the need to reduce Hg as much as possible to be able to continue to promote fish as a healthful food alterative.

Authorship statement

Maria Kippler: Methodology, Formal analysis, Data curation, Writing - original draft, Visualization, Project administration. Irina Gyllenhammar: Conceptualization, Investigation, Data curation, Writing - review & editing. Anders Glynn: Conceptualization, Writing - review & editing. Michael Levi: Formal analysis, Writing - review & editing. Sanna Lignell: Conceptualization, Investigation, Writing - review & editing. Marika Berglund: Conceptualization, Methodology, Investigation, Writing - review & editing, Supervision, Project administration.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115712.

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