## Urinary Arsenic Speciation in Children and Pregnant Women from Spain

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#### Abstract

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- 20 Inorganic arsenic (i-As) is a non-threshold human carcinogen that has been associated with
- 21 several adverse health outcomes. Exposure to i-As is of particular concern among pregnant
- women, infants, and children, as they are specifically vulnerable to the adverse health effects of
- i-As, and in utero and early-life exposure, even low to moderate levels of i-As, may have a
- 24 marked effect throughout the lifespan. Ion chromatography-mass spec- trometry detection (IC-
- 25 ICP-MS) was used to analyse urinary arsenic speciation, as an exposure biomarker, in samples
- 26 of 4-year-old children with relatively low-level arsenic exposure living in different regions in
- 27 Spain including Asturias, Gipuzkoa, Sabadell and Valencia. The profile of arsenic metabolites in
- urine was also determined in samples taken during pregnancy (1st trimester) and in the children from Valencia of 7 years old. The median of the main arsenic species found in the 4-year-old
- from Valencia of 7 years old. The median of the main arsenic species found in the 4-year-old children was 9.71 µg/l (arsenobetaine—AsB), 3.97 µg/l (dimethy- larsinic acid—DMA), 0.44 µg/l
- 31 (monomethylarsonic acid—MMA) and 0.35  $\mu$ g/l (i-As). Statistically significant differences were
- 32 found in urinary AsB, MMA and i-As according to the study regions in the 4-year-old, and in
- 33 DMA among pregnant women and their children. Spear- man's correlation coefficient among
- 34 urinary arsenic metabolites was calculated, and, in general, a strong methylation capacity to
- 35 methylate i-As to MMA was observed.

36 **Keywords**: Arsenic speciation; Children; Pregnant women; Biomarker; Urinary metabolites;

37 Inorganic arsenic

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#### Introduction

- 40 Arsenic (As) is a ubiquitous metalloid that is found in both inorganic arsenic (i-As) and organic
- forms, which can readily cross the placenta leading to foetal exposure (Concha et al. 1998;
- 42 Vahter 2009; Davis et al. 2014). Organic As, including arsenobetaine (AsB), arsenosugars and
- 43 arsenolipids, are often found in fish and seafood and considered relatively non-toxic (Navas-
- 44 Acien et al. 2011), while i-As, mainly found as arsenite and arsenate, has been classified as a
- group I, non-threshold, human carcinogen (IARC 2004). Other health effects have also been
- 46 attributed to i-As exposure such as neurological, cardiovascular, respiratory, and metabolic
- 47 diseases. Exposure to i-As is of particular concern among pregnant women, infants and
- 48 children, as they are specifically vulnerable to the adverse health effects of i-As, and in utero
- and early-life exposure, even low to moderate levels of i-As, may have a marked effect
- throughout the lifespan (Farzan et al. 2013; Davis et al. 2014; Farzan et al. 2015; Gilbert-
- 51 Diamond et al. 2016; Sanchez et al. 2016). However, further research on links between dietary
- factors and biomarkers of As is required from areas with relatively low-As exposure (Farzan et
- 53 al. 2013; Kordas et al. 2016).
- Rice is by far, the main dietary sources of i-As, when low i-As drinking water is available (Sohn
- 2014; Kippler et al. 2016). The higher levels of i-As in rice compared to other crops are due to
- anaerobic paddy field culture, which renders i-As highly available for rice plant uptake (Meharg
- and Zhao 2012). Significantly high levels of i-As have also been found in rice-based products
- widely consumed by infants and young children (Signes-Pastor et al. 2016b), which have been
- categorised as a particular sub-population that is more highly exposed to i-As due to higher
- 60 food consumption rates on a body weight basis than adults (EFSA 2009). Urinary As excretion is
- a biomarker of the dietary As exposure and a significant increase in urinary As levels has been
- reported for adults, pregnant women, infants and children after rice consumption (Cascio et al.
- 63 2011; Gilbert-Diamond et al. 2011; Davis et al. 2012; Meharg et al. 2014; Karagas et al. 2016).
- In this study, urinary As metabolites were analysed by ion chromatography with inductively
- 65 coupled plasma— mass spectrometric detection (IC-ICP-MS) and their relationship was
- 66 evaluated in a study population of 4-year-old children from Spain living in Asturias, Gipuzkoa,
- 67 Sabadell and Valencia (Figure 1). The differences in As species concentration in 4-year-old
- children's urine samples due to sex and living region were explored. Furthermore, As species
- and their relationship was studied in urinary samples of pregnant women—children pairs from
- Valencia, urinary samples of which were collected and evaluated in 4- and 7-year-olds.
- Likewise, the differences in As species concentrations in urinary samples from pregnant women
- and their children of 4 and 7 years old were explored.

#### Materials and Methods

#### 74 Study Population

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75 The population included in the study are mother-child pairs participants in the INMA—INfancia 76 y Medio Ambiente—Environment and Childhood project, a prospective population-based birth 77 cohort study conducted in several areas of Spain, www.proyectoinma.org (Guxens et al. 2012). 78 Women were recruited at the beginning of their pregnancy (2003–2006) and were followed up 79 until delivery (n = 2625). Their children were enrolled at birth and were followed up during 80 infancy. In this study, a subset of 100 urinary samples from 4-year-old children were randomly 81 (evenly distributed between boys and girls) selected from each study area: Asturias, Gipuzkoa, 82 Sabadell and Valencia. Additionally, urinary samples of the same children from the Valencia 83 cohort were selected at 7 years (n = 100) and paired with urinary samples of their mother at 1st 84 trimester of pregnancy (n = 100). Most of the participants in the study here were living in urban 85 (81%) and semiurban areas (16%) and only 3% were living in rural locations. 4-year-old children

#### 88 Sample Preparation

- The urine samples were stored at or below -20°C until analysed. Urinary samples were
- 90 centrifuged with a Sorvall Legend RT at 4500 g and diluted as appropriate before transferring a

had a median BMI of 15.99 ranging from 11.52 to 24.99. The median age of the pregnant

women was 31 ranging from 21 to 43 years old and 96% of them have Spanish nationality.

- 91 1 ml aliquot to a 2-ml polypropylene vial with 10 ll of analytical grade hydrogen peroxide to
- 92 convert any arsenite to arsenate to facilitate subsequent chromatographic detection. The urine
- 93 samples were analysed in different batches including blanks and replicate samples of the
- 94 certified reference material (CRM) ClinChek®—Control level I. Urinary samples were
- 95 normalised for urine dilution using specific gravity measured with a clinical refractometer.
- 96 Specific gravity measurement is suggested to normalise urine dilutions since urine creatinine
- 97 may vary with As-related kidney effects, age and other factors (Carlin et al. 2015).

#### 98 Chemical Analyses

- 99 A Thermo Scientific IC5000 ion chromatography system, with a Thermo AS7, 2 9 250 mm
- 100 column and a Thermo AG7, 2 9 50 mm guard column interfaced with a Thermo ICAP Q ICP-MS
- in collision cell mode was used to determine As speciation in urinary samples. A linear gradient
- mobile phase was carried out over 15 min starting at 100% mobile phase of 20 mM ammonium
- carbonate and finishing at 100% mobile phase of 200 mM ammonium carbonate. Authentic
- standards of AsB, DMA, tetratmethylarsonium, MMA and i-As were compared with the
- resulting chromatogram. DMA concentration series were used to calibrate the As present
- under each chromatographic peak.

#### Statistical Analyses

- 108 A descriptive of the different As species was first performed. A Kruskal–Wallis non-parametric
- test was used to determine any significant differences in the As species levels between groups
- according to region, sex and population category (pregnant women, 4- and 7-year-old

- children). Spearman's correlation coefficient (Rho) was determined to evaluate the relationship
- among As species logarithm concentration (log<sub>10</sub>), and the LOESS (local regression) method
- was used for non-linear regression smoothing. All statistical analyses and plots were performed
- using the R Statistical Software (R Core Team 2014). The limit of detection (LOD) was
- calculated as the mean of the blank concentrations plus three times the standard deviation of
- the blank concentrations multiplied by the dilution factor. The 1/2 LOD value was assigned for
- statistical analyses of the data when samples were below the LOD.

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#### Results

#### 120 CRM Recoveries

- 121 The mean ± SE concentration and recovery, calculated using the mean certified values, of the
- As species found in the urine CRM ClinChek®—Control level I, based on n = 33, were as
- follows:  $5.27 \pm 0.09 \,\mu\text{g/l}$  and  $115 \pm 2\%$  for i-As,  $2.44 \pm 0.04 \,\mu\text{g/l}$  and  $97 \pm 2\%$  for MMA,  $9.19 \pm 1.01 \,\mu\text{g/l}$
- 124 0.17  $\mu$ g/l and 94  $\pm$  2% for DMA and 15.17 $\pm$ 0.25  $\mu$ g/l and 90 $\pm$ 2% for AsB. The mean and range
- 125 concentrations of the As species certified in the urine CRM ClinChekO®—Control level I are as
- follows: 4.55 (2.73-6.37)  $\mu$ g/l for i-As, 2.50 (1.50-3.50)  $\mu$ g/l for MMA, 9.80 (5.88-13.7)  $\mu$ g/l for
- 127 DMA and 16.8 (12.6–21.0) µg/l for AsB. The limit of detection (LOD) for As speciation,
- 128 calculated from DMA calibration, was 0.011 µg/l.

#### 129 4-Year-Old Children

- 130 The main arsenic species in urine samples of 4-year-old children from Spain were AsB, DMA,
- 131 MMA and i-As. AsB was predominant with median of 9.71 µg/l and interguartile range (IQR)
- 132 from 2.58 to 34.92 μg/l followed by DMA (median: 3.97 μg/l and IQR: 2.06–6.20 μg/l), MMA
- 133 (median:  $0.44 \mu g/l$  and IQR:  $0.25-0.69 \mu g/l$ ) and i-As (median:  $0.35 \mu g/l$  and IQR: 0.21-0.56
- 134 µg/l). There were no significant statistical differences in As species concentration between 4-
- year-old boys and girls. Statistically significant differences were observed for AsB, MMA and i-
- As according to the study area at 4-year-old. Children living in Gipuzkoa had the lowest i-As
- concentration with a median of 0.22  $\mu$ g/l and IQR from 0.13 to 0.37  $\mu$ g/l (p<0.001), whereas
- they had the highest concentration of MMA (median: 0.52 µg/l and IQR: 0.37–0.79 µg/l; p <
- 139 0.001) and AsB (median: 16.32  $\mu$ g/l and IQR: 6.25–54.64  $\mu$ g/l; p < 0.001). The region of origin
- did not affect DMA concentration in the urine samples of 4-year- old children (p = 0.131)
- 141 (Table 1).
- Spearman correlation between log<sub>10</sub> i-As and log<sub>10</sub> MMA concentration in urine samples
- showed very strong correlation in Asturias (Rho = 0.809), strong correlation in Gipuzkoa (Rho =
- 144 0.747) and Valencia (Rho = 0.765) and moderate correlation in Sabadell (Rho = 0.571). A strong
- 145 correlation was found between  $log_{10}$  i-As and  $log_{10}$  DMA in samples from Valencia (Rho =
- 146 0.607) compared to the other regions that had a moderate correlation with a Spearman
- 147 coefficient ranging from 0.491 to 0.511. There was a strong correlation between log<sub>10</sub> MMA
- and  $log_{10}$  DMA in samples from Asturias (Rho = 0.707), Sabadell (Rho = 0.651) and Valencia

- 149 (Rho = 0.666) compared to a moderate one in Gipuzkoa (Rho = 0.520). A moderate correlation
- was found in urine samples from all regions between log<sub>10</sub> AsB and log<sub>10</sub> DMA. There were no
- significant correlations in log<sub>10</sub> AsB versus log<sub>10</sub> i-As and log<sub>10</sub> AsB versus log<sub>10</sub> MMA in
- 152 Gipuzkoa, Sabadell and Valencia; on the contrary, a moderate correlation was shown between
- those urinary As metabolites in samples from Asturias (Figure 2).

#### Pregnant Women-Children of 4- and 7-Year-Old Pairs

- 155 DMA concentration was higher in urine samples of pregnant women (median: 5.69 µg/l and
- 156 IQR:  $2.93-10.89 \,\mu g/l$ ; p < 0.003) than in the urine samples of their children of 4 and 7 years
- old. There were no statistically significant differences in AsB, MMA and i-As concentration in
- urine samples between pregnant women and their children of 4 and 7-years old (**Table 1**). The
- 159 correlation between log<sub>10</sub> i-As and log<sub>10</sub> MMA in 7-year-old children from Valencia was high
- 160 (Rho = 0.864), a bit lower when they were 4 years old (Rho = 0.765). The correlation between
- the other As species was similar in urine samples from Valencian children of 4 and 7 years old.
- A cluster of 6 maternal urine samples with much higher levels of i-As, ranging from 8.76 to
- 163 12.86 µg/l, was found, which affected the correlation between log<sub>10</sub> i-As and the other As
- species. Despite this cluster of urine samples from pregnant women with higher i-As levels,
- good correlation was found between  $log_{10}$  i-As and  $log_{10}$  MMA (Rho = 0.559), which was higher
- than that found in the 4- and 7-year-old children urine samples when that cluster of samples
- was not included in the Spearman correlation analysis (Rho = 0.875). There was a moderate
- 168 correlation between  $log_{10}$  MMA and  $log_{10}$  DMA in pregnant women urine samples (Rho =
- 169 0.529). A moderate correlation was also found between log<sub>10</sub> i-As and log<sub>10</sub> DMA in pregnant
- women urine samples when the cluster of samples with high levels of i-As was not included in
- the Spearman correlation analysis (Rho = 0.475), which was lower than that found for the 4- and
- 7-year-old children (Figure 3).

#### Discussion

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- Almost all pregnant women, 4- and 7-year-old children urinary samples contained detectable
- 176 (i.e. [LOD) AsB (99, 100 and 100 %, respectively), MMA (95, 93 and 100 %, respectively), DMA
- 177 (99, 98 and 100 %, respectively) and i-As (100, 96 and 99 %, respectively). Since seafood is the
- main source of AsB and the presence of this organic As compound in the urine samples
- suggests that seafood is in the diet of the pregnant women and children, the consumption of
- which has been estimated to be 26.40 kg/person/year in Spain (Magrama 2015). AsB is
- excreted unchanged in the urine, while arsenosugars and arsenolipids, also found in seafood,
- are metabolised predominantly to DMA before being excreted (Choi et al. 2010; Navas-Acien
- et al. 2011). Each autonomous regional public health administration in Spain monitors the
- 184 quality of drinking water and there are no reported cases of contamination with i-As in the
- qeographical areas where this study was carried out. Therefore, the i-As levels in drinking water
- are assumed to be below the EU regulation—10 μg/l (The Council of the European Union
- 187 1998). It is expected that i-As exposure comes largely from foodstuffs such as rice and rice-
- based products due to their high i-As content and regular consumption by the Spanish

189 population (Carbonell-Barrachina et al. 2012; Food and Authority 2014; Meharg et al. 2014; 190 Magrama 2015; Signes-Pastor et al. 2016a). The metabolism of i-As in the human body com-191 prises a series of reduction and methylation reactions, resulting in the formation of MMA (10-192 20%) and DMA (60–70%), which are excreted in urine together with unchanged i-As (10–30%) 193 (Vahter 2002). DMA is, therefore, a metabolite that reflects exposure of both i-As and organic 194 compounds (Navas-Acien et al. 2011). DMA may also be excreted in the urine unchanged after 195 rice consumption because significant levels of DMA have been reported in rice from certain 196 areas, including rice from Spain (Meharg et al. 2009; Signes-Pastor et al. 2016a). Indeed, 90% 197 of urinary As excretion after a rice diet containing both i-As and DMA at a 1:1 ratio has been 198 reported to be DMA (Meharg et al. 2014). In general terms, biomethylation is considered the 199 major detoxification process. However, the intermediate metabolites MMAIII and DMAIII are 200 highly toxic and considered to be toxic species of ingested i-As (Bredfeldt et al. 2006; Lo 2pez-201 Carrillo et al. 2014).

202 The sum of arsenic species concentration in urine samples of pregnant women in their 1st 203 trimester of pregnancy found here for the Valencia cohort was lower than the total As level 204 reported previously for pregnant women in their 1st trimester of pregnancy from the Sabadell 205 cohort, for whom seafood was identified as the main source of As (Forns et al. 2014, 2014a, 206 2014b). However, the urinary arsenic concentration (i-As + MMA + DMA) in pregnant women, 207 found in this study, was almost double than that found in the urine of pregnant women in their 208 2nd trimester of pregnancy living in the state of New Hamp- shire in the US (Gilbert-Diamond 209 et al. 2016). DMA concentration in pregnant women was 1.4-fold higher than that found in 210 their children's urinary samples of both 4- and 7-year-olds, which could be justified with a major 211 exposure to dietary DMA or due to the detoxification process, which has been associated with 212 a higher methylation capacity for women in the early stages of pregnancy (Concha et al. 1998; 213 Vahter 2009). The urinary summation of the As species for 4- and 7-year-old children found in 214 this study was much higher compared to the median of total As previously reported for 6- to 9-215 year-old children and 5- to 17-year-old children (1.17 and 1.72 µg/l, respectively) living in the 216 Southwest Spain (Huelva, Andalusia) (Aguilera et al. 2010; Rodríguez-Barranco et al. 2016). 217 Children aged 5–8 years from Montevideo, Uruguay, with low-level As exposure from drinking 218 water (median of 0.45 µg/l), had urinary median i-As, MMA and DMA values of 1.01, 0.95 and 219 7.9 µg/l, respectively (Kordas et al. 2016), which are about double the concentration found in 220 this study. A similar level of urinary i-As to that found here has been reported for native and 221 immigrant children aged 2 months-16 years living in Barcelona, Spain (median 0.4 µg/l) (Piñol 222 et al. 2015). There were no significant differences in any of the urinary As metabolites in 223 relation to sex in keeping with previous studies that did not find sex differences in total urinary 224 As concentration (Aguilera et al. 2010; Rodríguez-Barranco et al. 2016).

All 4-year-old children cohorts had strong methylation capacity to methylate i-As to MMA, especially those from Asturias, Gipuzkoa and Valencia that had 1.35-fold stronger correlation coefficients on average between log<sub>10</sub> i-As and log<sub>10</sub> MMA than that for those from Sabadell. The correlation coefficients between log<sub>10</sub> i-As and log<sub>10</sub> MMA for 7-year-old children from Valencia were 1.13-fold higher compared to themselves at 4-year-old, which may suggest an

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increase of capacity to methylate i-As to MMA with age. Indeed, the pregnant women had the highest correlation coefficient between log<sub>10</sub> i-As and log<sub>10</sub> MMA when the cluster of samples with high levels of i-As was not included in the calculations. Although lower capacity to further methylate MMA to DMA is suggested by the inferior correlation coefficient between log<sub>10</sub> MMA and log<sub>10</sub> DMA compared to the coefficient between log<sub>10</sub> i-As and log<sub>10</sub> MMA this is difficult to ascertain as both pregnant women and children may have been consuming seafood, which may affect urinary DMA concentration as implied with a moderate correlation coefficient found between log<sub>10</sub> AsB and log<sub>10</sub> DMA. Children from Asturias also had a moderate correlation between log<sub>10</sub> AsB and log<sub>10</sub> MMA and between log<sub>10</sub> AsB and log<sub>10</sub> i-As. This may indicate consumption of seafood products with significant i-As concentration such as some species of brown algae and bivalves (EFSA 2009; Amlund and Sloth 2011).

## Conclusions

In this study, it is shown that AsB, DMA, MMA and i-As are the main As metabolites in urine of pregnant women, 4- and 7-year-old children living in Spain with low-level As exposure from drinking water. Although further studies are required to identify the main dietary sources of As for the Spanish population, it is expected that seafood and rice items contribute significantly to organic As and i-As exposure, respectively. 4-year-old children from Asturias, Sabadell and Valencia had higher urinary i-As than those from Gipuzkoa. There were also significant differences in urinary AsB and DMA among regions. The similar levels of i-As found in urinary samples of pregnant women and their children of 4 and 7 years old suggest a long-term relatively low i-As dietary exposure. Correlation analyses between urinary As metabolites show a strong ability to methylate i-As to MMA, especially for pregnant women and 7-year-old children.

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#### 269 References

- 270 Aguilera I, Daponte A, Gil F et al (2010) Urinary levels of arsenic and heavy metals in children
- and adolescents living in the industri- alised area of Ria of Huelva (SW Spain). Environ Int
- 272 36:563–569. doi:10.1016/j.envint.2010.04.012
- 273 Amlund H, Sloth JJ (2011) Arsenic exposure from seafood consumption. In: Nriagu JO (ed)
- 274 Encyclopedia of environmental health. Elsevier, Burlington, pp 145–149
- 275 Bredfeldt TG, Jagadish B, Eblin KE et al (2006) Monomethylarson- ous acid induces
- transformation of human bladder cells. Toxicol Appl Pharmacol 216:69–79.
- 277 doi:10.1016/j.taap.2006.04.011
- 278 Carbonell-Barrachina AA, Wu X, Ramírez-Gandolfo A et al (2012) Inorganic arsenic contents in
- rice-based infant foods from Spain, UK, China and USA. Environ Pollut 163:77–83.
- 280 doi:10.1016/j. envpol.2011.12.036
- 281 Carlin DJ, Naujokas MF, Bradham KD et al (2015) Arsenic and environmental health: state of
- the science and future research opportunities. Environ Health Perspect.
- 283 doi:10.1289/ehp.1510209
- 284 Cascio C, Raab A, Jenkins RO et al (2011) The impact of a rice based diet on urinary arsenic. J
- 285 Environ Monit 13:257–265. doi:10. 1039/c0em00482k
- 286 Choi BS, Choi SJ, Kim DW et al (2010) Effects of repeated seafood consumption on urinary
- 287 excretion of arsenic species by volun- teers. Arch Environ Contam Toxicol 58:222–229.
- 288 doi:10.1007/s00244-009-9333-8
- 289 Concha G, Vogler G, Lezcano D et al (1998) Exposure to inorganic arsenic metabolites during
- 290 early human development. Toxicol Sci 44:185–190
- 291 Davis MA, Mackenzie TA, Cottingham KL et al (2012) Rice consump-tion and urinary arsenic
- concentrations in U.S. children. Environ Health Perspect 120:1418–1424.
- 293 doi:10.1289/ehp.1205014
- Davis MA, Li Z, Gilbert-Diamond D et al (2014) Infant toenails as a biomarker of in utero arsenic
- 295 exposure. J Expo Sci Environ Epidemiol 24:467–473. doi:10.1038/jes.2014.38
- 296 EFSA (2009) European food safety authority. Scientific opinion on arsenic in food. EFSA panel
- on contaminants in food chain (CONTAM), Parma
- 298 Farzan SF, Karagas MR, Chen Y (2013) In utero and early life arsenic exposure in relation to
- 299 long-term health and disease. Toxicol Appl Pharmacol 272:384–390.
- 300 doi:10.1016/j.taap.2013.06.030
- 301 Farzan SF, Li Z, Korrick SA et al (2015) Infant infections and respiratory symptoms in relation to
- arsenic exposure in a U.S. cohort. Environ Health. doi:10.1289/ehp.1409282

- Food E, Authority S (2014) Dietary exposure to inorganic arsenic in the European population, 1.
- 304 doi: 10.2903/j.efsa.2014.3597 Forns J, Fort M, Casas M et al (2014) Exposure to metals
- duringpregnancy and neuropsychological development at the age of 4
- 306 years. Neurotoxicology 40:16–22. doi:10.1016/j.neuro.2013.10.006 Fort M, Cos 🗓 in-Toma 🗓 s
- 307 M, Grimalt JO et al (2014a) 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.
- 309 Environ Sci Pollut Res 21:9234-9241. doi:10.1007/s11356-014-2827-6
- Fort M, Grimalt JO, Casas M, Sunyer J (2014b) Food sources of arsenic in pregnant
- 311 Mediterranean women with high urine concentrations of this metalloid. Environ Sci Pollut Res
- 312 1-10:11689-11698. doi:10.1007/s11356-014-2614-4
- Gilbert-Diamond D, Cottingham KL, Gruber JF et al (2011) Rice consumption contributes to
- arsenic exposure in US women. Proc Natl Acad Sci USA 108:20656–20660.
- 315 doi:10.1073/pnas.1109127108
- 316 Gilbert-Diamond D, Emond JA, Baker ER et al (2016) Relation between in utero arsenic
- 317 exposure and birth outcomes in a cohort of mothers and their newborns from new hampshire.
- 318 Environ Health Perspect. doi:10.1289/ehp.1510065
- 319 Guxens M, Ballester F, Espada M et al (2012) Cohort profile: the INMA-INfancia y Medio
- 320 Ambiente-(environment and child-hood) project. Int J Epidemiol 41:930–940. doi:10.1093/ije/
- 321 dyr054
- 322 IARC (2004) International agency for research on cancer monographs on the evaluation of
- 323 carcinogenic risks to humans. Some drinking- water disinfectants and contaminants, including
- 324 Arsenic
- 325 Karagas MR, Punshon T, Sayarath V et al (2016) Association of rice and rice-product
- 326 consumption with arsenic exposure early in life. JAMA Pediatr 03766:1–8.
- 327 doi:10.1001/jamapediatrics.2016.0120
- 328 Kippler M, Skröder H, Mosh S et al (2016) Elevated childhood exposure to arsenic despite
- reduced drinking water concentra- tions—a longitudinal cohort study in rural Bangladesh. Env
- 330 Inter 86:119–125. doi:10.1016/j.envint.2015.10.017
- Kordas K, Queirolo El, Mañay N et al (2016) Low-level arsenic exposure: nutritional and dietary
- predictors in first-grade Uruguayan children. Environ Res 147:16–23. doi:10.1016/j.
- 333 envres.2016.01.022
- López-Carrillo L, Hernández-Ramírez RU, Gandolfi AJ et al (2014) Arsenic methylation capacity
- is associated with breast cancer in northern Mexico. Toxicol Appl Pharmacol 280:53–59. doi:10.
- 336 1016/j.taap.2014.07.013
- 337 Magrama (2015) Informe del Consumo de Alimentación en España. Ministerio de Agricultura
- 338 Alimentación y Medio Ambiente Meharq AA, Zhao FJ (2012) Arsenic & rice. Springer-Verlag,

- 339 Berlin Meharg AA, Williams PN, Adomako E et al (2009) Geographical variation in total and
- inorganic arsenic content of polished (white) rice. Environ Sci Technol 43:1612–1617.
- 341 doi:10.1021/es802612a
- Meharg AA, Williams PN, Deacon CM et al (2014) Urinary excretion of arsenic following rice
- 343 consumption. Environ Pollut 194:181–187. doi:10.1016/j.envpol.2014.07.031
- Navas-Acien A, Francesconi KA, Silbergeld EK, Guallar E (2011) Seafood intake and urine
- concentrations of total arsenic, dimethylarsinate and arsenobetaine in the US population.
- 346 Environ Res 111:110–118. doi:10.1016/j.envres.2010.10.009
- Piñol S, Sala A, Guzman C et al (2015) Arsenic levels in immigrant children from countries at risk
- of consuming arsenic polluted water compared to children from Barcelona. Environ Monit
- 349 Assess 187:661. doi:10.1007/s10661-015-4869-8
- 350 R Core Team (2014) R: A language and enrionment for statistical computing. R Foundation for
- 351 Statisical Computing, Vienna Rodr 🛭 guez-Barranco M, Gil F, Hernández AF et al (2016)
- Postnatal arsenic exposure and attention impairment in school children.
- 353 Cortex. doi:10.1016/j.cortex.2014.12.018 Sanchez TR, Perzanowski M, Graziano JH (2016)
- Inorganic arsenic and respiratory health, from early life exposure to sex-specific effects: a
- 355 systematic review. Environ Res 1–19:537–555. doi:10. 1016/j.envres.2016.02.009
- 356 Signes-Pastor AJ, Carey M, Carbonell-Barrachina AA et al (2016a) Geographical variation in
- inorganic arsenic in paddy field samples and commercial rice from the Iberian Peninsula. Food
- 358 Chem 202:356–363. doi:10.1016/j.foodchem.2016.01.117
- 359 Signes-Pastor AJ, Carey M, Meharg AA (2016b) Inorganic arsenic in rice-based products for
- infants and young children. Food Chem 191:128–134. doi:10.1016/j.foodchem.2014.11.078
- 361 Sohn E (2014) The toxic side of rice. Nat Outlook, pp 5–6 The Council of the European Union
- 362 (1998) Council directive 98/83/ EC of 3 November 1998 on the quality of water intended for
- 363 human consumption. Off J Eur Commun L 330:32–54 Vahter M (2002) Mechanisms of arsenic
- 364 biotransformation. Toxicology 181–182:211–217. doi:10.1016/S0300-483X(02)00285-8 Vahter
- 365 M (2009) Effects of arsenic on maternal and fetal health. Annu Rev Nutr 29:381–399.
- 366 doi:10.1146/annurev-nutr-080508-141102

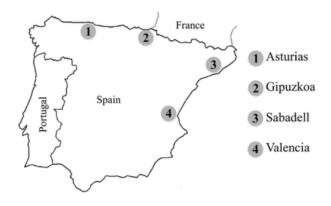
**Table 1**: Arsenic speciation (median [25–75 %ile]) in urinary pregnant women, 4-year-old and 7-year-old children samples from each cohort study

Category	N	AsB (µg/l)	DMA (μg/l)	MMA (µg/l)	i-As (μg/l)
Children—4 years	400	9.71 (2.58–34.92) <sup>A</sup>	3.97 (2.06-6.20)	0.44 (0.25-0.69)	0.35 (0.21-0.56)
Girls—4-year-old	200	9.21 (2.44-28.25)	3.82 (1.85-6.13)	0.40 (0.24-0.69)	0.32 (0.21–0.57)
Boys—4-year-old	200	10.51 (2.89–40.41)	4.07 (1.85–6.27)	0.45 (0.25–0.70)	0.37 (0.21–0.56)
P value		0.778	0.376	0.568	0.796
Asturias—4-year–old	100	9.07 (1.68–25.31) <sup>b</sup>	3.76 (2.19–5.67)	0.35 (0.18-0.60) <sup>b</sup>	0.38 (0.25-0.54)
Gipuzkoa—4-year-old	100	16.32 (6.25–54.64) <sup>a</sup>	4.23 (2.27–8.92)	0.52 (0.37-0.79)	0.22 (0.13-0.37) <sup>b</sup>
Sabadell—4-year-old	100	5.72 (1.74–20.49) <sup>b</sup>	3.73 (1.62–5.45)	0.49 (0.25–0.84) <sup>ab</sup>	0.39 (0.23-0.65) <sup>a</sup>
Valencia—4-year-old	100	9.01 (2.43–48.78) <sup>ab</sup>	4.19 (2.34–6.39)	0.36 (0.22-0.60) <sup>b</sup>	0.44 (0.28-0.59) <sup>a</sup>
<i>P</i> -value		<0.001	0.131	<0.001	< 0.001
4-year-old-valencia	100	9.01 (2.43–48.78)	4.19 (2.34-6.39) <sup>b</sup>	0.36 (0.22-0.60)	0.44 (0.28-0.59)
7-year-old-valencia	100	6.81 (1.63–27.61)	4.14 (2.57–6.49) <sup>b</sup>	0.35 (0.22-0.50)	0.40 (0.23-0.57)
Pregnant women-valencia	100	11.96 (4.14–50.34)	5.69 (2.93–10.89) <sup>a</sup>	0.32 (0.19-0.63)	0.42 (0.27-0.62)
<i>P</i> -value		0.115	0.003	0.594	0.518

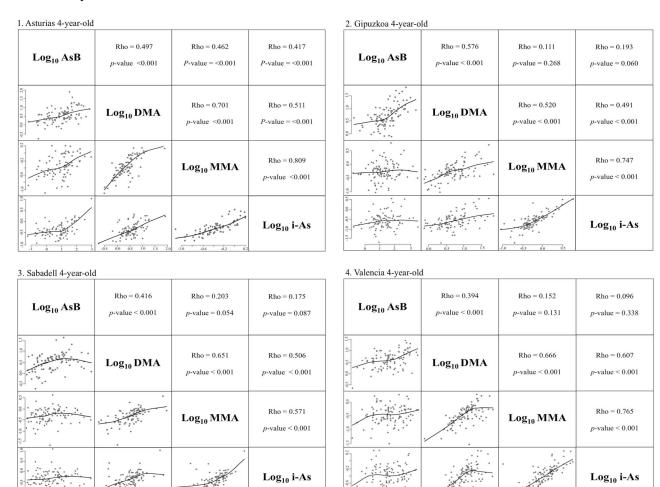
Median (25–75 %ile); values with the same letters were not significantly different at p-value < 0.05 for the variable studied.

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# Figure 1: Geographical location of the cohorts in Spain included in this study.



# **Figure 2**: Correlation between arsenic species in urinary 4-year-old samples from each cohort study



# Figure 3: Correlation between arsenic species in urinary pregnant women and 7-year-old children samples from Valencia

1. Valencia 7-year-ole	d	-	
Log <sub>10</sub> AsB	Rho = $0.481$ <i>p</i> -value < $0.001$	Rho = 0.270  p-value = 0.004	Rho = $0.248$ <i>p</i> -value = $0.013$
8	Log <sub>10</sub> DMA	Rho = 0.707  p-value < 0.001	Rho = 0.684  p-value < 0.001
0 0 0		Log <sub>10</sub> MMA	Rho = 0.864  p-value < 0.001
\$\text{Q} \\ \text{Q} \\ \text	0.0 0.5 10	15 10 05 00	Log <sub>10</sub> i-As

2. Valencia pregnant	mothers		
Log <sub>10</sub> AsB	Rho =0.457  p-value <0.001	Rho = 0.278  p-value =0.006	Rho = $-0.030$ p-value = $0.766$
8 8 8	Log <sub>10</sub> DMA	Rho = 0.529  p-value < 0.001	Rho =0.249  p-value = 0.012
80 0		Log <sub>10</sub> MMA	Rho = 0.559  p-value < 0.001
01 50 000 50 00 50 00 50 00 50 00 50 00 50 00 50 00 50 00 50 00 50 00 50	00 05 10 15	15 10 05 00	Log <sub>10</sub> i-As