Assessment of exposure to trace metals in a cohort of pregnant women from an urban center by urine analysis in the first and third trimesters of pregnancy

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Abstract

Background: Prenatal exposure to trace metals, whether they are essential, non-essential or toxic, must be assessed for their potential health effects in the offspring. Herein is reported a preliminary approach to this end which involved collection of urine samples during the first and third trimesters of pregnancy from 489 mothers from Sabadell (Catalonia, Spain), a highly industrialized town. These samples were analyzed for cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), arsenic (As), molybdenum (Mo), cadmium (Cd), antimonium (Sb), cesium (Cs), thallium (Th) and lead (Pb).

Results: An acid digestion method was developed and validated for Q-ICP-MS analysis of these 12 metals. The median concentrations of metals ranged from 0.13 to 290 μg/g creatinine, the highest levels were found for Zn and the lowest for Th. The mean concentrations of most metals except As, Ni, Th and Pb showed statistically significant differences between both trimesters. The concentrations of Mo, Se, Cd, Cs and Sb were higher in the first than in the third trimester, whereas the opposite was found for Co, Cu and Zn. The concentrations of all metals in both sampling periods showed statistically significant correlations ($p<0.01$ for Mo and Cu, $p<0.001$ for the others).

Conclusions: The significant correlations of metal urine concentrations in the first and third trimesters of pregnancey suggest that the observed differences between both periods are related to physiological changes. Accordingly, the measured urine concentrations during either the first or third trimesters can be used as estimates of exposure during pregnancy and can serve as markers for prenatal intake of these metals in the studied cohort.

1. Introduction

Humans are regularly exposed to metals present in air, water, food, soil and domestic materials. Although the banning of lead from petrol has proven beneficial, many other sources of potentially hazardous metals present in the human environment still remain to be controlled. Whereas certain metals are essential for life, e.g. zinc (Zn), copper (Cu) and iron (Fe), others are toxic, even at low concentrations, e.g. mercury (Hg), lead (Pb), arsenic (As), thallium (Th), chromium (Cr) or cadmium (Cd). Given the increasing use of these toxic metals in new technologies and the increasing inputs of them from road traffic and other sources, there is growing concern over the public health implications of continued exposure to them (Järup 2003; Lauwerys and Lison 1994; Rodriguez and Diaz 1995; Schulz et al. 2007; Wells et al. 2011; Zubero et al. 2010).

The study of metal concentrations in humans is of high interest because of the essential metabolic functionality of some of them and the toxic properties of others. Moreover, exposure to metals at the onset of life, both in the fetal period and during the first years, can be associated with negative health effects in later stages (Vahter 2008). Accordingly, assessing the exposure to a large number of metals, particularly in the earliest stages of life, may provide the knowledge necessary for identifying public health problems and implementing prevention policies early on.

Mothers constitute a source of heavy metals for their infants during pregnancy and lactation. However, only a few studies on prenatal exposure to trace metals have been published, most of which focused on a small number of these elements (Messiha et al., 1988; Vahter et al., 2008; Wright and Baccarelli 2007; Al-Saleh et al. 2011; Kippler et al. 2009; Shirai et al. 2010). In some cases, animal models have been used to assess the prenatal effects of these pollutants (Liu et al. 2009; Tokar et al. 2010). However, specific measurements at the individual level can enable a better understanding of the possible influence of exposure to metals on health. Such measurements also facilitate the identification of sources and routes of metal contamination at both individual and the general population levels. These aspects are even more important when dealing with prenatal exposure.

Unfortunately, there are no generally accepted methods for physiologically assessing exposure to metals. Urine is the preferred source of information for heavy metals biomonitoring, can be collected without invasive methods and has been widely used in large environmental studies such as the German Environmental Survey for Children (GerES) and the National Health and Nutrition Examination (NHANES) (Esteban and Castaño 2009). However, one basic requirement for biomonitoring of metals with urine analysis concerns the reproducibility of different urine measurements from each individual in samples collected at different time periods. This aspect is particularly significant for women during pregnancy in which children are in-utero exposed to metals and other compounds by maternal transmission. Thus, analysis of metals in urine collected at different pregnancy periods may provide useful information for assessment of the efficacy of the use of urine samples as exposure markers.

Therefore, the objectives of this study are: i) to develop an analytical method using acid digestion prior to analysis by inductively coupled plasma quadruple mass spectrometry (Q-ICP-MS) for the simultaneous analysis of 12 metals in maternal urine and ii) to determine the concentrations of 12 metals, namely cobalt (Co), nickel (Ni), Cu, Zn, selenium (Se), As, molybdenum (Mo), Cd, antimonium (Sb), cesium (Cs), Th and Pb, in urine samples of pregnant women living in a highgly industrialized urban town (Sabadell, Catalonia, Spain), which were collected during their first and third trimester of pregnancy. This approach affords an assessment of the steadiness of the concentrations of these metals in urine during the pregnancy period and their usefulness for epidemiological studies, maternal and prenatal exposure estimates.

2. Materials and methods

2.1. Urine samples

As part of the INMA research network (Childhood and Environment) (Guxens et al. 2012) 657 pregnant women were recruited in their 12th week medical visit in the Sant Fèlix Primary Care Center II (Sabadell), between 2004 and 2006. Recruitment involved only those women that lived in Sabadell, were older than 16 years, had a singleton pregnancy, volunteered for the program and wanted to give birth at the Hospitals of Sabadell or Terrassa (a nearby city). Women suffering from chronic diseases, having impaired communication or that become pregnant by assisted reproduction were excluded. After obtaining the consent from the admitted women, questionnaires were administered by trained interviewers in the 12th and 32th weeks of pregnancy.

Urine samples were collected in 100 mL polypropylene containers in the first and third trimester of pregnancy from 489 pregnant women of this cohort. The samples were stored in polyethylene tubes at -20ºC until further processing. This research project was approved by the Research Ethics Committee of the CREAL. To maintain confidentiality, participant information was encoded.

2.2. Analysis of urine samples

Prior to Q-ICP-MS analysis, the samples were digested and diluted to oxidize and remove organic matter and to minimize the concentrations of inorganic solids (Castillo et al. 2008; Krachler 1996). Three mL of each urine sample, 3 mL of Instra-Analysed 65% HNO_3 (J.T. Baker, Germany) and 1.5 mL of Instra-Analysed 30% H_2O_2 (Baker) were introduced in Teflon vessels. The mixtures were left in an oven at 90ºC overnight. After cooling, the vessels were opened and then placed on a heating plate at 250°C to evaporate off the nitric acid. Once dried, the resulting solid samples were dissolved in 3 mL of 4% HNO_3, placed in 7 mL glass bottles and subsequently stored in a refrigerator until instrumental analysis. Before analysis, an internal standard of 10 ppb of In was introduced and depending on sample density they were diluted to 30 mL or 60 mL with MilliQ water to avoid non-spectral interference.

Q-ICP-MS analysis was performed by an X-SERIES II (Thermo Fisher Scientific) instrument. Specific isotope ions for Co and Ni were selected in order to avoid potential calcium interferences from the sample matrix. Cl atoms may also potentially interfere in the determination of As and Se. In these cases the collision/reaction cell technique should be added to the instrumental methods but no interferences were observed in the present samples and these cells were not used. Instrumental limit of detection (LOD) for all metals was 0.2 ng/mL attending to the most reliable lowest calibration point. The two samples corresponding to the first and third trimesters of each subject were digested and analyzed at the same time. One MilliQ water blank was processed in each batch of samples to control for possible contamination. If there was any contamination, thorough cleaning of all material was performed and digestion was repeated. Field samples were also obtained by analysis of Milli Q water which was previously stored in the containers used for maternal urine bottles and transported together with the samples.

A Bio-Rad Level 1 (Lyphochek Urine Metals Control 1-69131; Marnes-la-Coquette, France) urine reference was extensively used to evaluate the developed methodology, as it contains metal concentrations close to those in the urine samples from the study cohort. This reference material provided certified values for As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Sb, Tl, Zn and Se. Prior to digestion, the lyophilized reference urine samples were reconstituted with 25 mL of MilliQ water as recommended by the manufacturer.

All glassware and polypropylene material was thoroughly cleaned by soaking in 10% nitric acid for 24 h, followed by rinsing three times with MilliQ water. The Teflon vessels were cleaned after each use by rinsing with 10% nitric acid three times, and, following the last rinse, leaving them in the oven at 90ºC overnight. Finally, the vessels were rinsed with a large volume of MilliQ water.

Creatinine was determined at the Echevarne laboratory of Barcelona by the Jaffé method (kinetic with target measurement, compensated method) with Beckman Coulter© reactive in AU5400 (IZASA®).

2.4. Statistical analyses

Descriptive statistical parameters were initially computed. Values for mean, standard deviation (SD), median and P91 were calculated for the metal concentrations. Normality was checked by the Kolmogorov-Smirnov test. The metal concentrations between the first and the third trimesters were compared using Spearman correlations and paired Mann-Whitney hypothesis tests. The individual ratios between the metal concentrations during the third and first trimesters were also calculated. Mean, standard deviation and median values for these ratios were computed. All statistical analyses were performed using Stata 12.0 software (Stata Corporation, College Station, Texas).

3. Results

3.1. Population characteristics

The median age of the mothers at the time of their last menstrual period was 31 years, ranging between 20 and 40 years. Their mean body mass index before pregnancy was 23.77 kg/m2 (standard deviation 4.53 kg/m^2, median 22.44 kg/m^2, range 16.69-40.77 kg/m^2). Overweight and obese women encompassed 18.1% and 8.4%, respectively. The proportion of primiparous mothers was 49%, 41% had another infant and 10.2% had more than two infants.

3.2. Method validation

The developed analytical method was tested by analysis of the aforementioned reference material. The analyses were performed in different time periods, on three replicates per period (Table 1). The observed mean results are within the acceptable range of assigned values. The average concentrations of some metals, such as Cd, were close to the lower limit. The relative standard deviations varied between 5% (As and Cd) and 18% (Zn). Repeatibility ranged between 1% (As) and 17% (Zn). Reproducibility ranged between 1% (Tl) and 17% (Cu; Table 1).

3.3. Metal concentrations

The metal concentrations and statistics of the samples collected in the first and third trimester normalized to creatinine content (µg/g creatinine) are shown in Table 2. The metal concentrations were not normally distributed but skewed to the right in both trimesters.

Ni, Cu, Zn, As, Se, Mo, Cd, Cs and Pb were detected in more than 90% of samples, whereas Co and Sb were detected in more than 65% in the first and the third trimester (Table 2). Tl was the only element detected in less than 20% of the samples. The differences in metal concentrations between the urine samples collected in both periods were statistically significant for all metals except Ni, As, Tl and Pb (median values: 32 (1st)/35 (3rd) µg/g creatinine for As, 3.9/3.9 for Ni, 0.14/0.13 for Tl, 3.8/3.9 for Pb; Table 2). The concentrations of Co, Cu and Zn were higher during the third trimester (median values: 0.45 (1st)/1.3 (3rd) µg/g creatinine for Co, 12/15 for Cu, 256/290 for Zn; Table 2). The opposite was found for the concentrations of Mo, Se, Cd, Sb and Cs (median values: 55 (1st)/44 (3rd) µg/g creatinine for Mo, 10/8.7 for Se, 0.61/0.54 for Cd, 0.36/0.28 for Sb and 8.0/6.8 for Cs; Table 2).

Consistently with these differences, comparison of the individual concentrations revealed that more mothers had higher concentrations of Se, Mo, Cd, Sb, Cs and Tl in the first than in the third trimester (53-64%; Table 3). On the other hand, more mothers exhibited higher concentrations of Co, Ni, Cu and Zn in the third than in the first trimester (55-82%; Table 3). The concentration ratios between the third and first trimesters were consistent with these observed differences (Table 3). Co, Cu and Zn showed the higher third-first trimester median concentration ratios and Mo, Se, Cd and Sb had the lower.

The first and third trimester concentrations of all metals were significantly correlated (Table 3). The Spearman coefficients ranged between 0.16 (Mo) and 0.60 (Zn). The degree of significance of these correlations was $p < 0.001$ in most cases (Co, Ni, Zn, Se, As, Cd, Sb, Cs and Pb) and $p < 0.01$ in others (Cu, Mo).

4. Discussion

4.1. Concentrations of trace metals in the Sabadell cohort

The median concentrations found in the Sabadell cohort (n = 489) were compared with those reported in previous studies worldwide (Table 4). Most literature data are reported in µg/g creatinine but in some cases they are reported in µg/L (Afridi et al. 2009; Link et al. 2007; Minoia et al. 1990; Callan et al., 2013). Despite these unit differences, in healthy populations the average values measured using either of these two methods generally differed by less than 10% (NHANES 2009). Thus, all results in Table 4 should be comparable. Most previous studies have dealt with the general population; only a few specifically encompass pregnant women as in the present study, such as those from

Pakistan (Afridi et al. 2009), Korea (Moon et al. 2003) or Germany (Callan et al. 2013). Accordingly, it should be taken into account that, once levels are normalized to creatinine content, the values for women tend to be higher than those for men (NHANES 2009; Schuhmacher et al. 1994). Furthermore, pregnancy may involve increases or decreases in the concentrations of certain metals, as discussed later.

The concentrations of Ni and As were higher than those reported in previous studies (Link et al., 2007; Ohashi et al., 2006; NHANES 2009; Banza et al., 2009; Alimonti et al., 2000; Seifert et al., 2000; Minoia et al., 1990; Callan et al, 2013; Table 4). On the other hand, Zn, Se and Tl showed lower urine concentrations in Sabadell than in studies from other population groups (Banza et al., 2009; Paschal et al., 1998; Schuhmacher et al., 1994; Minoia et al., 1990; Afridi et al., 2009; Callan et al., 2013; Table 4). The other metals showed intermediate concentrations when comparing with previous studies.

The median concentrations of Co in the first trimester, 0.45 µg/g creatinine, were intermediate between those reported in previous studies (Table 4) but those in the third trimester, 1.3 µg/g creatinine, were higher than in most previous studies except one from a mining polluted area of Congo (mean 15.7 µg/g creatinine) (Banza et al. 2009) and were similar to those described in a maternal population from Australia (Callan et al., 2013). The strong difference in the concentrations of this metal in the first and third trimesters of pregnancy levels is consistent with a significant increase in Co excretion during pregnancy. These concentrations during the last stage of pregnancy may not be comparable to those from general population.

The observed concentrations of Pb, 3.8-3.9 µg/g creatinine (Table 4), were significantly lower than those reported in populations from Italy from a time period in which Pb was still used as additive in gasoline, 17 µg/L (Minoia et al. 1990), or Korea (5.1 µg/g creatinine; Moon et al. 2003). However, they were higher than those recently reported in the US, 0.63-0.72 µg/g creatinine (NHANES 2009), or 1.9 µg/g creatinine (Paschal et al. 1998). These concentrations were consistent with the effects of Pb withdrawal from gasoline, although the Sabadell levels may be consistent with predicted increases of Pb mobilization from the bone tissues during pregnancy (Gulson et al. 2004).

4.2. Comparison of levels in the first and third trimesters

Some previous studies have considered changes in metal concentrations during pregnancy but they essentially compared blood or serum samples and only analyzed a few metals such as Cu and Zn (Huang et al. 1999; Liu et al. 2010; Izquierdo-Álvarez et al. 2007; Hernandez et al. 1996), Se, Cu and Zn (Kilinc et al. 2010) or Pb and Cd (Bonithon-Kopp et al. 1986). A very small number of studies on metals during pregnancy have considered urine samples, e.g. As (Gardner et al. 2010) or Cd (Hernandez et al. 1996). Thus, there is limited precedent with which to compare the findings of the present study.

As mentioned above, the concentrations of all metals except Ni, As, Th and Pb were statistically different between the first and third trimesters. Most of the metals with differences between the two stages of pregnancy showed higher concentrations in the urine collected in the first trimester than in the third trimester, except in the case of Co, Cu and Zn. Some previous studies considering the Zn and Cu in plasma or blood samples also found significant differences in concentration at different periods of pregnancy (Izquierdo-Álvarez et al. 2007; Liu et al. 2010; Hernandez et al. 1996), although in one study on Zn, no difference was found (Huang et al. 1999). On the contrary, no significant differences for Se in serum samples during pregnancy were observed in a southeastern Mediterranean region of Turkey (Kilinc et al. 2010) nor for Cd in Aragon (Spain) (Izquierdo-Álvarez et al. 2007) or Southern Catalonia (Spain)

(Hernandez et al., 1996), while significant differences in the concentrations of these metals in the first and the third trimesters were found in our study. Some of these studies did not take into account paired samples of the same mothers at different stages and they involved a lower number of samples.

Different changes and adaptations occur in the women during pregnancy, such as increase of plasma volume. This may explain why most metals show a decrease in concentration during pregnancy. However, despite of this higher volume the concentrations of some minerals and vitamins also increase (King 2000). These metabolic changes may also influence the amount of metal released into urine. For instance, the increase in glomerular filtration rate during pregnancy has been related with observed concentration increments of Zn in urine during this maternal period (Swanson and King 1987). This observation is also consistent with the increase of Zn between the first and third trimesters found in the Sabadell cohort (Table 2) and could also explain the significant increases of other metals such as Co and Cu (Table 2). In any case, there must be specific factors affecting each metal separately that must be considered in specific studies. This is the first study in which differences in exposure during pregnancy have been studied for a large number of metals over a large number of paired samples (n = 489).

The concentrations of trace metals in urine collected in the first and third trimesters of pregnancy were significantly correlated in all metals studied, with high statistical significance in most cases. These significant correlations likely reflect an absence of major changes in metal exposure during pregnancy and the differences between both trimesters observed for most metals may reflect metabolic changes during this period. Accordingly, the findings from this studied cohort indicate that the measurements of these trace metals at any stage of pregnancy provide a representative estimate of the exposure to these compounds during the whole period. Sampling in specific stages may be chosen according to logistics or specific study purposes and the observed concentrations must always be interpreted in reference to this selected phase.

5. Conclusions

Exposure to metals in pregnant women has been assessed from their urine composition collected in the first and third trimesters of pregnancy using a newly developed digestion protocol for Q-ICP-MS analysis. All metals except Ni, As, Th and Pb showed statistically significant concentration differences between these two periods. The concentrations of all metals in the first and third trimesters were significantly correlated which reflect the absence of major changes of metal inputs in the studied women during pregnancy. The significant concentration differences between these two sampling periods may respond to metabolic pregnancy changes. Accordingly, the measurements of the studied trace metals in urine provide representative estimates of exposure during the whole pregnancy period.

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TABLES

Table 1. Concentrations obtained with the developed method for analysis of trace metals in urine samples in the analysis of Bio-Rad Level 1 urine reference standard (ng/mL).

			All values		Repeatability		Reproducibility	
Element	Reference value	Acceptable range (20%)	Mean ± SD[a]	RSD[b]	Mean ± SD[b]	RSD[b]	Mean ± SD[c]	RSD[b]
As	65	52 - 78	59 ± 3.1	5%	58 ± 0.35	1%	56 ± 2.0	4%
Cd	9.0	7.2 - 11	7.2 ± 0.37	5%	7.1 ± 0.11	2%	7.2 ± 0.55	8%
Co	6.8	5.4 - 8.2	6.8 ± 0.67	10%	6.8 ± 0.67	10%	6.5 ± 0.75	12%
Cu	10	8.1 – 12	10 ± 1.5	16%	9.4 ± 0.81	9%	10 ± 1.7	17%
Ni	4.5	2.3 - 6.7	4.0 ± 0.6	16%	4.3 ± 0.8	18%	3.6 ± 0.36	10%
Pb	14	11 – 16	14 ± 1.6	11%	14 ± 2.2	15%	13 ± 1.6	13%
Sb	9.5	7.5 -11	9.7 ± 1.3	13%	9.1 ± 0.81	9%	10 ± 0.4	4%
Se	72	57 – 86	69 ± 7.5	11%	69 ± 7.5	11%	69 ± 7.5	11%
Tl	8.5	6.8 – 10	8.4 ± 0.52	6%	8.0 ± 0.22	3%	8.3 ± 0.09	1%
Zn	381	305 - 457	363 ± 67	18%	370 ± 65	17%	305 ± 19	6%

[a] mean ± standard deviation (n = 8) . [b] RSD: relative standard deviation (in %)

Table 2. Descriptive statistics of the concentrations of trace metals in the maternal urine samples collected in the first and third trimesters of pregnancy (µg/g creatinine). p-value for difference according to Mann-Whitney paired test

	% Detected		Mean (SD)		Median		P90		p-value
	1st	3rd	1st	3rd	1st	3rd	1st	3rd	
Co	73.6	84.4	0.73 (1.4)	1.6 (2.5)	0.45	1.3	1.4	2.9	$p < 0.001$
Ni	98.4	98.1	4.9 (5.3)	4.8 (4.5)	3.9	3.9	9.0	8.6	
Cu	100	100	14 (11)	17 (13)	12	15	24	30	$p < 0.001$
Zn	100	100	315 (313)	342 (224)	256	290	552	631	$p < 0.001$
Se	99.8	99.3	12 (11)	9.9 (6.3)	10	8.7	20	16	$p < 0.001$
As	99.8	99.8	69 (120)	62 (79)	32	35	147	136	
Mo	100	100	64 (49)	50 (32)	55	44	106	81	$p < 0.001$
Cd	90.1	87.5	0.77 (0.79)	0.67 (0.48)	0.61	0.54	1.3	1.3	$p < 0.01$
Sb	73.7	64.8	0.81 (1.7)	0.56 (2.0)	0.36	0.28	1.4	0.84	$p < 0.001$
Cs	100	100	9.3 (11)	7.4 (4.1)	8.0	6.8	13	11	$p < 0.001$
Tl	19.7	17.2	0.18 (0.16)	0.18 (0.43)	<LOD	<LOD	0.30	0.30	
Pb	98.9	100	4.8 (4.3)	5.2 (4.9)	3.8	3.9	8.1	8.9	

Table 3. Comparisons of the metal concentrations in maternal urine collected in the first and third trimesters of pregnancy (µg/g creatinine)

	Spearman coeficients of the correlations	Concentration ratios between the 3rd and 1st trimestres		Concentrations in the 3rd and 1st trimesters	
		Mean (SD)	Median	% 3rd > 1st	% 1st > 3rd
Co	0.39***	4.5 (7.0)	2.2	84	16
Ni	0.39***	1.4 (2.0)	1.1	55	45
Cu	0.21**	1.6 (1.3)	1.2	62	38
Zn	0.60***	1.3 (1.0)	1.1	59	41
Se	0.43***	0.97 (0.71)	0.84	37	63
As	0.24***	2.6 (4.6)	1.1	52	48
Mo	0.16**	1.0 (1.1)	0.80	36	64
Cd	0.57***	1.1 (0.87)	0.92	42	57
Sb	0.40***	1.6 (6.7)	0.75	38	62
Cs	0.26***	0.97 (0.61)	0.89	37	63
Tl	0.22***	1.3 (2.6)	0.91	46	53
Pb	0.46***	1.4 (1.3)	1.0	50	50

** p-value < 0.01; *** p-value < 0.001

Table 4. Comparison of trace metal concentrations from the Sabadell cohort and previous studies (concentrations in µg/g creatinine unless otherwise indicated)

Reference	Sampling Years	Location	N	Co	Ni	As	Zn	Sb	Cd	Pb	Cu	Cs	Tl	Se
Present work[a]	2004-06	Sabadell – 1st trim	489	0.45	3.9	32	256	0.36	0.61	3.8	12	8.0	0.13	10
Present work[a]	2004-06	Sabadell – 3rd trim	489	1.3	3.9	35	290	0.28	0.54	3.9	15	6.8	0.13	8.7
Link et al., 2007[a]	2002-03	South Germany	500	…	…	4.6[d]	…	…	…	…	…	…	…	…
NHANES report, 2009[a]	1999-00	USA (99-00)	2500	0.35	…	…	…	0.12	0.18	0.72	…	4.1	…	…
NHANES report, 2009[a]	2001-02	USA (01-02)	2500	0.36	…	…	…	0.13	0.20	0.64	…	4.5	…	…
NHANES report, 2009[a]	2003-04	USA (03-04)	2500	0.31	…	8.2	…	…	0.21	0.63	…	4.6	…	…
Ohashi et al., 2006[b]	2000-05	Japan	1000	0.60	1.8	…	…	…	…	…	13	…	…	…
Banza et al., 2009[b]	2006-07	DR Congo	179	15.7	3.3	18	306	0.07	0.75	3.2	17	…	…	17
Paschal et al., 1998[b]	1988-94	USA	496	0.78	…	…	…	0.67	…	1.9	…	1.0	0.24	…
Moon et al., 2003[b]	2000	Korea	38	…	…	…	…	…	1.6	5.1	…	…	…	…
Batáriová et al. 2006	2001-03	Czech Republic	160	…	…	…	…	…	0.33	…	…	…	…	…
Alimonti et al. 2000[b]	nr	Rome	131	…	0.39	…	…	…	…	…	…	…	…	…
Schuhmacher et al., 1994[b]	nr	Tarragona	434	…	…	…	699	…	…	…	27	…	…	…
Seifert et al., 2000[b]	1990-92	Germany (1990/92)	4000	…	…	4.6	…	…	0.21	…	9.5	…	…	…
Zubero et al., 2008[b]	2006-08	Bizkaia	29	…	…	…	…	…	0.36	…	…	…	…	…
Minoia et al., 1990[b]	nr	Italy	11-900	0.57[d]	0.9[d]	17[d]	456[d]	0.79[d]	0.86[d]	17[d]	23	…	0.42[d]	22[d]
Afridi et al., 2009[c]	nr	Pakistan	93 (pregnant)	…	…	…	1150[d]	…	…	…	…	…	…	…
Afridi et al., 2009[c]	Nr	Pakistan	115 (non pregnant)	…	…	…	850[d]	…	…	…	…	…	…	…
Callan et al., 2013[ad]	2008-11	Australia	173	1.2	2.3	13	396	…	…	…	…	10.4	…	26
Hinwood et al., 2013 [ae]	2008-11	Australia	173	…	…	…	…	…	<0.3	0.7	…	…	…	…

[a]Median. [b]Geometric mean. [c]Arithmetic mean. [d]µg/L [e] Pregnant women (3rd trimester)