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## Mercury speciation in meconium and associated factors

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### ABSTRACT

Meconium is formed early in gestation and it is normally not excreted until after birth. Thus it may provide a longer and cumulative record of exposure to mercury (Hg). The present study aims to speciate Hg in meconium samples (N = 488) from Slovenian and Croatian new-borns prenatally exposed to low levels of methyl-Hg (MeHg) from maternal seafood intake and to Hg<sup>0</sup> from maternal dental amalgam fillings. We had complete data of total Hg (THg) and MeHg in meconium and THg in maternal hair (MH), while THg and MeHg in maternal blood (MB) were available only for Croatian mothers. Personal data namely maternal seafood intake, age, pre-pregnancy BMI, parity, smoking, estimated gestational age at birth, sex, and birth weight were available for the majority of participants, except the number of dental amalgams which was in most cases missing for Croatian mothers. The median THg concentration in meconium was 11.1 (range: 0.41–375.2) ng/g and inorganic Hg (Hg(II)) presented 98.8% (range: 82%–100%, CV: 2%) of THg. We observed significant correlation between meconium and MH Hg levels, with the highest correlation between hair THg and meconium MeHg. Correlation analysis including MB (available only for Croatian population) showed a significant positive correlation between THg in meconium and THg in MB ( $R_s = 0.642$ ). Additionally, MeHg from MB was correlated with MeHg in meconium ( $R_s = 0.898$ ), while the correlation between Hg(II) in MB and meconium was positive, but not significant. Maternal seafood intake was significantly correlated with meconium MeHg ( $R_s = 0.498$ ) and Hg(II) ( $R_s = 0.201$ ). Multiple linear regression (performed on the Slovenian population, N = 143) confirmed a positive association between meconium MeHg and seafood intake. Furthermore, meconium Hg(II) was positively associated with the number of maternal dental amalgam fillings, but linear regression models did not confirm correlation between seafood intake and meconium Hg(II) levels. We assume that Hg<sup>0</sup> released from maternal dental amalgam fillings and MeHg from seafood intake were both transported through the placental barrier and partitioned between different foetal compartments including meconium. Weak correlation between maternal seafood intake and Hg(II) levels in meconium suggests that there is certain evidence of MeHg demethylation. However, because this correlation was not confirmed by the multiple regression, MeHg demethylation during prenatal life cannot be neither confirmed nor excluded. Further investigations at higher level of exposure are needed to confirm this observations. We can conclude that meconium is a suitable biomarker for MeHg and Hg<sup>0</sup> exposure during pregnancy. However, comparability of the results reported in meconium in different studies is hindered by a lack of standardized sampling protocols, storage, and analysis.

### 1. Introduction

Meconium is formed early in gestation, presumably from the 13th week of gestation, and it is normally not excreted until after birth, usually in the first 72 h in amount of 20–70 g (Bearer, 2003; Concheiro and Huestis, 2018). It is a dark viscous material that mostly consists of eliminated mucosa epithelia, water, bile, bile acids, epithelium cells,

and other lipids swallowed with the amniotic fluid (Concheiro and Huestis, 2018). The total weight of meconium increases exponentially with gestational age; about 1 g of meconium accumulated in the foetal gut during 23–26 weeks of gestation and about 5 g after 27–32 weeks. According to recent data ~80% of meconium accumulates after 38 weeks of pregnancy (Bakdash et al., 2010; Concheiro and Huestis, 2018). Accordingly, meconium analysis provides an overview of the

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exposure primarily during the last trimester of pregnancy. In the case of prenatal low-level and long-term exposure to various pollutants, meconium analysis was shown to be more sensitive compared with blood as meconium is cumulative and repository matrix (Park and Lee, 2014). Furthermore, meconium represents foetal tissue and is therefore a direct measure of exposure, by contrast to maternal blood (MB) or hair (MH), which provide only indirect estimations of exposure (Ostrea et al., 2006).

Few studies have reported mercury (Hg) levels in meconium (Gundacker et al., 2010; Jiang et al., 2014; Knezović et al., 2016; Ostrea et al., 2002; Peng et al., 2015; Ramirez et al., 2003; Rothenberg et al., 2019; Unuvar et al., 2007). Nonetheless, Hg concentrations in meconium are known to generally be higher than those in MB (Ramirez et al., 2000). Except the recent one (Rothenberg et al., 2019) none of the studies so far performed Hg speciation. On a small sample size (N = 14) Rothenberg et al. reported 99%–100% of meconium Hg being in inorganic (Hg(II)) form (Rothenberg et al., 2019). Based on listed studies we cannot conclude what are Hg kinetics and biotransformation mechanisms in meconium during prenatal life. The general population is usually exposed to low levels of different Hg species, including organic Hg from seafood intake (mostly as monomethyl-Hg,  $\text{CH}_3\text{Hg}^+$ , MeHg), elemental Hg ( $\text{Hg}^0$ ) vapour from dental amalgam fillings, and, potentially, inorganic Hg (Hg(II)) from cosmetics and food. Placental transport of  $\text{Hg}^0$  vapour released from dental amalgam and inhaled from the atmosphere has already been confirmed before (Clarkson et al., 1972; Yoshida, 2002). Further, MeHg readily penetrates placental and blood-brain barriers (Kajiwara et al., 1996) where it can enter foetal circulation. However, the exact MeHg dynamics (including individual variations) remain unclear (Caito et al., 2018). At the same time, it was long believed that Hg(II) is accumulated in the placenta and therefore limits the amount of Hg reaching the foetus (Al-Saleh et al., 2011). However, researchers showed that in rats, although having slightly different Hg kinetics than humans, Hg(II) can also pass the placental barrier in small amounts (Oliveira et al., 2015). Hg transport from MB to foetal tissues is further supported by some studies that reported positive correlations between meconium total Hg (THg) levels with fish consumption during pregnancy (Jiang et al., 2010; Knezović et al., 2016) or with dental amalgams (Gundacker et al., 2010); however, all studies do not concur on this point (Unuvar et al., 2007). Because meconium is not usually used in studies assessing prenatal Hg exposure, little is known about the possible kinetic mechanisms of different Hg species in meconium.

The present study aims to speciate Hg in meconium samples (N = 488) from Slovenian and Croatian new-borns prenatally exposed to low levels of MeHg from maternal seafood intake and to  $\text{Hg}^0$  from maternal dental amalgam fillings. We used existing datasets which contained information on participants' lifestyle and personal characteristics and THg and MeHg concentrations in MH and MB (Miklavčič et al., 2013, 2011; Trdin et al., 2019). In this study, we performed Hg speciation in meconium and evaluated the associations between Hg species in meconium with Hg concentrations in MH and MB as well as with specific participants' characteristics and habits that could contribute to Hg levels in meconium.

## 2. Materials and methods

### 2.1. Study population and sampling

Our study population included healthy pregnant women and their new-borns from Slovenia (the city of Ljubljana and its surroundings up to 50 km) and from the Adriatic coastal region of Croatia (the city of Rijeka and its county Primorsko-goranska). They were recruited in 2007–2009 as a part of the PHIME (Public Health Impact of Long-term Low-level Mixed Element Exposure in Susceptible population Strata; EU 6th Framework Programme) birth cohort study. The study was conducted in accordance with the Declaration of Helsinki; the Republic of

Slovenia National Medical Ethics Committee approved the protocol (No. 98/05/06) for the Slovenian participants and the Ethics Committee of University Hospital Centre Rijeka approved the protocol (No. 2170-29-02/1-07-1) for the Croatian participants. All subjects gave their informed consent.

Valent et al. (2013b) and Miklavčič et al. (2013) described the study design, recruitment, sampling, and questionnaires in detail. MH samples were stored at room temperature in a zip-lock plastic bag and then analysed without any cleaning. MB and meconium were stored in a freezer at temperature below  $-24^\circ\text{C}$ . Unfortunately, not all women who gave their informed consent answered precisely to all questions from questionnaires and not all of them were sampled for listed matrices. Therefore, the number of included participants in statistical analysis varies throughout the text; precise number of included participants have been provided in the tables and figures.

Pregnant women were recruited and sampled in the 3rd trimester (28–41 weeks of gestation). MB was available only for the Croatian part of the population, but maternal hair was available for both populations. In most cases meconium was sampled on the day of birth. However, there were cases where meconium was sampled in the first few days ( $\leq 3$  days) after birth. Unfortunately, the information when meconium was sampled was not available for all of the collected samples. However, in all cases meconium was sampled (5–10 g) from the diaper and was frozen (below  $-24^\circ\text{C}$ ) until analysis were carried out.

### 2.2. Data collected with questionnaires

During 3rd trimester women filled out short questionnaire for assessment of their individual characteristics. Approximately one month after delivery, women filled out the long questionnaire which provided more detailed information about the socio-demographic and health status of the whole family, smoking habits, food intake frequency, number of dental amalgams, etc. Women reported smoking before or during pregnancy, and we generated the new variable of 'ever-smokers' to include all smokers regardless of the period of smoking. Seafood intake during pregnancy was estimated from the long questionnaire. This questionnaire included seven questions on seafood that addressed the frequency of consumption of 150-g servings of fish, crustaceans, and molluscs and tuna, mackerel, or sardines in oil. We converted and combined all listed categories to estimate daily seafood intake (Miklavčič et al., 2013; Trdin et al., 2019; Valent et al., 2013a). To assess the number of dental amalgams, women were divided into four classes (< 3 amalgams, 3–5 amalgams, 6–9 amalgams, 10 + amalgams). Information on dental amalgam fillings was incomplete, especially for the Croatian part of the population. Women also reported the number of births with gestational age of at least 24 weeks regardless of whether the babies were born alive or stillborn. According to their answer, we divided them into two groups: nulliparous and parous women.

### 2.3. Mercury measurements methods

All analyses of THg and MeHg in biological samples were performed at the Jožef Stefan Institute, Ljubljana, Slovenia. Total mercury in MH, MB, and meconium of Croatian population was determined using a Direct Mercury Analyser (DMA; Milestone, USA). The method is described in detail elsewhere (EPA Method 7473, 1998; Miklavčič et al., 2011). Meconium samples of the Slovenian population were digested with the addition of 2 mL of a mixture of 65%  $\text{HNO}_3$  + 70%  $\text{HClO}_4$  (1:1, v/v) and 5 mL of 96%  $\text{H}_2\text{SO}_4$ . Samples were heated to  $220^\circ\text{C}$  for 20 min. For determining THg in the digested samples, a semi-automated Mercury Analyser based on cold vapour atomic absorption spectrometry (CVAAS, Model Hg-201; Sanso Seisakusho Co. Ltd., Japan) was used. The method is described in detail elsewhere (Akagi, 1997). All measurements were performed under strict quality control procedures and gave comparable results (Miklavčič et al., 2011; Trdin et al., 2019).

**Table 1**  
Basic demographic characteristics of studied population.

Participant's characteristics	Slovenia (N = 287)	Croatia (N = 201)	All (N = 488)	p-value
Seafood intake (meals per day)	0.30 (0–4.5), N = 225	0.44 (0–2.21), N = 183	0.37 (0–4.5), N = 408	0.000 <sup>b</sup>
Dental amalgam (% of < 3 amalgams/3–5 amalgams/6–9 amalgams/≥ 10 + amalgams)	7/34/40/19, N = 212	–	–	–
Ever smoking (% of smokers)	35, N = 98	42, N = 85	38, N = 183	0.002 <sup>c</sup>
Pre-pregnancy BMI (kg/m <sup>2</sup> )	24.3 (17.1–44.5), N = 277	22.9 (16.8–40.7), N = 200	23.7 (18.8–44.5), N = 477	0.001 <sup>b</sup>
Maternal age (years)	30.6 (20–45), N = 278	30.0 (19–43), N = 190	30.4 (19–45), N = 468	0.285 <sup>d</sup>
Parity (% of nulliparous)	49, N = 132	44, N = 88	47, N = 220	0.080 <sup>c</sup>
Child's sex (% of boys/girls)	53/47	50/50	52/48	0.728 <sup>c</sup>
Gestational age (weeks)	39.5 (28–42), N = 224	39.3 (34–41), N = 154	39.4 (28–42), N = 378	0.302 <sup>a</sup>
Birth weight (kg)	3.4 (2.0–4.8), N = 224	3.5 (2.4–4.8), N = 180	3.5 (2.0–4.8), N = 404	0.037 <sup>a</sup>

<sup>a</sup> Mann Whitney U.<sup>b</sup> Kruskal-Wallis.<sup>c</sup>  $\chi^2$ .<sup>d</sup> ANOVA.

To check the accuracy of the meconium measurement method, we used the NIST SRM 2781 Domestic sludge. The assigned value ( $3.68 \pm 0.14 \mu\text{g/g}$ ) was in good agreement with the determined value ( $3.62 \pm 0.08 \mu\text{g/g}$ ,  $k = 2$ ;  $n = 12$ ).

Unfortunately, in some cases there was not enough sample to perform both measurements for THg and MeHg. Therefore, MeHg in meconium was measured in samples that were available. For this purpose, the samples were first homogenised and then  $\sim 0.2 \text{ g}$  was weighed into glass tubes. Then, 10 mL of 4 M HNO<sub>3</sub> was added and the mixture was heated at 72 °C for 24 h (Hammerschmidt et al., 2013). After cooling, the digested samples were diluted to 30 mL with MQ water. An aliquot of the digested sample was transferred to measuring glass vials, and neutralised with 0.7 mL of 13 M KOH and the pH was adjusted to 4.6 with the addition of 1 mL of citrate buffer. Then, 50  $\mu\text{L}$  of ethylating reagent (1% NaBEt<sub>4</sub> in 1% KOH) (Liang et al., 1994) was added to the measuring vials. The vials were capped and placed into the Tekran 2700 automated system with an auto sampler. Ethylated MeHg as ethylmethylmercury was purged onto a Tenax trap and thermally desorbed (180 °C) onto an isothermal gas chromatography (GC) column. Hg species were converted to Hg<sup>0</sup> by pyrolysis at 600 °C and measured by cold vapour atomic fluorescence detector (CVAFS, Tekran Instruments Corporation, Canada). To check the accuracy of MeHg measurements, we analysed lyophilised whole human blood PT-WB1 obtained from a non-exposed population as a reference material. MeHg in PT-WB1 was determined in the PHIME project through interlaboratory comparisons. The assigned value ( $5.8 \pm 0.5 \mu\text{g/kg}$ ) was in good agreement with the determined value ( $5.76 \pm 0.25 \mu\text{g/kg}$ ,  $k = 2$ ;  $n = 12$ ).

Total Hg and MeHg concentrations were determined on a wet weight basis because we observed some MeHg degradation during lyophilisation. The average H<sub>2</sub>O content calculated on a subsample ( $N = 10$ ) was  $75.8 \pm 2\%$ . Inorganic mercury levels (presumably the majority was in the form of Hg(II)) were calculated by subtracting MeHg from THg.

The estimated measured uncertainty for Hg determination in meconium was 9.8% ( $k = 2$ ) for THg measurements, 11.4% ( $k = 2$ ) for MeHg measurements, and 15.0% ( $k = 2$ ) for Hg(II) levels. The limits of detection (LODs) calculated as three times the standard deviation of the blank sample were 0.11 ng/g for THg and 0.02 ng/g for MeHg measurements. The limits of quantifications (LOQs) calculated as ten times the standard deviation of the blank sample were 0.36 ng/g for THg and 0.05 ng/g for MeHg.

#### 2.4. Statistical analysis

Principal component analysis (PCA) was performed to summarise the main patterns of variations between Hg in meconium, seafood intake, and number of dental amalgams as active variables and personal characteristics and habits as supplementary variables (Spearman rank correlation with Varimax rotation;  $N = 100$ ). PCA was performed using the XLSTAT statistical package.

Further statistical analysis were performed using STATA 12 software. The distribution of measured Hg species in samples was checked using the Shapiro-Wilk test. Hg concentrations in all measured samples were not normally distributed. Therefore, median values were used to describe the original data. For comparison between populations, we used the Mann Whitney U; chi-square ( $\chi^2$ ); ANOVA; and Kruskal-Wallis tests, depending on the variable used.

In the next step, Spearman correlations between meconium Hg species and Hg in MB was performed only for Croatian population, while other correlations (meconium Hg levels and MH Hg, dental amalgams, seafood intake, BMI, age, gestational age, birth weight) included both populations. To confirm observed Spearman's correlations we performed multiple linear regression on Slovenian population, as they had complete information on dental amalgams and seafood intake. Before performing multiple linear regression analysis, the Hg

**Table 2**

Results of total Hg (THg), methyl-Hg (MeHg as Hg), and inorganic Hg (Hg(II)) in ng/g wet weight; and percentage of Hg(II) (%) in the samples of meconium from our study; and Hg concentrations in meconium as reported in the selected literature. Median values, range, and number observed are shown below.

Meconium Hg		Our study			Literature					
		Slovenia	Croatia	All	Philippines, Manila <sup>a</sup>	USA <sup>b</sup>	Austria <sup>c</sup>	Philippines, Tagum <sup>d</sup>	Taiwan <sup>e</sup>	Turkey <sup>f</sup>
THg [ng/g]	Median	11.5	10.1	11.1	3.17	3.9	4.0	48.6	82.6	9.4 (µg/g)
	Range	0.41–375.2	1.18–359.8	0.41–375.2	0.43–71.6	0.64–11.0	0.4–128	20–200		0–66.5
	N	287	201	488	426	14	36	36	545	143
	CV (%)	170	165	169						
MeHg [ng/g]	Median	0.07	0.19	0.10		0.0078				
	Range	0.005–1.39	0.005–3.13	0.005–3.13		< MDL-0.078				
	N	204	145	349		17				
	CV (%)	139	124	147						
Hg(II) [ng/g]	Median	10.6	9.98	10.4		3.9				
	Range	0.40–375.2	1.63–359.7	0.40–375.2		0.64–11.0				
	N	204	145	349		14				
	CV (%)	195	179	189						
%Hg(II) [%]	Median	99.2	98.1	98.8		100				
	Range	81.9–100	88.2–100	81.9–100		99–100				
	N	204	145	349		14				
	CV (%)	2.2	1.9	2.1						

All-combined Slovenian and Croatian population; CV-coefficient of variation.

<sup>a</sup> Ostrea et al., 2002, in ng/ml.

<sup>b</sup> Rothenberg et al. (2019).

<sup>c</sup> Gundacker et al. (2010).

<sup>d</sup> Ramirez et al. (2000).

<sup>e</sup> Jiang et al. (2014).

<sup>f</sup> Unuvar et al. (2007), in µg/g.

concentrations in meconium were log-transformed. Models were designed to study associations between meconium Hg species and possible covariates. Models were adjusted for selected personal characteristics and habits (seafood intake, number of dental amalgam fillings, smoking status, pre-pregnancy body mass index (BMI), maternal age, parity, gestational age, child's sex, and birth weight) and were included to provide the highest  $R^2$ . They were designed to cover and include all possible variables that could have an impact on meconium Hg levels. The level of significance ( $p$ ) was set at 0.05.

### 3. Results and discussion

Our studied population included pregnant women and their newborns from central Slovenia ( $N = 287$  pairs) and coastal Croatia ( $N = 201$  pairs). The average age of mothers at delivery was 30.4 years, their pre-pregnancy BMI was 23.7 kg/m<sup>2</sup>, and for 47% of them having their first pregnancy. Approximately 3% of women self-reported never eating any kind of seafood. We identified 38% of women as 'ever-smokers'. New-borns' average gestational age was 39 weeks and their average birth weight was 3.5 kg, and 52% of them were boys. Table 1 lists all collected demographic data, including  $p$  value to determine the difference between both populations.

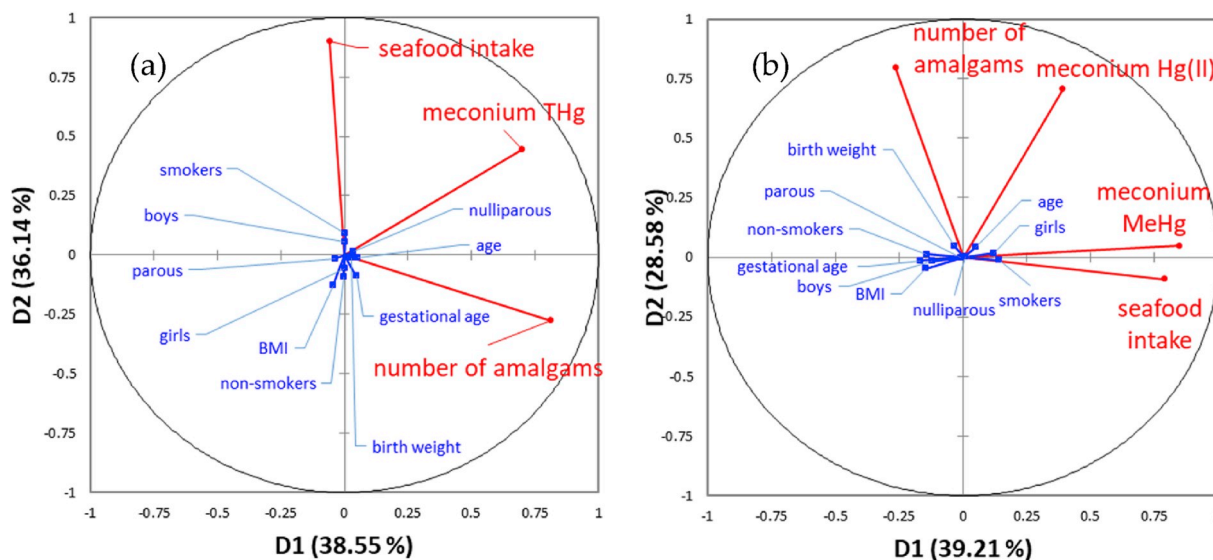
In this issue we have presented the Hg speciation results in MB and cord blood from the same population (Trdin et al., 2019). Table 2 lists the results of Hg measurements in meconium. Median THg concentrations in meconium (reported in wet weight) are on average higher than those reported previously in MB and cord blood of the same participants, namely 2.04 and 1.84 ng/g, respectively (Miklavčič et al., 2013). Median THg concentrations measured in meconium from our study are higher compared to those found in Manila, the Philippines, USA, and Austria, and lower than those reported in Tagum, the Philippines, Taiwan, and Turkey (Table 2). The study from Tagum, Philippines (Ramirez et al., 2000) reported much higher levels of meconium Hg in comparison with study from Manila, Philippines (Ostrea et al., 2002). It could be that results differ because the Tagum study included participants from artisanal gold mining and processing community. In contrast, study from Manila included randomly selected newborns from different hospitals, which were included to present socioeconomic

cross-section of hospitals in Manila.

On the other hand, our data showed wider range of exposure with higher maximum values (Table 2). Only one study from Istanbul reported much higher Hg levels with average value of 9.4 µg/g (Unuvar et al., 2007). We did not observe a statistically significant difference between Slovenian and Croatian population in meconium THg levels ( $p = 0.601$ ) or Hg(II) levels ( $p = 0.437$ ), however, there was a statistically significant difference between populations in meconium MeHg ( $p < 0.001$ ) and % Hg(II) ( $p < 0.001$ ) levels. The MeHg level in the Croatian population is higher because pregnant women from Croatia ate seafood more frequently than did Slovenian women (Table 1). Levels of meconium THg were significantly correlated with meconium MeHg ( $R_s = 0.345$ ,  $p < 0.001$ ) and Hg(II) ( $R_s = 0.999$ ,  $p < 0.001$ ), and also correlation between meconium MeHg and Hg(II) was significant ( $R_s = 0.329$ ,  $p < 0.001$ ).

As observed from Table 2, Hg(II) presented the majority (82%–100%) of measured THg in meconium. To the best of our knowledge, this is the first study to perform Hg speciation in meconium with such a large sample size. Median values of meconium Hg are ~2-fold higher compared to study from USA. Additionally, our results showed wider range of exposure to all Hg species (Table 2). The latter is presumably related to the fact that they performed Hg speciation on a very limited sample size ( $N = 14$ ) and median THg concentrations found in blood of participating women were also lower, compared with ours (0.48 vs. 2.04 ng/g) (Rothenberg et al., 2019; Trdin et al., 2019).

Intestinal MeHg demethylation and biotransformation to inorganic Hg are clearly important steps in MeHg excretion from the adult body (Caito et al., 2018; Rowland et al., 1977), but in some cases higher proportions of MeHg in faeces indicated that MeHg can also be trapped in faeces until defecation (Rand et al., 2016). In comparison with MeHg, Hg(II) in faeces has lower (re)absorption capacity (~95% vs. ~7%) and the major part of excreted Hg(II) is retained in faeces until defecation (Horvat et al., 2012). According to literature data, we hypothesised that MeHg demethylation makes the highest contribution to Hg(II) content not only in faeces (Ou et al., 2014) but also in meconium (Rothenberg et al., 2019), although intestinal demethylation mechanisms are not fully characterised (Rothenberg et al., 2017). For comparison, Hg(II) content in human stool samples varies from 68% to >



**Fig. 1.** PCA between active variables (marked in red), namely, (a) seafood intake, number of dental amalgams, and meconium THg levels; as well as (b) seafood intake, number of dental amalgams, meconium Hg(II), and meconium MeHg; and supplementary variables (marked in blue), namely, maternal smoking status, maternal age, pre-pregnancy BMI, parity, gestational age, sex, and birth weight for the Slovenian population. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

95% of THg (Rand et al., 2016). In addition to MeHg demethylation, high Hg(II) content also results from Hg<sup>0</sup> oxidation released from dental amalgam fillings and inhaled from the atmosphere which is oxidised by catalase in blood and other tissues (Clarkson, 1997) and from accumulation of Hg(II) from food, cosmetics and other sources (Ou et al., 2014). According to all possible sources of Hg(II) it is difficult to find a reliable estimation of contribution for each one.

PCA was performed to test the most probable origin of meconium Hg, the factors that could contribute to high Hg(II) content in meconium, and how our data is correlated. Fig. 1a shows results of PCA between main (active) variables (marked in red), namely, meconium THg, maternal seafood intake, and number of dental amalgams, and covariate (supplementary) variables (marked in blue), namely, maternal smoking status, maternal age, pre-pregnancy BMI, parity, gestational age, sex, birth weight). The first PCA covered 74.69% of variability (N = 100). In the second PCA (Fig. 1b) we included Hg speciation in meconium, and therefore the active variables include meconium Hg(II) and MeHg, maternal seafood intake, and number of dental amalgams and the supplementary variables are the same as in Fig. 1a. The second PCA covered 67.79% of variability (N = 100). Based on the results in Fig. 1a, we can assume that meconium THg levels are correlated with both, maternal seafood intake and number of dental amalgams. Furthermore, Fig. 1b shows that MeHg in meconium was highly related with maternal seafood intake. By contrast, Hg(II) in meconium was more related to number of dental amalgam fillings than to seafood intake. However, the correlation between meconium Hg(II) and amalgams is not as strong as that between meconium MeHg and seafood intake. The reason can be that not all Hg(II) is accumulated in meconium, a part is circulating between foetal swallowing of amniotic fluid and foetal urine excretion (Gilbert and Brace, 1989; Stigter et al., 2011).

Beside the PCA we further examined the Spearman correlation coefficients, which are presented in Table 3. Results of Spearman's correlation analysis are consistent with correlations observed from PCA. We found a significant correlation between seafood intake and Hg species in meconium, with the highest correlation in case of meconium MeHg levels ( $R_s = 0.498$ ). Maternal seafood intake was also significantly correlated with meconium Hg(II) levels ( $R_s = 0.201$ ). Furthermore, number of dental amalgam fillings were weakly correlated with THg ( $R_s = 0.175$ ) and Hg(II) ( $R_s = 0.145$ ) in meconium but not

with MeHg (negative, nonsignificant correlation). Correlations between Hg species in meconium and maternal blood were additionally performed as rough estimations, because we sampled MB at only one point during pregnancy, but meconium starts to form at the beginning of the 2nd trimester and MB was available only for Croatian population. However, all Hg that can be passed to the foetal side originates from maternal blood (plasma). The correlation between MeHg in MB and meconium was high and significant ( $R_s = 0.898$ ); however Hg(II) levels in MB and meconium were not significantly correlated. These results are consistent with the recently published study (Rothenberg et al., 2019). Moreover, Table 3 showed correlation between meconium and MH Hg levels, as the latter, like meconium, indicates, a long-term exposure (Horvat et al., 2012). The highest correlation was observed between THg in hair and MeHg in meconium ( $R_s = 0.829$ ). Despite the discrepancy regarding the time of sampling as described above, the observed correlations obviously reflect constant dietary habits of the mothers during the pregnancy period.

To confirm the correlations shown in Table 3, we performed multiple linear regression analyses (Table 4). In linear regression modelling we did not adjust for MB. The major reason for exclusion of blood is to avoid misinterpretations, as blood was taken only once in the 3rd trimester; further, we did not have the exact time of the collection of meconium after birth, and in literature it was shown that Hg concentrations measured in meconium depend on the time of its collection (Ortega Garcia et al., 2006). Furthermore, blood was available only for the Croatian population, which also had a lot of missing values for dental amalgams. Therefore, we decided to include only the Slovenian population in the regression modelling (N = 143) and to include only those variables (e.g. seafood intake, dental amalgam), which present exposure to Hg and presumably did not change throughout the pregnancy or could modify Hg kinetics mechanisms (BMI, age, smoking status, parity, etc.).

As shown in Table 4, meconium THg and Hg(II) levels were positively associated with the number of dental amalgams. Furthermore, meconium MeHg levels were associated with maternal seafood intake. The meconium ratio between Hg(II) and MeHg levels suggested that this ratio is negatively associated with maternal seafood intake and positively associated with the number of dental amalgams. Girls had statistically higher levels of MeHg in meconium (0.24 vs. 0.20 ng/g); however, this difference is probably not physiologically important. By

**Table 3** Spearman's correlations (Rs) between Hg species in meconium and Hg levels in maternal hair (MH), maternal blood (MB), and personal characteristics and habits.

	MH		MB <sup>s</sup>		MeHg	Hg(II)	%MeHg	Seafood intake	Dental amalgam	BMI	Age	Gestational age	Birth weight
	THg	THg	THg	THg									
THg in meconium	0.314*** (N = 471)	0.642*** (N = 198)	0.573*** (N = 198)	0.075 (N = 198)	0.267*** (N = 198)	0.184*** (N = 408)	0.175** (N = 255)	-0.108* (N = 477)	0.112* (N = 468)	-0.015 (N = 378)	-0.001 (N = 404)		
MeHg in meconium	0.829*** (N = 337)	0.857*** (N = 142)	0.898*** (N = 142)	-0.287** (N = 142)	0.646*** (N = 142)	0.498*** (N = 289)	-0.094 (N = 181)	-0.233*** (N = 341)	0.144** (N = 336)	-0.183** (N = 268)	-0.042 (N = 286)		
Hg(II) in meconium	0.297*** (N = 337)	0.614*** (N = 142)	0.539*** (N = 142)	0.112 (N = 142)	0.192* (N = 142)	0.201*** (N = 289)	0.145* (N = 181)	-0.130* (N = 341)	0.084 (N = 336)	-0.050 (N = 268)	-0.007 (N = 286)		

MH-maternal hair; MB-maternal blood; \*p < 0.05; \*\*p < 0.005; \*\*\*p < 0.0001; <sup>s</sup>available for Croatian mothers only.

combining the results from Tables 3 and 4, we can suggest, that both MeHg from seafood (Kajiwara et al., 1996) and Hg<sup>0</sup> vapour from dental amalgams (Clarkson et al., 1972) were transported through the placental barrier and accumulated in meconium.

Regression modelling showed that meconium MeHg was highly associated with maternal seafood intake; however, statistically significant correlation (Table 3) between seafood intake and meconium Hg (II) was not confirmed in linear regression modeling (Table 4). Therefore, MeHg demethylation process could not be entirely confirmed nor excluded. As multiple regression (Table 4) was performed only on Slovenian population, where seafood intake is lower compared with Croatian participants (Table 1), it is possible, that at higher levels of exposure the results of statistical analysis would be different. However, results from Rothenberg et al. (2019) which observed no correlation between fish servings and meconium Hg(II) during late gestation could be consistent with our study. On the other hand, the lack of an association in the study of Rothenberg et al. could be explained by their limited sample size (N = 14) or with low seafood intake. Although MeHg demethylation in the environment and biota is well established (Du et al., 2019), and gut demethylation of MeHg in adults was confirmed as an essential step in reducing its bioavailability (Li et al., 2018; Rand et al., 2016), based on our data, we cannot confirm nor exclude the hypothesis of intestinal MeHg demethylation during prenatal life. If MeHg demethylation occurs we expect that seafood intake, as a major source of MeHg in MB, would be positively associated with Hg(II) in meconium. This agrees with an in vitro study indicating that human neonate and infant cannot demethylate MeHg in the gut (Rowland et al., 1983). Furthermore, MeHg demethylation was shown to be initiated postnatally (Byczkowski and Lipscomb, 2001). It is assumed that with increased maternal seafood intake the MeHg concentration in maternal plasma increases, resulting in increased placental transport of MeHg (Kajiwara et al., 1996; Kuhnert et al., 1981). As we found relatively low MeHg concentrations in meconium, we hypothesise that the major part of MeHg is distributed throughout foetal tissues and partially returned in maternal blood system, and that only a small part is excreted to the meconium. Once inside the meconium, MeHg is probably there accumulated without any biotransformation, given that intestinal functions are not initiated during the prenatal period and that the processes of bile excretion, gut absorption, and gut demethylation are usually assumed to be inactive for physiologically based pharmacokinetic modelling (Ou et al., 2018). Clearly, further studies are needed, to confirm this observations and to establish mechanisms at higher levels of exposure.

If we focus on the statistical model with meconium Hg(II) levels (Table 4), the results showed weak, but significant association between Hg(II) in meconium and the mother's number of dental amalgam fillings. Similarly, Rothenberg et al. reported significant positive correlation between meconium THg and maternal amalgam fillings (Rothenberg et al., 2019). Consistently, Gundacker et al. reported positive association between both THg in meconium and Hg(II) levels in placenta and the number of dental amalgam fillings (Gundacker et al., 2010). Two mechanisms may be responsible for such association. The first is the Hg<sup>0</sup> transport through the placental barrier (Clarkson et al., 1972; Yoshida, 2002) and oxidation of Hg<sup>0</sup> to Hg(II) on the foetal side (Takahashi et al., 2003) and the second is Hg<sup>0</sup> oxidation in MB (Magos et al., 1978) and Hg(II) transport through the placenta (Oliveira et al., 2015; Ou et al., 2014).

Hg<sup>0</sup> vapour is readily absorbed by the lungs and diffused into the blood (Park and Zheng, 2012). Hg<sup>0</sup> is, to some extent, dissolved in blood; a minor part is bound to haemoglobin; and the majority is oxidised to Hg(II) by catalase (Magos et al., 1978). Although the oxidation process occurs in a relatively short time, it is still long enough for lipid-soluble Hg<sup>0</sup> to penetrate the blood-brain and placental barriers (Park and Zheng, 2012). The exact mechanism and location of Hg<sup>0</sup> oxidation is currently unknown; however, it was shown that as in maternal tissues foetal tissues could also convert Hg<sup>0</sup> to Hg(II). An animal study

**Table 4** Results of multiple regression models (N = 143) for Slovenian population. Levels of ln Hg species in meconium adjusted for seafood intake, number of dental amalgams and some other personal characteristics and habits.

MODELS	ln THg β; p (95% CI) model: p = 0.05, R <sup>2</sup> = 0.119	ln MeHg β; p (95% CI) model: p < 0.001, R <sup>2</sup> = 0.263	ln Hg(II) β; p (95% CI) model: p < 0.05, R <sup>2</sup> = 0.119	ln Hg(II)/MeHg β; p (95% CI) model: p < 0.001, R <sup>2</sup> = 0.222
Seafood intake (meals per day)	0.22; 0.602 (-0.62; 1.07)	<b>2.15; 0.000 (0.29; 3.01)</b>	0.19; 0.659 (-0.67; 1.05)	<b>-1.83; 0.000 (-2.75; -0.92)</b>
Number of dental amalgams	<b>0.31; 0.002 (0.12; 0.50)</b>	0.07; 0.489 (-0.13; 0.26)	<b>0.32; 0.002 (0.12; 0.51)</b>	<b>0.24; 0.011 (0.06; 0.43)</b>
Smoking (non-smokers vs. smokers)	-0.33; 0.075 (-0.68; 0.03)	-0.10; 0.588 (-0.46; 0.26)	-0.33; 0.074 (-0.69; 0.03)	-0.22; 0.263 (-0.62; 0.17)
Pre-pregnancy BMI <sup>a</sup> (kg/m <sup>2</sup> )	-0.02; 0.211 (-0.06; 0.01)	-0.02; 0.359 (-0.05; 0.02)	-0.02; 0.211 (-0.09; 0.01)	-0.001; 0.976 (-0.04; 0.04)
Mother's age (years)	-0.01; 0.648 (-0.05; 0.03)	0.02; 0.323 (-0.02; 0.06)	-0.01; 0.643 (-0.05; 0.03)	-0.03; 0.207 (-0.08; 0.02)
Parity (nulliparous vs. parous)	0.02; 0.907 (-0.32; 0.36)	-0.23; 0.192 (-0.57; 0.12)	0.02; 0.885 (-0.32; 0.37)	0.29; 0.131 (-0.09; 0.67)
Estimated gestational age (weeks)	-0.06; 0.211 (-0.16; 0.04)	-0.08; 0.104 (-0.18; 0.02)	-0.06; 0.219 (-0.16; 0.04)	0.03; 0.591 (-0.08; 0.14)
Child's sex (boys vs. girls)	0.23; 0.201 (-0.59; 0.12)	<b>0.48; 0.009 (0.12; 0.84)</b>	-0.24; 0.186 (-0.60; 0.12)	<b>-0.64; 0.001 (-1.03; -0.25)</b>
Birth weight (g)	0.0001; 0.864 (-0.0004; 0.001)	7.8 × 10 <sup>-6</sup> ; 0.971 (-0.0004; 0.0004)	0.00003; 0.887 (-0.0004; 0.0005)	0.0001; 0.727 (-0.001; 0.00004)

Bolded are significant associations.  
a -body mass index.

**Table 5**

Average contents (with range and number observed) of inorganic Hg (%Hg(II)) content in different biological matrices as reported in literature and herein. For MB and meconium are presented median values, and for placenta and amniotic fluid arithmetic means.

	MB <sup>a</sup>	placenta <sup>b</sup>			amniotic fluid <sup>c</sup>	meconium <sup>d</sup>
		central	amnion	chorion		
% Hg(II)	14.1	79	65	75	75	98.8
	0-95.7	43-95	38-90	52-94	65-88	81.9-100
	225	17	17	17	57	349

<sup>a</sup> Trdin et al. (2019).  
<sup>b</sup> Horvat et al., 1991.  
<sup>c</sup> Suzuki et al. (1977).  
<sup>d</sup> Present study.

indicated that the distribution of Hg<sup>0</sup> and oxidation to Hg(II) is determined by tissue-specific factors such as perfusion rate and the concentration of Hg-binding ligands (Morgan et al., 2002). Literature on Hg speciation in human placenta and amniotic fluid is limited. To compare levels of Hg species in different biological matrices we took data from Hg speciation in maternal blood from the same population (Trdin et al., 2019) and data on Hg speciation in placental tissue performed on Slovenian population (central Slovenia) (Horvat et al., 1991). Unfortunately, Suzuki et al. did not report on Hg levels in maternal blood, but to the best of our knowledge, this is the only study which performed Hg speciation in amniotic fluid (Suzuki et al., 1977). However, Table 5 shows that the Hg(II) content is relatively low only in MB. By contrast, in placenta and amniotic fluid, Hg(II) presents more than half of measured THg, and in meconium, the majority of THg is in Hg(II) form. From these data, we assumed that Hg<sup>0</sup> oxidation occurs in the placenta or amniotic fluid (Bogdanović Pristov et al., 2009). During gestation, the placenta increases in mass and perfusion to provide nutrients and gases to the foetus, and consequently, the amount of Hg<sup>0</sup> reaching the foetus is increased. At this time the formation of reactive oxygen species is an unavoidable consequence of aerobic metabolism and accordingly, the activity of enzymes required for antioxidant defence (superoxide dismutase, catalase, and peroxidase) increases (Watson et al., 1998). Therefore, we can assume that although the influx of Hg<sup>0</sup> is increased, the activity of catalase, a major factor required for Hg<sup>0</sup> oxidation, is also increased. This would lead to rapid oxidation of Hg<sup>0</sup> to Hg(II) and the majority of Hg(II) is probably complexed in placenta (Kuhnert et al., 1981). The placenta has key factors, glutathione (Mover and Ar, 1997), metallothioneins (Itoh et al., 1996), and selenium (Khan and Wang, 2009) that all have high affinity for Hg(II), and they may limit the amount of Hg(II) reaching the foetus. As shown recently, some of Hg(II) can also pass through placenta (Ou et al., 2014). However, due to high perfusion some Hg<sup>0</sup> is transported through the placental barrier and can also be oxidised in amniotic fluid or in foetal tissues. This is supported by a study on pregnant rats that showed that Hg<sup>0</sup> from dental amalgams was accumulated in the placenta but some part also crossed the placenta and was oxidised in the foetal liver (Takahashi et al., 2003). However, Hg(II) present in amniotic fluid could be from foetal urine and swallowed by the foetus combined with other nutrients (Underwood et al., 2005). If Hg<sup>0</sup> is transported and oxidised in foetal liver, we assume, that some part of Hg(II) is also transported to the meconium. Once in the meconium Hg(II) is unlikely to re-cross the membranes and is probably accumulated in the meconium until defecation after birth.

The main drawback of our study is the lack of precise data on dental amalgam fillings (as one of the important sources of Hg(II) in meconium) for the Croatian population, meaning that we were unable to include both populations in linear regression models. Further, MB was not available for the Slovenian participants. Additionally, we did not have the exact date and hour of meconium collection after birth; this is



problematic, as it was shown, that Hg concentrations depend on the time of meconium collection (Ortega Garcia et al., 2006). To precisely follow the Hg kinetics in meconium and investigate occurrence of demethylation process, it would be reasonable to sample MB several times during gestation.

#### 4. Conclusions

This is the first study to perform Hg speciation in meconium on such large sample size. Our results showed that Hg(II) presented the majority (82%–100%) of measured THg in the meconium. To identify possible sources of Hg species in meconium we examined the correlations between Hg in meconium with Hg species in MH, MB, and personal characteristic obtained from the questionnaires. We observed significant correlation between meconium and MH Hg levels, as the latter, like meconium, indicates a long-term exposure. Furthermore, from the observed associations, we can suggest that high Hg(II) content in the meconium results from the transport of Hg<sup>0</sup> vapour from maternal dental amalgam fillings through the placental barrier and the subsequent partitioning of Hg(II) in foetal tissues, body fluids, and meconium. Correlation between maternal seafood intake and Hg(II) levels in meconium was not confirmed with linear regression, therefore there is certain evidence of MeHg demethylation, but in our study it is unlikely that MeHg biotransformation would significantly contribute to high Hg(II) content found in meconium.

Observed associations between meconium MeHg and seafood intake, and meconium Hg(II) and dental amalgams were significant only when meconium Hg species were taken into account in contrast to meconium THg measurements. Based on our data we can conclude that meconium is a suitable biomarker to assess prenatal Hg exposure to MeHg and Hg<sup>0</sup> under the condition that Hg speciation analysis is performed. With this approach, we will characterize the levels of prenatal Hg exposure from different sources. Sampling of meconium is non-invasive and simple, however, methods used so far are not standardized, therefore the results obtained in different studies are not entirely comparable. Further studies are also needed to test the hypothesis of MeHg demethylation, which would limit the use of meconium as biomarker of prenatal MeHg exposure.

#### Author contributions

A.T. wrote the paper and performed all meconium Hg measurements. I.F. contributed to the protocols and interpretation of the data. V.F. helped with Hg measurements. I.Ž. helped with statistics and performed PCA analysis. J.S.T. helped with statistics. I.P. and Z.Š. contributed to the study design and sampling in Croatia. M.H. contributed to the study design and protocols, supervised all Hg measurements, and interpreted the data.

#### Conflicts of interest

The authors declare no conflict of interest.

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#### References

- Akagi, H., 1997. Analytical method for evaluating human exposure to mercury due to gold mining. In: Proceedings of the International Workshop on Health and Environmental Effects of Mercury Due to Mining Operations. National Institute for Minamata Disease, Manila.
- Al-Saleh, I., Shinwari, N., Mashhour, A., El, G., Mohamed, D., Rabah, A., 2011. Heavy metals (lead, cadmium and mercury) in maternal, cord blood and placenta of healthy women. *Int. J. Hyg Environ. Health* 214, 79–101. <https://doi.org/10.1016/j.ijheh.2010.10.001>.
- Bakdash, A., Burger, P., Goecke, T.W., Fasching, P.A., Reulbach, U., Bleich, S., Hastedt, M., Rothe, M., Beckmann, M.W., Pragst, F., Kornhuber, J., 2010. Quantification of fatty acid ethyl esters (FAEE) and ethyl glucuronide (EtG) in meconium from newborns for detection of alcohol abuse in a maternal health evaluation study. *Anal. Bioanal. Chem.* 396, 2469–2477. <https://doi.org/10.1007/s00216-010-3474-5>.
- Bearer, C.F., 2003. Meconium as a biological marker of prenatal exposure. *Ambul. Pediatr.* 3, 40–43. [https://doi.org/10.1367/1539-4409\(2003\)003<0040:MAABMO>2.0.CO;2](https://doi.org/10.1367/1539-4409(2003)003<0040:MAABMO>2.0.CO;2).
- Bogdanović Pristov, J., Spasojević, I., Miković, Ž., Mandić, V., Cerović, N., Spasić, M., 2009. Antioxidative defense enzymes in placenta protect placenta and fetus in inherited thrombophilia from hydrogen peroxide. *Oxid. Med. Cell Longev.* 2, 14–18.
- Byczkowski, J.Z., Lipscomb, J.C., 2001. Physiologically based pharmacokinetic modeling of the lactational transfer of methylmercury. *Risk Anal.* 21.
- Caito, S.W., Jackson, B.P., Punshon, T., Scrimale, T., Grier, A., Gill, S.R., Love, T.M., Watson, G.E., Wijngaarden, E. Van, Rand, M.D., 2018. Variation in methylmercury metabolism and elimination status in humans following fish consumption. *Toxicol. Sci.* 161, 443–453. <https://doi.org/10.1093/toxsci/kfx226>.
- Clarkson, T.W., 1997. The toxicology of metal. *Crit. Rev. Clin. Lab. Sci.* 34, 369–403.
- Clarkson, T.W., Magos, L., Greenwood, M.R., 1972. The transport of elemental mercury into fetal tissue. *Biol. Neonate* 21, 239–244.
- Concheiro, M., Huestis, M.A., 2018. Drug exposure during pregnancy: analytical methods and toxicological findings. *Bioanalysis* 2. <https://doi.org/10.4155/bio-2017-0260>.
- Du, H., Ma, M., Igarashi, Y., Wang, D., 2019. Biotic and abiotic degradation of methylmercury in aquatic ecosystems: a review. *Bull. Environ. Contam. Toxicol.* 102, 605–611. <https://doi.org/10.1007/s00128-018-2530-2>.
- EPA Method 7473, 1998. Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry. [WWW Document]. URL: <https://www.epa.gov/homeland-security-research/epa-method-7473-sw-846-mercury-solids-and-solutions-thermal-decomposition>.
- Gilbert, W.M., Brace, R.A., 1989. The missing link in amniotic fluid volume regulation: intramembranous absorption. *Obstet. Gynecol.* 74, 748–754.
- Gundacker, C., Fröhlich, S., Graf-Rohrmeister, K., Eibenberger, B., Jessenig, V., Gicic, D., Prinz, S., Wittmann, K.J., Zeisler, H., Vallant, B., Pollak, A., Husslein, P., 2010. Perinatal lead and mercury exposure in Austria. *Sci. Total Environ.* 408, 5744–5749. <https://doi.org/10.1016/j.scitotenv.2010.07.079>.
- Hammerschmidt, C.R., Finiguerra, M.B., Weller, R.L., Fitzgerald, W.F., 2013. Methylmercury accumulation in plankton on the continental margin of the Northwest Atlantic Ocean. *Environ. Sci. Technol.* 47, 3671–3677.
- Horvat, M., Prosenč, A., Smrke, J., Konda, D., Byrne, A.R., Stegnar, P., Bergic, I., 1991. Trace element levels in the hair, blood, cord blood and placenta of pregnant women from central Slovenia. In: Aitio, A., Aro, A., Järvisalo, J., Vainio, H. (Eds.), *Trace Elements in Health and Disease. The Royal Society of Chemistry*, pp. 83–94.
- Horvat, M., Snoj Tratnik, J., Miklavčič, A., 2012. Mercury: biomarkers of exposure and human biomonitoring. In: Knudsen, L.E., Merlo, D.F. (Eds.), *Biomarkers and Human Biomonitoring. Volume 1: Ongoing Programs and Exposures. The Royal Society of Chemistry, Cambridge, UK*, pp. 381–417.
- Itoh, N., Fujita, Y., Nakanishi, H., Kawai, Y., Mayumi, T., Hwang, G.S., Min, K., Onosaka, S., Muto, N., Tanaka, K., 1996. Binding of Cd to metallothionein in the placenta of Cd-treated mouse. *J. Toxicol. Sci.* 21, 19–27.
- Jiang, C., Hsi, H., Fan, C., Chien, L., 2014. Fetal exposure to environmental neurotoxins in Taiwan. *PLoS One* 9. <https://doi.org/10.1371/journal.pone.0109984>.
- Jiang, C.B., Yeh, C.Y., Lee, H.C., Chen, M.J., Hung, F.Y., Fang, S.S., Chien, L.C., 2010. Mercury concentration in meconium and risk assessment of fish consumption among pregnant women in Taiwan. *Sci. Total Environ.* 408, 518–523. <https://doi.org/10.1016/j.scitotenv.2009.10.043>.
- Kajiwara, A., Yasutake, Y., Adachi, K., Hirayama, T., 1996. Methylmercury transport across the placenta via neutral amino acid carrier. *Arch. Toxicol.* 70, 310–311. <https://doi.org/10.1007/s002040050279>.
- Khan, M. A. K., Wang, F., 2009. Mercury-selenium compounds and their toxicological significance: toward a molecular understanding of the mercury-selenium antagonism. *Environ. Toxicol. Chem.* 28, 1567–1577. <https://doi.org/10.1897/08-375.1>.
- Knezović, Z., Trgo, M., Sultović, D., 2016. Monitoring mercury environment pollution through bioaccumulation in meconium. *Process Saf. Environ. Prot.* 101, 2–8. <https://doi.org/10.1016/j.psep.2016.01.013>.
- Kuhnert, P., Kuhnert, B.R., Erhard, B.S., 1981. Comparison of mercury levels in maternal blood, fetal cord blood, and placental tissues. *Am. J. Obstet. Gynecol.* 139, 209–213. [https://doi.org/10.1016/0002-9378\(81\)90448-8](https://doi.org/10.1016/0002-9378(81)90448-8).
- Li, H., Lin, X., Zhao, J., Cui, L., Wang, L., Gao, Y., Li, B., Chen, C., Li, Y.F., 2018. Intestinal methylation and demethylation of mercury. *Bull. Environ. Contam. Toxicol.* <https://doi.org/10.1007/s00128-018-2512-4>.
- Liang, L., Bloom, N.S., Horvat, M., 1994. Simultaneous determination of mercury

- speciation in biological materials by GC/CVAFS after ethylation and room-temperature precooling. *Clin. Chem.* 40, 602–607.
- Magos, L., Halbach, S., Clarkson, T.W., 1978. Role of catalase in the oxidation of mercury vapor. *Biochem. Pharmacol.* 27, 1373–1377.
- Miklavčič, A., Casetta, A., Snoj Tratnik, J., Mazej, D., Krsnik, M., Mariuz, M., Sofianou, K., Špirič, Z., Barbone, F., Horvat, M., 2013. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. *Environ. Res.* 120, 7–17. <https://doi.org/10.1016/j.envres.2012.08.010>.
- Miklavčič, A., Cuderman, P., Mazej, D., Snoj Tratnik, J., Krsnik, M., Planinšek, P., Osredkar, J., Horvat, M., 2011. Biomarkers of low-level mercury exposure through fish consumption in pregnant and lactating Slovenian women. *Environ. Res.* 111, 1201–1207. <https://doi.org/10.1016/j.envres.2011.07.006>.
- Morgan, D.L., Chanda, S.M., Price, H.C., Fernando, R., Liu, J., Brambila, E., O'Connor, R.W., Beliles, R.P., Barone, S., 2002. Disposition of inhaled mercury vapor in pregnant rats: maternal toxicity and effects on developmental outcome. *Toxicol. Sci.* 66, 261–273. <https://doi.org/10.1093/toxsci/66.2.261>.
- Mover, H., Ar, A., 1997. Antioxidant enzymatic activity in embryos and placenta of rats chronically exposed to hypoxia and hyperoxia. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 117, 151–157.
- Oliveira, C.S., Joshee, L., Zalups, R.K., Pereira, M.E., Bridges, C.C., 2015. Disposition of inorganic mercury in pregnant rats and their offspring. *Toxicology* 335, 62–71. <https://doi.org/10.1016/j.tox.2015.07.006>.
- Ortega Garcia, J.A., Carrizo Gallardo, D., Ferris i Tortajada, J., Garcia, M.M.P., Grimalt, J.O., 2006. Meconium and neurotoxicants: searching for a prenatal exposure timing. *Arch. Dis. Child.* 91, 642–646. <https://doi.org/10.1136/adc.2005.084129>.
- Ostrea Jr., E., Bielawski, D.M., Posecion Jr., N.C., 2006. Meconium analysis to detect fetal exposure to neurotoxicants. *Arch. Dis. Child.* 91, 628–629. <https://doi.org/10.1136/adc.2006.095059>.
- Ostrea Jr., E.M., Morales, V., Ngoumna, E., Prescilla, R., Tan, E., Hernandez, E., Ramirez, G.B., Cifra, H.L., Manlapaz, M.L., 2002. Prevalence of fetal exposure to environmental toxins as determined by meconium analysis. *Neurotoxicology* 23, 329–339. [https://doi.org/10.1016/S0161-813X\(02\)00077-3](https://doi.org/10.1016/S0161-813X(02)00077-3).
- Ou, L., Chen, L., Chen, C., Yang, T., Wang, H., Tong, Y., Hu, D., Zhang, W., Long, W., Wang, X., 2014. Associations of methylmercury and inorganic mercury between human cord blood and maternal blood: a meta-analysis and its application. *Environ. Pollut.* 191, 25–30. <https://doi.org/10.1016/j.envpol.2014.04.016>.
- Ou, L., Wang, H., Chen, C., Chen, L., Zhang, W., Wang, X., 2018. Physiologically based pharmacokinetic (PBPK) modeling of human lactational transfer of methylmercury in China. *Environ. Int.* 115, 180–187. <https://doi.org/10.1016/j.envint.2018.03.018>.
- Park, B.Y., Lee, B.K., 2014. Use of meconium in perinatal epidemiology: potential benefits and pitfalls. *Ann. Epidemiol.* 24, 878–881. <https://doi.org/10.1016/j.annepidem.2014.09.009>.
- Park, J., Zheng, W., 2012. Human exposure and health effects of inorganic and elemental mercury. *J. Preven. Med. Public Health* 45, 344–352.
- Peng, S., Liu, L., Zhang, X., Heinrich, J., Zhang, J., Schramm, K., Huang, Q., Tian, M., Ali, S., Akber, M., Eqani, S., Shen, H., 2015. A nested case-control study indicating heavy metal residues in meconium associate with maternal gestational diabetes mellitus risk. *Environ. Health* 14, 1–10. <https://doi.org/10.1186/s12940-015-0004-0>.
- Ramirez, G.B., Pagulayan, O., Stat, M.S., Akagi, H., Rivera, A.F., Eng, C., Lee, L.V., Berroya, A., Cristina, M., Cruz, V., Casintahan, D., 2003. Tagum Study II: follow-up study at two years of age after prenatal exposure to mercury. *Pediatrics* 111, 289–295. <https://doi.org/10.1542/peds.111.3.e289>.
- Ramirez, G.B., Vince Cruz, M.C., Pagulayan, O., Ostrea, E., Dalisay, C., 2000. The Tagum study I: analysis and clinical correlates of mercury in maternal and cord blood, breast milk, meconium, and infants' hair. *Pediatrics* 106, 774–781.
- Rand, M.D., Vorobjekina, D., van Wijngaarden, E., Jackson, B.P., Scrimale, T., Zareba, G., Love, T.M., Myers, G.J., Watson, G.E., 2016. Methods for individualized determination of methylmercury elimination rate and de-methylation status in humans following fish consumption. *Toxicol. Sci.* 149, 385–395. <https://doi.org/10.1093/toxsci/kfv241>.
- Rothenberg, S.E., Keiser, S., Ajami, N.J., Wong, M.C., Gesell, J., Petrosino, J.F., Johs, A., 2017. The role of gut microbiota in fetal methylmercury exposure: insights from a pilot study. *Toxicol. Lett.* 3, 60–67. <https://doi.org/10.1016/j.toxlet.2015.11.022>.
- Rothenberg, S.E., Wagner, C.L., Hamidi, B., Alekseyenko, A.V., Azcarate-Peril, M.A., 2019. Longitudinal changes during pregnancy in gut microbiota and methylmercury biomarkers, and reversal of microbe-exposure correlations. *Environ. Res.* 172, 700–712. <https://doi.org/10.1016/j.envres.2019.01.014>.
- Rowland, I.R., Davies, M.J., Grasso, P., 1977. The effect of elimination of the gastrointestinal flora on the accumulation of methylmercuric chloride by the rat. *Biochem. Soc. Trans.* 5, 423–425.
- Rowland, I.R., Robinson, R.D., Doherty, R.A., Landry, T., 1983. Are developmental changes in methylmercury metabolism and excretion mediated by the intestinal microflora? In: Clarkson, T.W., Nordberg, G.F., Sager, P.R. (Eds.), *Reproductive and Developmental Toxicity of Metals*. Springer, Boston, MA, pp. 745–758. [https://doi.org/10.1007/978-1-4615-9346-1\\_32](https://doi.org/10.1007/978-1-4615-9346-1_32).
- Stigter, R.H., Mulder, E.J.H., Bruinse, H.W., Visser, G.H.A., 2011. Fetal urine production in late pregnancy. *Obstet. Gynecol.* <https://doi.org/10.5402/2011/345431>. 2011.
- Suzuki, T., Takemoto, T.-I., Shishido, S., Kani, K., 1977. Mercury in human amniotic fluid. *Scand. J. Work Environ. Health* 3, 32–35.
- Takahashi, Y., Tsuruta, S., Arimoto, M., Tanaka, H., Yoshida, M., 2003. Placental transfer of mercury in pregnant rats which received dental amalgam restorations. *Toxicology* 185, 23–33. [https://doi.org/10.1016/S0300-483X\(02\)00588-7](https://doi.org/10.1016/S0300-483X(02)00588-7).
- Trdin, A., Snoj Tratnik, J., Mazej, D., Fajon, V., Krsnik, M., Osredkar, J., Prpič, I., Špirič, Z., Petrovič, O., Marc, J., Neubauer, D., Kodrič, J., Kobal, A.B., Barbone, F., Falnoga, I., Horvat, M., 2019. Mercury speciation in prenatal exposure in Slovenian and Croatian population - PHIME study. *Environ. Res.* <https://doi.org/10.1016/j.envres.2019.108627>.
- Underwood, M.A., Gilbert, W.M., Sherman, M.P., 2005. State of the art amniotic fluid: not just fetal urine anymore. *J. Perinatol.* 341–348. <https://doi.org/10.1038/sj.jp.7211290>.
- Unuvar, E., Ahmadov, H., Riza Kiziler, A., Aydemir, B., Toprak, S., Ulker, V., Ark, C., 2007. Mercury levels in cord blood and meconium of healthy newborns and venous blood of their mothers: clinical, prospective cohort study. *Sci. Total Environ.* 374, 60–70. <https://doi.org/10.1016/j.scitotenv.2006.11.043>.
- Valent, F., Mariuz, M., Bin, M., Little, D., Mazej, D., Tognin, V., Tratnik, J., McAfee, A.J., Mulhern, M.S., Parpinel, M., Carrozzini, M., Horvat, M., Tamburlini, G., Barbone, F., 2013a. Associations of prenatal mercury exposure from maternal fish consumption and polyunsaturated fatty acids with child neurodevelopment: a prospective cohort study in Italy. *J. Epidemiol.* 23, 360–370. <https://doi.org/10.2188/jea.JE20120168>.
- Valent, F., Horvat, M., Sofianou-Katsoulis, A., Spiric, Z., Mazej, D., Little, D., Prasouli, A., Mariuz, M., Tamburlini, G., Nakou, S., Barbone, F., 2013b. Neurodevelopmental effects of low-level prenatal mercury exposure from maternal fish consumption in a mediterranean cohort: Study rationale and design. <https://doi.org/10.2188/jea.JE20120030>.
- Watson, A.L., Skepper, J.N., Jauniaux, E., Burton, G.J., 1998. Changes in concentration, localization and activity of catalase within the human placenta during early gestation. *Placenta* 19, 27–34. [https://doi.org/10.1016/S0143-4004\(98\)90095-9](https://doi.org/10.1016/S0143-4004(98)90095-9).
- Yoshida, M., 2002. Placental to fetal transfer of mercury and fetotoxicity. *Tohoku J. Exp. Med.* 196, 79–88.