# Profile of Urinary Arsenic Metabolites during Pregnancy

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**Chronic exposure to inorganic arsenic (In-As) from drinking water is associated with different health effects, including skin, lung, bladder, and kidney cancer as well as vascular and possibly reproductive effects. In-As is metabolized through the process of methylation, resulting in the production and excretion of methylated species, mainly monomethylarsenate (MMA) and dimethylarsenate (DMA). Because a large percentage of the dose is excreted in urine, the distribution of urinary In-As, MMA, and DMA is considered a useful indicator of methylation patterns in human populations. Several factors affect these patterns, including sex and exposure level. In this study, we investigated the profile of urinary In-As, MMA, and DMA of pregnant women. Periodic urine samples were collected from early to late pregnancy among 29 pregnant women living in Antofagasta, Chile, who drank tap water containing 40 μg/L In-As. The total urinary arsenic across four sampling periods increased with increasing weeks of gestation, from an initial mean value of 36.1 to a final value of 54.3 μg/L. This increase was mainly due to an increase in DMA, resulting in lower percentages of In-As and MMA and a higher percentage of DMA. Our findings indicate that among women exposed to moderate arsenic from drinking water during pregnancy, changes occur in the pattern of urinary arsenic excretion and metabolite distribution. The toxicologic significance of this is not clear, given recent evidence suggesting that intermediate methylated species may be highly toxic. Nevertheless, this study suggests that arsenic metabolism changes throughout the course of pregnancy, which in turn may have toxicologic effects on the developing fetus.** *Key words:* **arsenic, arsenic metabolism, arsenic methylation, Chile, pregnancy, urinary arsenic.** *Environ Health Perspect* **111:1888–1891 (2003). doi:10.1289/ehp.6254 available via *http://dx.doi.org/* [Online 4 September 2003]**

Chronic exposure to inorganic arsenic (In-As) is known to cause characteristic skin lesions; cancers of the skin, bladder, kidney, and lung; and vascular health effects [National Research Council (NRC) 2001; World Health Organization (WHO) 2001]. Evidence suggests that In-As may also increase the risk of diabetes, hypertension, and other internal cancers (WHO 2001). In addition, In-As exposure may be associated with various reproductive and developmental effects (Hopenhayn et al. 2003; Hopenhayn-Rich et al. 2000; WHO 2001). Worldwide, the most common source of human exposure is through drinking water from sources that are naturally high in In-As. Populations in a number of countries have been identified as having high exposures and elevated health risks (WHO 2001).

The metabolism of In-As is mainly through methylation to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Until recently, methylation was regarded as the main metabolic detoxification pathway by which the highly toxic In-As species were converted to the less toxic and more easily excreted methylated species [U.S. Environmental Protection Agency (EPA) 1988; Vahter and Concha 2001). Because most In-As, MMA, and DMA (hereafter referred to as arsenic species) is eliminated in the urine, the sum of urinary arsenic species has been considered a good measure of In-As exposure, and the relative proportion of urinary species, particularly the methylated forms, has been considered an appropriate indicator of methylation efficiency and detoxification capacity (Hopenhayn-Rich et al. 1996a, 1996b; Vahter 1999; Vahter and Concha 2001). Recently, this notion has been changing because of the isolation of trivalent methylated species ($MMA^{+3}$ and $DMA^{+3}$), the evidence that $MMA^{+3}$ is a necessary intermediate in the methylation process ($In\text{-}As^{+5} \rightarrow In\text{-}As^{+3} \rightarrow MMA^{+5} \rightarrow MMA^{+3} \rightarrow DMA^{+5} \rightarrow DMA^{+3}$), and findings from laboratory studies showing that trivalent methylated species, particularly $MMA^{+3}$, may be more toxic than In-As (Styblo et al. 2000, 2002). However, the degree of tissue exposure to these intermediates is not yet clear, trivalent MMA and DMA are unstable in solution (e.g., urine), and the methodology for their measurement in urine has only recently been developed (Del Razo et al. 2001). Issues of sampling and stability of species during storage, as well as interindividual variation in the stability of trivalent species, affect the differentiation and the relative proportions of $MMA^{+3}$ and $DMA^{+3}$ (Del Razo et al. 2001). Therefore, further development is needed before separation of all urinary species becomes a common monitoring tool in epidemiologic studies.

The relative distribution of urinary In-As, MMA, and DMA is still considered an adequate indicator of methylation capacity after exposure to In-As, although the interpretation of the findings in terms of detoxification versus toxification is currently an area of active investigation (Styblo 2002; Vahter and Concha 2001). Under the detoxification hypothesis of In-As through methylation, a relatively higher proportion of methylated species, particularly DMA, in the urine has been viewed as an indicator of higher methylation capacity or efficiency. Later studies of various populations exposed to contrasting arsenic exposures found that a relatively small variability could be attributed to exposure, but other factors also contributed to urinary distribution of arsenic metabolites, such as age, smoking, and genetic susceptibility (Vahter 1999). In addition to these factors, several studies have reported gender differences in the distribution of arsenic metabolites, with women having a higher percentage of methylated metabolites compared with men, even after adjusting for other factors. A study in the Atacama Desert in Chile compared methylation patterns in residents from two villages with distinct arsenic water concentrations (600 μg/L vs. 15 μg/L). In both locations, women were found to have lower percentages of In-As and higher percentages of DMA compared with men (Hopenhayn-Rich 1996a, 1996b). Studies in Taiwan (Hsueh et al. 1998), Finland (Kurttio 1998), and the United States (Kalman et al. 1990) found similar gender differences.

A study conducted among women living at high altitude in the Andean region of Argentina (Concha et al. 1998) reported on

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the effect of pregnancy on arsenic methylation. Ten pregnant women provided urine samples for arsenic speciation analysis shortly before giving birth and then at 2.8 weeks, 2.5 months, and 4.4 months postpartum. A higher proportion of methylated species was observed during late pregnancy compared with after delivery (87%, 78%, 64%, and 69% DMA, respectively). These results led us to investigate the distribution of urinary arsenic species (In-As, MMA, and DMA) to evaluate potential changes in arsenic methylation patterns during the course of pregnancy. For this purpose, we measured arsenic species in sequential urine samples obtained during pregnancy from women exposed to moderately elevated arsenic levels in drinking water.

## Materials and Methods

In the present methylation study we examined a subgroup of women who participated in a pregnancy cohort study investigating several reproductive outcomes in relation to arsenic exposure from drinking water. A detailed description of the parent study has been published (Hopenhayn 2003). For the purpose of the present study, a brief description of the overall methodology follows.

The cohort study enrolled more than 900 women in two Chilean cities: Antofagasta, with arsenic water levels averaging around 40 μg/L, and Valparaiso, with low exposure to arsenic in water (< 1 μg/L). Women were eligible to enter the study if they were at least 18 years of age, were between 16 and 36 weeks of gestation, and used tap water for drinking and cooking purposes. All women agreed to participate in an in-person interview that provided information regarding their sociodemographic characteristics, dietary and other lifestyle habits, fluid consumption, exercise, medical history, and other topics. Cohort participants provided a urine sample at entry into the study, which took place at one of their regularly scheduled prenatal clinic appointments. The participants were recruited from three public health clinics in Antofagasta and two in Valparaiso.

A subgroup of 29 women from Antofagasta enrolled in the pregnancy cohort study was selected to also participate in this study. The selection of women invited into this study was based on their entering the cohort investigation relatively early in gestation, but otherwise the group was a convenience sample attending one of the three Antofagasta clinics used in the overall study. Participants in this study agreed to provide additional urine samples throughout their pregnancy at relatively regular intervals, about a month apart. To obtain their samples, they were contacted by a midwife who was on the cohort study staff and was also assigned to the methylation study sample collection. Sequential urine samples were obtained either at later prenatal visits or at the participants' home, depending on whether the midwife was able to be at the clinic when the study participants had their prenatal appointments. Each woman provided three to five samples, depending on the weeks of gestation at enrollment and on practical considerations. Because most women ($n$ = 27) provided at least four samples, for the sake of consistency we selected up to four per participant for laboratory analysis. For women who had provided five samples, we used the first and last, plus two additional ones so that the time spacing between any two samples was similar. For the purpose of the analysis presented here, we excluded two women for whom we obtained only three samples and one woman who had very low total arsenic concentration (2.4 μg/L, with nondetectable levels of both MMA and DMA).

The study protocol was reviewed and approved by the University of Kentucky Institutional Review Board and by the participating Chilean institutions. The women participating in this study signed two consent forms: one for the overall cohort study and one for the added participation in this methylation study.

The sample collection included spot urine samples voided into a plastic cup, which was immediately poured into two 30-mL propylene bottles. These were placed promptly on ice until they were transferred to a –20°C freezer later the same day. After all the samples were collected, they were shipped to the University of Washington, where they were analyzed by batch hydride generation cryogenic trapping and atomic absorption spectophotometry (Kalman 1988). The laboratory personnel were unaware of the identity of the study subjects and the timing of pregnancy of each sample. The quantitation limits for the arsenic species analyzed were 0.2 ppb for In-As and MMA and 0.4 ppb for DMA. Quality control analysis included two blanks per batch, benchmark control samples before each batch and every five samples, and replicate analysis on 10% of the samples. The maximum allowable control sample variation permitted was 15%, and the average variability for each species was around 10%. Creatinine measurements were also performed on all samples to adjust the arsenic concentration to the creatinine content.

As explained above, the women included in this methylation study were part of a larger reproductive study that collected data for many demographic and personal characteristics of participants. For this report, we present only the general characteristics of the subgroup and concentrate mainly on their methylation profile throughout pregnancy.

We used univariate analyses to describe general demographic, lifestyle, and pregnancy characteristics of the study group. The four urine samples analyzed per woman were labeled sequentially as $S_1$, $S_2$ , $S_3$, and $S_4$, with $S_1$ denoting the first sample and $S_4$ denoting the last one. The corresponding week of gestation at which each sample was obtained was derived from the date of sample collection and an algorithm developed to estimate the gestational age at birth for the entire cohort study (Hopenhayn et al. 2003). Briefly, the algorithm was based on comparisons among three measures: date of last menstrual period (LMP), ultrasound during pregnancy, and clinical examination of the infant at birth. The LMP was the basis for the determination of weeks of gestation for 92% of the overall cohort participants, and similarly, for 90% of the 26 women in this methylation study.

We calculated the total urinary In-As metabolites (Tot-As) by adding across the three species measured (In-As, MMA, and DMA) and deriving the relative proportions of each species. We used graphical representation to examine the distribution of metabolites and the Tot-As for the four sampling periods across pregnancy. We assessed differences across the groups by one-way analyses of variance and by nonparametric test for trend across ordered groups (Cuzick 1985). Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA) and STATA (Stata Corporation, College Station, TX, USA).

## Results

Table 1 presents selected characteristics of the present study group, including age, parity, weight gain, and education, among others. In general, the women who participated in this methylation study, as characterized in

**Table 1.** General characteristics of the study group.

| Characteristic | No. | Frequency (%) |
|---|---|---|
| Education | | |
| None or primary school | 9 | 35 |
| Middle school | 12 | 46 |
| Post middle school | 5 | 19 |
| Monthly income (in Chilean pesos)[a] | | |
| $0–200,000 | 16 | 62 |
| ≥ $200,001 | 10 | 38 |
| Parity | | |
| No birth | 9 | 35 |
| 1 | 8 | 31 |
| 2 | 6 | 23 |
| ≥ 3 | 3 | 12 |
| Race | | |
| Indigenous | 5 | 19 |
| Nonindigenous | 21 | 81 |
| Kessner[b] | | |
| Inadequate | 1 | 4 |
| Intermediate | 6 | 24 |
| Adequate | 18 | 72 |
| Tobacco smoking | | |
| No | 22 | 85 |
| Yes[c] | 4 | 15 |
| Maternal age (years) | 26 | 23.7 ± 5.5[d] |
| Weight gain (kg) | 23 | 11.3 ± 4.7[d] |
| Weeks of gestation at first visit | 25 | 9.9 ± 3.4[d] |

[a]During study period, $1 U.S. was ~ 400 Chilean pesos. [b]Index of prenatal care. [c]Range, 0.14–2.0 cigarettes per day. [d]Values shown are mean ± SD.

Table 1, were similar to the overall cohort participants in Antofagasta, from where they were subsampled (Hopenhayn et al. 2003). Most had been pregnant before (65% had a previous birth), started prenatal care adequately early (mean gestation at first prenatal visit, 9.9 weeks), had adequate prenatal care (72%), and did not have post-high school education (19% went to college). Only four women reported smoking during pregnancy, and the number of cigarettes smoked per day was low (0.14–2.0/day).

Table 2 summarizes the weeks of gestation of the study participants at each of the four sampling periods, and the corresponding Tot-As, urinary creatinine, and total creatinine-adjusted arsenic measurements. Although the number of weeks of gestation across the different sampling periods slightly overlapped, the 95% confidence intervals of the means indicate that they represent quite distinct periods (18.6–20.6, 25.5–26.5, 30.2–31.6, 34.8–36.5 weeks). The sampling periods showed a steady increase in the weeks of gestation from $S_1$ to $S_4$, starting at < 20 weeks and ending around 36 weeks, thus covering most of the second half of pregnancy. This time frame corresponds to the period where most fetal growth and maturation, as well as maternal physical changes, take place (a pregnancy is considered full term if delivery occurs at ≥ 37 completed weeks, with 40 weeks being used for estimation of a woman's expected due date).

The mean Tot-As (sum of species) levels show a clear increasing trend across pregnancy, with $S_4$ having an overall mean 51.2% greater than $S_1$ (from 36.1 to 54.3 µg/L; Table 2). The creatinine-adjusted values showed a similar trend, with an even steeper gradient (from 35.6 to 63.5 µg As/g creatinine). The medians show a similar effect, increasing from 30.3 to 61.7 µg As/g creatinine.

Table 2 also presents the percentage of In-As, percentage of MMA, percentage of DMA, and MMA:DMA for each of the four sampling periods. The mean proportion of In-As decreased by 44.1% from the $S_1$ to $S_4$ (from 11.8% to 8.1%). The proportion of MMA also decreased steadily (from 7.3% to 5.6%), and the proportion of DMA correspondingly increased (from 80.9% to 86.3%). This resulted in a decrease of the MMA:DMA ratio (from 0.095 to 0.066). The tests for trend produced significant results for Tot-As as well as for each of the metabolites analyzed across the four time periods. In addition to the trend of the group overall, we observed a decrease in percentage of In-As and an increase in percentage of DMA in most of the study subjects (22 of the 26). However, the change in Tot-As was mostly accounted for by the increases in DMA (which changed from a mean of 30.2 µg/L for $S_1$ to 46.9 µg/L for $S_4$; compare with the change in In-As from 3.8 µg/L to 4.4 µg/L and in MMA from 2.1 µg/L to 3.0 µg/L). Correspondingly, most of the change in the relative distribution of metabolites was due to the increase in DMA.

In Figure 1 we present the decrease observed for the interindividual variability in the proportions of In-As, MMA, and DMA across pregnancy ($S_1$–$S_4$).

It is also interesting that the two participants with the highest percentages of In-As in $S_1$ (who also had the highest percentages of MMA and lowest percentages of DMA) are the two women who reported smoking one or two cigarettes per day. Aside from this observation, we did not find other variables to noticeably affect the distribution of arsenic urinary metabolites, such as month of the year sample was taken (to examine seasonal variations), maternal age, weight gain, or education (analysis not shown).

## Discussion

The results of this study indicate that, among women exposed to arsenic from drinking water, changes occur in the pattern of arsenic excretion and metabolite distribution during the course of pregnancy. These changes are likely to

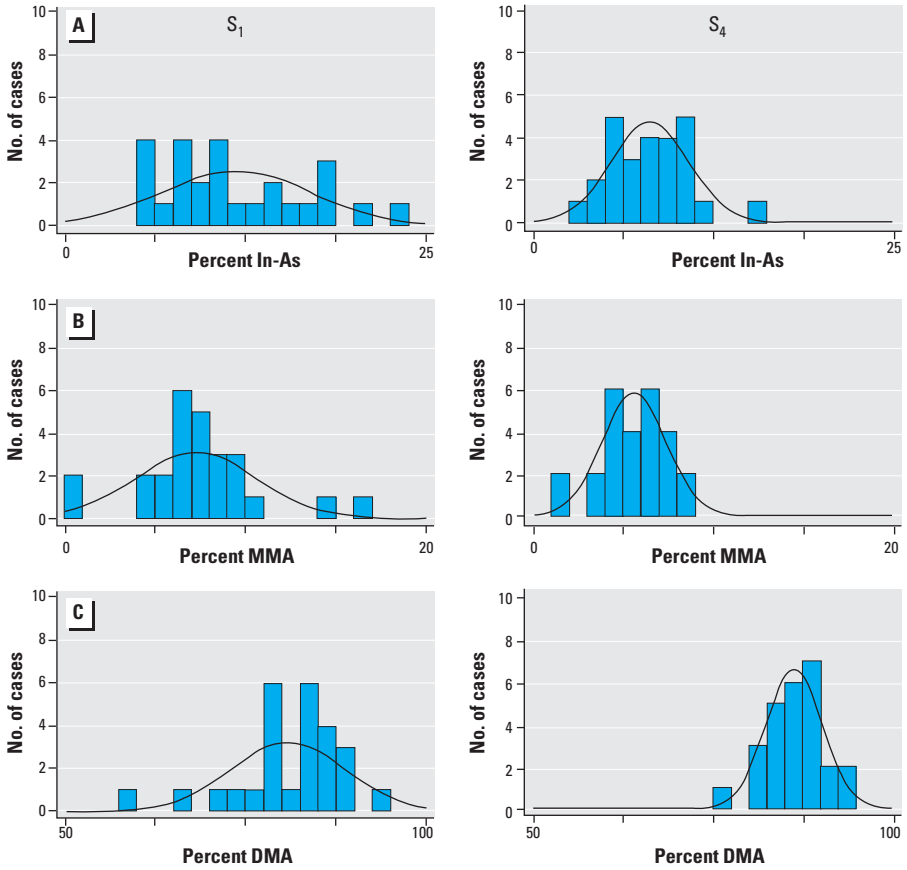


**Figure 1.** Distribution of the percentages of (*A*) In-As, (*B*) MMA, and (*C*) DMA in urine, at the first ($S_1$) and last ($S_4$) sampling periods, for all the study participants.

**Table 2.** Gestational weeks, creatinine, and arsenic species for each sampling period.

| | Gestational age (weeks) | Tot-As (µg/L) | Tot-As adjusted for creatinine (µg/g) | | Percent In-As | Percent MMA | Percent DMA | MMA:DMA |
|---|---|---|---|---|---|---|---|---|
| | | | Mean ± SD | Median | | | | |
| Sample 1 | 19.6 ± 2.4 | 36.1 ± 24.6 | 35.6 ± 19.6 | 30.3 | 11.8 ± 5.1 | 7.3 ± 3.4 | 80.9 ± 7.9 | 0.10 ± 0.06 |
| Sample 2 | 26.0 ± 1.3 | 45.2 ± 27.5 | 52.5 ± 29.8 | 42.3 | 9.6 ± 3.6 | 6.1 ± 2.4 | 84.3 ± 5.7 | 0.08 ± 0.04 |
| Sample 3 | 30.9 ± 1.8 | 54.2 ± 28.9 | 60.0 ± 16.4 | 58.5 | 9.5 ± 5.1 | 5.9 ± 2.1 | 84.6 ± 6.0 | 0.07 ± 0.03 |
| Sample 4 | 35.7 ± 2.0 | 54.3 ± 21.9 | 63.5 ± 19.4 | 61.7 | 8.1 ± 2.7 | 5.6 ± 1.8 | 86.3 ± 3.9 | 0.07 ± 0.02 |
| *p*-Value (test for trend) | — | < 0.01 | < 0.01 | | 0.01 | 0.02 | 0.01 | 0.01 |

Values shown are mean ± SD except where noted.

represent changes in arsenic metabolism. The Tot-As levels systematically increased, mainly due to a rise in DMA concentration. In turn, this also changed the relative distribution of arsenic species, decreasing the percentage of In-As and MMA and increasing the proportion of DMA, as well as decreasing the MMA:DMA ratio.

It is not clear why the Tot-As concentration increased. It is possible that the exposure dose increased if the women in the study consumed more tap water–based drinks as their pregnancy progressed. Because we did not take repeated dietary or fluid consumption histories at the time of each urine sampling, we cannot evaluate potential changes in total fluid or arsenic intake. However, examination of the entire pregnancy cohort of the parent study, which had a much larger study size and a wider range in weeks of gestation at entry into the study, did not show women interviewed later in pregnancy (gestation ≥ 30 weeks) reporting higher consumption of water-based fluids compared with women interviewed earlier in pregnancy (gestation ≤ 20 weeks).

Because the Tot-As increase was mainly due to an increase in total DMA, it is possible that with the progression of pregnancy there is an induction of methylation (Vahter et al. 1995), which facilitates arsenic excretion (Concha et al. 1998; Vahter and Concha 2001). Given all the hormonal and other changes that occur throughout pregnancy (e.g., in fluid volume and distribution), it is feasible that these may affect the metabolism of arsenic. This hormonal hypothesis has some support in the gender differences observed in the profile of urinary arsenic species. Another study in Chile found that women had a greater proportion of methylated metabolites compared with men, particularly DMA (3.5% difference), even after controlling for other factors such as age, smoking, and exposure level (Hopenhayn-Rich et al. 1996b). Gender differences in the distribution of urinary arsenic metabolite distribution were also reported in other arsenic studies in Finland (Kurttio et al. 1998), Taiwan (Hsueh et al. 1998), and the United States (Kalman et al. 1990), although the magnitude of the differences varies and the presentation of results does not allow direct comparisons.

It is also possible that during pregnancy there is an increased release of tissue-bound arsenic, as DMA, or as In-As or MMA, which is further methylated and excreted as DMA. Individuals exposed to high arsenic levels who were given an arsenic-chelating agent (2,3-dimercaptopropane-1-sulfonic acid) showed a quick increase in the concentration of urinary arsenic, particularly methylated species (Aposhian et al. 1997), supporting the hypothesis that arsenic is bound to tissues and can be released through chemical induction. The activity of endogenous methylases in human placenta increased steadily with weeks of gestation, suggesting that increased formation of methyltransferases might occur during pregnancy (Paik et al. 1991). Therefore, there is some empirical support for the induction of methylation during pregnancy. However, it is not clear whether the observed results reflect an increase in the methylation and excretion of arsenic from current exposure, or from release of arsenic bound to tissues, or both.

It remains to be investigated whether this increased excretion of arsenic, and in particular of methylated metabolites, can be viewed as an enhanced detoxification process or as an increased risk of exposure to highly toxic intermediate trivalent methylated arsenicals. Concha et al. (1998) obtained urine samples from infants shortly after birth and found they had an average of 90% DMA, similar to the 87% of the mothers. It would be interesting to investigate the correlation of urinary total arsenic and the methylation profile of mothers in pregnancy with those of their infants.

Previous studies of the distribution of arsenic metabolites in urine have consistently found large interindividual variability across different populations and exposure levels. Interestingly, in this study we observed a decrease in interindividual variation among the study subjects as pregnancy progressed towards full term. In particular, the highest percentages of In-As and MMA in earlier pregnancy disappeared, as the lower percentages of DMA diminished. We have no explanation for this finding at this time. Although the size of our study was relatively small, having repeated measures of the same individuals across time reduces some of the problems associated with interindividual variability and makes comparison possible across repeated periods.

To our knowledge, this is the first study in humans to investigate changes in arsenic methylation during pregnancy. Concha et al. (1998) compared full-term pregnancy with postpartum patterns of urinary arsenic species. Although their comparison covered different time periods than ours, the results are consistent in showing highest total arsenic excretion and percentage of DMA shortly before delivery, with an apparent return to normal metabolite distribution after the postpartum period. The urine samples from the newborns of these women had a similar metabolite distribution to that of the mothers before delivery (9.2% In-As, 90% DMA), but the correlation between mothers and infants was not given. To give a complete description of how pregnancy affects the arsenic methylation process, a study covering the entire pregnancy period, the postpartum period, and the newborn infant is warranted. Given the changes in methylation patterns found throughout the second half of pregnancy, levels in full-term newborns may also differ from premature infants, which could potentially reflect different arsenic species exposure and metabolism.

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