

# Metabolism of Inorganic Arsenic in Children with Chronic High Arsenic Exposure in Northern Argentina

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This study concerns the metabolism of inorganic arsenic (As) in children in three villages in northern Argentina: San Antonio de los Cobres and Taco Pozo, each with about 200 µg As/l in the drinking water, and Rosario de Lerma, with 0.65 µg As/l. Findings show that the concentrations of As in the blood and urine of the children in the two As-rich villages were on average 9 and 380 µg/l, respectively, the highest ever recorded for children. The concentrations were about 10 and 30 times higher for blood and urine, respectively, than in Rosario de Lerma. Total As in urine was only slightly higher than the sum of metabolites of inorganic As (U-As<sub>met</sub>), i.e., inorganic As, methylarsonic acid (MMA), and dimethylarsinic acid (DMA); this shows that inorganic As was the main form of As ingested. In contrast to previous studies on urinary metabolites of inorganic As in various population groups, the children and women in the present study excreted very little MMA. Thus, there seems to be a polymorphism for the enzymes (methyltransferases) involved in the methylation of As. Interestingly, the children had a significantly higher percentage of inorganic As in urine than the women, about 50% versus 32%. Also, the percentage of inorganic As in the children is considerably higher than in previous studies on children (about 13% in the two studies available) and adults (about 15–25%) in other population groups. This may indicate that children are more sensitive to As-induced toxicity than adults, as the methylated metabolites bind less to tissue constituents than inorganic As. In the children, the percentage inorganic arsenic in urine decreased, and the percentage of DMA increased with increasing U-As<sub>met</sub>, indicating an induction of As methylation with increasing exposure. *Key words:* age, bio-transformation, blood, drinking water, exposure, methylation, urine. *Environ Health Perspect* 106:355–359 (1998). [Online 15 May 1998]

<http://ehpnet1.niehs.nih.gov/docs/1998/106p355-359concha/abstract.html>

A large number of people in different parts of the world are exposed to arsenic via drinking water. Such exposure has, in several studies, been associated with an increased risk for skin cancer and various internal cancers (1–3). Other adverse health effects associated with chronic exposure to As include hyperkeratosis, skin pigmentation, effects on the circulatory system (sometimes leading to gangrene), neurotoxicity, and hepatotoxicity (1,3,4). Although exposure via drinking water implies lifelong exposure beginning in early childhood, there are essentially no studies focused on children. There are only a few observations, particularly of As-induced hyperpigmentation and hyperkeratosis in children (5–8). As to acute and subacute effects, an incidence of arsenic poisoning due to contaminated formula that gave rise to ingestion of 1.3–3.6 mg As/day in 12,000 infants resulted in hundreds of intoxicated infants and 130 fatalities (4,9). This would indicate that young children are more sensitive to As than adults.

In general, very little is known about factors that influence the toxicity and metabolism of arsenic in humans. Inorganic As is methylated to the more readily excreted and less toxic metabolites methylarsonic acid (MMA) and dimethylarsinic acid (DMA), but there is considerable variation

between species and population groups (10,11). Experimental studies have indicated that the methylation of As is influenced by the dose level, mode of administration, form of As administered, and nutritional status of the subject (12–14). However, the influence of age is essentially unknown.

We have previously reported on elevated exposure to As via drinking water in native women in an Andean village in northern Argentina (11,15,16). In contrast to all previous studies on population groups from Europe, America, and Asia (17), the Andean women were found to excrete very little MMA in the urine, about 2% on average.

The aim of the present study was to investigate the metabolism of As in children in the same area, the Salta and Chaco Provinces of northeastern Argentina. We studied two villages with As-rich water (one populated predominately with indigenous people and the other with descendants of the Spanish immigrants) and one area with low water concentrations of As. For evaluation of differences in metabolism between children and adults, some women from the same villages were included.

## Materials and Methods

### *Study area and investigated population.*

One of the areas investigated was San Antonio de los Cobres (hereafter called S. A.

Cobres), a village of about 5,000 inhabitants, mainly indigenous (18). The village is located at an altitude of 3,800 m above sea level in the western part of the Salta Province, in the arid Puna region of the middle Andes. In this area, the volcanic bedrock has a high content of As. There is no industrial source of As pollution in the area. The local economy is based on a rudimentary agriculture (potatoes and other vegetables) and breeding of llamas, goats, and sheep. The basic diet consists of meat, maize, rice, and vegetables. According to the statistics of the local Primary Health Care Committee (work area 29, Los Andes, Gerardo Vogler, Head), about 47% of the population is below 14 years of age. About 12% of the children 2–5 years of age suffer from malnutrition. The mortality rate is 52/1,000 for children under 1 year of age. No signs of chronic As intoxication, such as palmo-plantar hyperkeratosis, pigmentation changes, or skin cancer, have been observed by the local physician.

Taco Pozo is located on the plains of the Chaco Province, east of Salta. The village has 6,600 inhabitants, mainly “criollos” (descendants of Spanish immigrants). The As in the drinking water is of geological origin (19). There are no mines in the region, and the most important industries are related to cotton production and to the breeding of cattle, goats, and pigs. According to the local physician (Luis A. Verón, personal communication), signs of palmo-plantar hyperkeratosis are quite common in the area, also in children, but no statistics are available. In the present study, only one 13-year-old boy and one 43-year-old woman showed palmo-hyperkeratosis.

For comparison, a group of children in Rosario de Lerma, belonging to the Salta Province, was studied. The village is located

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We acknowledge Ricardo Maderna, CIGIBA, National University of la Plata; the staff of the hospital in San Antonio de los Cobres, in particular Gerardo Vogler, Director of the hospital; Gloria de Telechea, Hugo Telechea, and Alfredo Corrado of the hospital in Rosario de Lerma; and Luis Alberto Verón of the El Quebrachal Hospital, Salta Province. This project was financed by the Commission of the European Community, contracts CT 93 0305 and PL 9324313.

Received 10 November 1997; accepted 10 March 1998.

at an altitude of 1,000 m above sea level, has 18,000 inhabitants, mainly indigenous, and is known to have a low concentration of As in the drinking water.

The study comprised 24 children and 15 women in S. A. Cobres, 12 children and 12 women in Taco Pozo, and 21 children and 12 women in Rosario de Lerma. The participants were recruited by the physicians at the local hospitals and via the local radio or television. For investigation of As exposure and metabolism, samples of the local drinking water, food, urine, and blood were collected in each village. All the children and women were interviewed about their water consumption and dietary habits. Children below 10 years of age were interviewed with the help of their mothers.

**Sampling and chemical analysis.** Drinking water from the public water distribution systems in each village was collected in 100-ml acid-washed polyethylene bottles. In S. A. Cobres, the sampling of blood and urine was carried out at the hospital in April 1994. Because some of the urine samples were lost, the sampling was repeated in November 1994. In Rosario de Lerma, the sampling was carried out at the local hospital in November 1994. In Taco Pozo, samples were collected in the homes of the women and children in September 1995. Blood samples were collected from the arm vein (cubital) using Venoject tubes (VP-100SDK; Terumo, Leuven, Belgium), containing EDTA ( $K_2$ ) as an anticoagulant. Spot urine samples were collected in acid-washed plastic

containers and transferred to 100-ml acid-washed polyethylene bottles. The pH of the urine and the presence of glucose and protein were tested using N-Combur-Test (Boeinger Mannheim, GmbH, Germany). Concentrated HCl (1 ml to 100 ml urine) was added to prevent bacterial growth. All samples were frozen directly and kept at  $-20^\circ\text{C}$  until they were transported (on wet ice) to Sweden for analysis.

The concentrations of total As in drinking water, food, blood, and urine were determined using hydride generation atomic absorption spectrophotometry (HG-AAS) following dry ashing (20–22). The As concentrations in urine were adjusted for variation in density, determined by a Hand refractometer (Atago, Japan). Because the concentration of total As in blood and urine may be highly influenced by dietary As of seafood origin, mainly arsenobetaine, we also measured the sum of the metabolites of inorganic As in the urine samples ( $U\text{-As}_{\text{met}}$ ) using direct HG-AAS after addition of HCl to 0.6 M (23,24). Separation of the different metabolites of inorganic As in urine was performed using ion-exchange chromatography on columns of AG 50 WX-8 resin (Bio-Rad, Hercules, CA), according to the method of Tam et al. (25), by which inorganic As, MMA, and DMA are eluted sequentially with 0.5 M HCl, deionized water, 1 M  $\text{NH}_4\text{OH}$ , and 4 M  $\text{NH}_4\text{OH}$  (11). The As concentrations in the urine samples from Rosario de Lerma were too low for speciation. The detection limit of the HG-AAS method was on average  $2.1 \text{ ng} \pm 0.91$  [mean  $\pm$  standard deviation (SD)]. Selenium in serum was analyzed in the Analytica AB laboratory, Stockholm, Sweden, by a method of Sanders and Brodie (26).

For quality control (QC) purposes, the Standard Reference Water [1643c, National

**Table 1.** Concentrations of total arsenic in drinking water ( $\mu\text{g/l}$ ) and food ( $\mu\text{g/kg}$  wet weight) collected at different places in San Antonio de los Cobres, Taco Pozo, and Rosario de Lerma

Town	Samples	Concentration
San Antonio de los Cobres	Tap water	
	Hospital kitchen	193
	From two private homes	219, 217
	Food	
	Soup (from three different restaurants)	322, 259, 427
	Empanada (meat pie) from street vendor	131
	Pasta (restaurant)	150
Taco Pozo	Rice (restaurant)	268
	Tap water	
	Restaurant	214
	Private home	216
	Food	
	Soup (restaurant)	281
	Rice (restaurant)	356
Rosario de Lerma	Maize porridge (private home)	418
	Tap water	
	Hospital	0.63
	From two different restaurants	0.73, 0.65
	Food	
	Soup (hospital kitchen)	<2
	Pasta (restaurant)	<8
Schnitzel with potatoes and green beans (restaurant)	<11	

**Table 2.** Median concentrations and range of total arsenic in blood and urine and the percentages of Inorg As, MMA, and DMA in urine of children and women

Town	Age (years)	B-As ( $\mu\text{g/l}$ )	U-As <sub>met</sub> ( $\mu\text{g/l}$ ) <sup>a</sup>	Urinary arsenic metabolites			
				% Inorg As	% MMA	% DMA	
San Antonio de los Cobres	Children	8 (3–15)	9.1 (6.0–15) <i>n</i> = 24	323 (125–578) <i>n</i> = 22	49 (21–76)	3.6 (0.9–12)	47 (22–69)
	Women	35 (20–47)	7.6 (2.7–18) <i>n</i> = 15	303 (127–481) <i>n</i> = 11	25 (6.5–42)	2.1 (0.6–8.3)	74 (55–93)
Taco Pozo	Children	9 (6–13)	9.1 (5.5–17) <i>n</i> = 12	440 (337–621) <i>n</i> = 12	42 (26–54)	3.4 (1.3–7.9)	54 (44–68)
	Women	41 (22–66)	11 (4.7–17) <i>n</i> = 12	386 (90–606) <i>n</i> = 12	39 (18–52)	2.2 (1.1–3.5)	58 (46–80)
Rosario de Lerma	Children	11 (5–14)	0.83 (0.27–1.5) <i>n</i> = 20	13 <sup>b</sup> (4.7–36) <i>n</i> = 21			
	Women	28 (18–53)	0.95 (0.69–1.8) <i>n</i> = 11	7.6 <sup>b</sup> (3.2–23) <i>n</i> = 12			

Abbreviations: B-As, total arsenic in blood; U-As<sub>met</sub>, sum of arsenic metabolites in urine; Inorg As, inorganic As; MMA, monomethylarsonic acid; DMA, dimethylarsonic acid.

<sup>a</sup>Adjusted to a density of 1.019 g/ml (average of all urine samples).

<sup>b</sup>No urinary arsenic speciation.

Institute of Standards and Technology (NIST), Gaithersburg, MD] and the Standard Reference Material 2670 (toxic metals in freeze-dried urine) were analyzed for total As (certified concentrations  $82 \pm 1.2 \mu\text{g/l}$  and  $480 \pm 100 \mu\text{g/l}$ , respectively) together with the collected water and urine samples. The obtained concentrations were  $87 \pm 6.2 \mu\text{g/l}$  (mean  $\pm$  SD;  $n = 97$ ) and  $520 \mu\text{g/l} \pm 50$  ( $n = 92$ ). The QC sample used in the determination of arsenic metabolites in urine consisted of a human urine sample spiked with  $55.2 \mu\text{g/l}$  of arsenate, MMA, and DMA in the ratio 20:20:60. The total concentration of the As metabolites was set to  $60 \mu\text{g/l}$ , based on several previous analyses at our laboratory and a reference laboratory (Analytica AB). When analyzed together with the collected urine samples, the average concentration of the sum of the As metabolites was  $63 \pm 6.2 \mu\text{g/l}$  ( $n = 43$ ). In addition, 10 urine samples of children with MMA concentrations below 3% were spiked with known concentrations of MMA and DMA. The average recovery was 88% (range 72–117%) for MMA and 108% (96–126%) for DMA. Participation in an interlaboratory comparison for As metabolites in human urine samples (27) showed good agreement between our results and the average values of the six participating laboratories (11). The food samples were analyzed together with the standard reference materials bovine liver 1577, and rice flour, with certified concentrations of  $0.055 \pm 0.005 \mu\text{g/g}$  dry weight (dw) and  $0.41 \pm 0.005 \mu\text{g/g}$  dw, respectively. The obtained results were  $0.054 \pm 0.005 \mu\text{g/g}$  ( $n = 4$ ) for bovine liver 1577 and  $0.47 \pm 0.01 \mu\text{g/g}$  ( $n = 3$ ) for rice flour. We also spiked, in duplicate, five food samples with  $17 \text{ ng As/g}$  (SD 0.27) using Standard Reference Water (1643c, NIST). The average recovery was 96% (range 90–102%).

Statistical analyses were performed using the Mann Whitney Rank Sum Test (comparison of groups) and Spearman Rank Order Correlation Test.

## Results

The concentration of As in the drinking water was about  $200 \mu\text{g/l}$  in both S. A. Cobres and Taco Pozo and less than  $1 \mu\text{g/l}$  in Rosario de Lerma (Table 1), whereas the concentration of selenium was less than  $2 \mu\text{g/l}$ . In the As-rich villages, the food contained high As concentrations (Table 1), especially soup, which was consumed twice a day by about 70% of the investigated individuals in S. A. Cobres and 30% of those in Taco Pozo. Also maize porridge, which was the most common food in Taco Pozo, contained high concentrations of As.

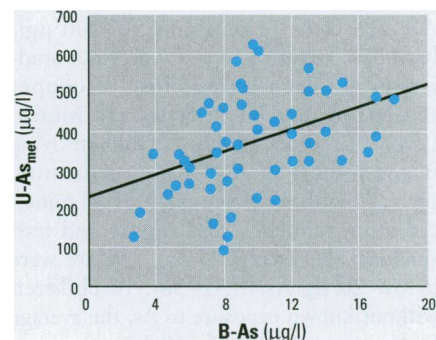
The concentrations of total As in blood (B-As) were about as high in the children as in the women in both S. A. Cobres and Taco Pozo, but these concentrations were about 10 times higher than in Rosario de Lerma (Table 2). The B-As was not influenced by sex or age. The selenium concentrations in blood plasma of three children and four women from S. A. Cobres were  $51\text{--}63 \mu\text{g/l}$  and  $67\text{--}88 \mu\text{g/l}$ , respectively.

The concentrations of the sum of U-As<sub>met</sub> in children and women from the As-rich areas were more than 30 times higher than in the control area (Table 2) and were about as high in the children as in the women. With the exception of the women in Taco Pozo, the concentrations of total As in urine were only slightly higher (median of children and women in S. A. Cobres  $327$  and  $307 \mu\text{g/l}$  and Taco Pozo  $457$  and  $464 \mu\text{g/l}$ , respectively) than those of the metabolites of inorganic As, indicating that the main exposure was to inorganic As. Probably, the women in Taco Pozo were exposed to more organic As compounds via food than inorganic As. U-As<sub>met</sub> was not influenced by sex or age. There was a significant positive correlation between the concentrations of B-As and U-As<sub>met</sub> in the As-rich areas ( $r = 0.42$ ;  $p = 0.002$ ; Fig. 1).

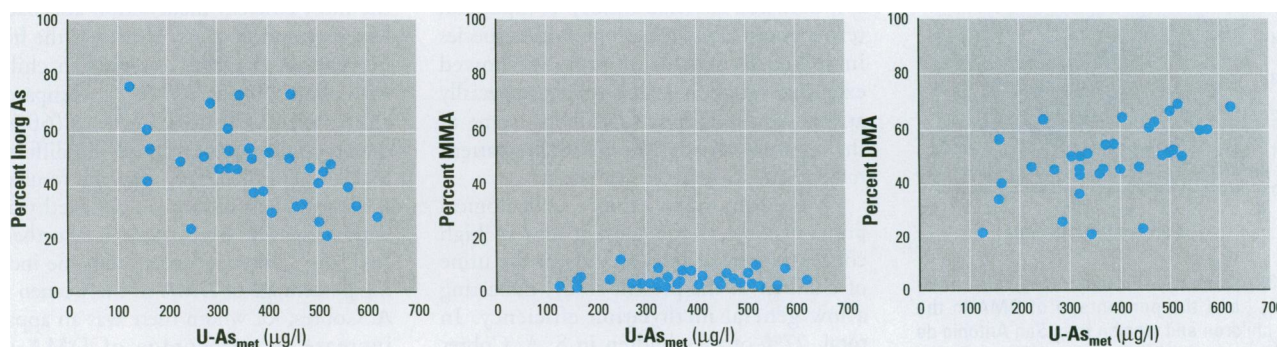
As shown in Table 2, most of the children and women had only low levels of MMA in the urine. However, children had significantly higher ( $p < 0.0001$ ) inorganic

As in the urine than the women. This difference was most apparent in S. A. Cobres, where six of the children had over 60% inorganic As. Among the children, there was no significant association between age and the percentage of inorganic As, MMA, or DMA in the urine. There was also no difference between girls and boys, with the exception of a higher percentage of MMA in boys (mean 6.0%) as compared to girls (mean 3.1%) in S. A. Cobres ( $p = 0.023$ ). As shown in Figure 2, there was a significant negative association between the percentage of inorganic As and U-As<sub>met</sub> ( $r = -0.54$ ;  $p = 0.001$ ) in children and a corresponding positive association between the percentage of DMA in urine and U-As<sub>met</sub> ( $r = 0.57$ ;  $p < 0.0001$ ). A similar association was not seen in the women.

To evaluate the effect of methylation (measured as the percentage of DMA in urine) on the retention and excretion of U-As<sub>met</sub>, we calculated the ratio B-As/U-As<sub>met</sub> for all individuals except the women in Taco Pozo who were found to have higher total U-As than U-As<sub>met</sub>, which would indicate exposure to other arsenic compounds than inorganic As, possibly via



**Figure 1.** Association between the concentrations of total arsenic in blood (B-As) and the sum of arsenic metabolites in the urine (U-As<sub>met</sub>) of children and women from San Antonio de los Cobres and Taco Pozo. The urine is adjusted to a density of  $1.019 \text{ g/ml}$  (average of all urine samples).  $Y = 224.7 + 14.5X$ ;  $r = 0.415$ .



**Figure 2.** Distribution of urinary arsenic metabolites in relation to the concentrations of arsenic in the urine [sum of arsenic metabolites (U-As<sub>met</sub>)] of children from San Antonio de los Cobres and Taco Pozo. Abbreviations: Inorg As, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsonic acid. The urine is adjusted to a density of  $1.019 \text{ g/ml}$  (average of all urine samples).

food (23). Obviously, such an exposure to other As compounds would influence also B-As, which we did not speculate.

Figure 3, shows a significant decrease of the ratio  $B\text{-As}/U\text{-As}_{\text{met}}$  with increasing percentage of DMA in the urine ( $r = -0.48$ ;  $p = 0.001$ ).

## Discussion

The concentrations of As in the drinking water collected at different sampling sites in S. A. Cobres and Taco Pozo were about 20 times higher than the provisional World Health Organization (WHO) drinking water guideline of  $10 \mu\text{g As/l}$  (28). There were also high As concentrations in the major foods, all of which were prepared using the local water. The intake of inorganic As via drinking water and food caused highly elevated concentrations in the blood and urine of the investigated children and women—about 10 and 30 times, respectively, the concentrations observed in the control area, Rosario de Lerma, in which the As concentration in the drinking water was below  $1 \mu\text{g/l}$ .

The concentrations of B-As (average  $9 \mu\text{g/l}$ ) and  $U\text{-As}_{\text{met}}$  (average  $380 \mu\text{g/l}$ ) are the highest ever reported for children. Average  $U\text{-As}_{\text{met}}$  of about  $50\text{--}200 \mu\text{g/l}$  have been reported in a few previous studies on children living close to copper smelters in the United States and Mexico (29,30). In other studies of children living in the vicinity of As-emitting industries, such as coal-fired power plants, coal mines, cadmium refineries, and copper and lead smelters, the average  $U\text{-As}_{\text{met}}$  values were below  $20 \mu\text{g As/l}$  (31–36). In children without known exposure to As, the average  $U\text{-As}_{\text{met}}$  were  $5\text{--}10 \mu\text{g/l}$  (30,35).

The children in the present study, especially those in S. A. Cobres, had a considerably higher percentage of inorganic As

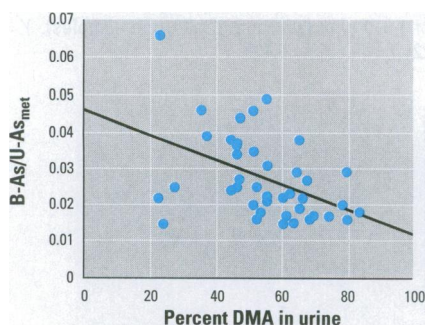
(about 47%) and a lower percentage of DMA (50%) in urine than the women, but both had similar low percentages of MMA. There are only two previous reports on the speciation of As metabolites in the urine of children. Buchet et al. (32) reported average values of 12% inorganic As, 28% MMA, and 60% DMA in children in Belgium ( $n = 14$ ), and Kalman et al. (31) reported average values of 13% inorganic As, 16% MMA, and 71% DMA in children in the United States ( $n = 158$ ). However, in both these studies, the  $U\text{-As}_{\text{met}}$  was low ( $<20 \mu\text{g/l}$ ), only slightly higher than the  $U\text{-As}_{\text{met}}$  in children from Rosario de Lerma (on average  $10 \mu\text{g/l}$ ), which was too low for reliable speciation with our methods. In the present study, only two children in S. A. Cobres had a pattern of As metabolites in the urine similar to the pattern reported by Buchet et al. (32) and Kalman et al. (31).

The low percentage of MMA in children and women was similar to that observed in our previous studies on As exposure in native Andean women (11,16). All other published reports on urinary As metabolites in human subjects (including populations in Europe, America, and Asia exposed to inorganic As in the general environment or occupationally) consistently show average values of 10–30% inorganic As, 10–20% MMA, and 60–80% DMA (17). However, a few studies of factory workers exposed to As (37,38) and subjects exposed to As in the drinking water (39) have reported low urinary percentages of MMA in a few individuals. Thus, there is a considerable interindividual variation in As methylation. Further research is needed to elucidate the reasons for such variations. The results indicate a genetic polymorphism for As methyltransferase. This enzyme is not fully characterized, but has been isolated from tissues of several mammalian species (40,41). It should also be noted that adequate quality control has not always been reported for  $U\text{-As}_{\text{met}}$ . A recently published interlaboratory comparison (experienced laboratories only) of As species in spiked human urine samples showed extensive variation in the results, especially at low concentrations (27). Thus, some of the reported variation in metabolite pattern may be due to analytical variation.

More important, from a toxicological point of view, is the finding of very high concentrations of inorganic As in the urine of children in the present study, indicating a low general methylation efficiency. In total, 27% of the children in S. A. Cobres had more than 60% inorganic As and less than 40% DMA in the urine. This may indicate that the children are more sensitive

to As-induced toxicity, as inorganic As is the main form interacting with tissue constituents (14). Further studies on the metabolism and effects of As in children are urgently needed. Unfortunately, there are no statistics available on As-related health effects in the areas studied in the present project. However, according to the local physicians, signs of palmo-plantar hyperkeratosis are common in Taco Pozo, but not in S. A. Cobres. Experimental studies have indicated that selenium may protect against As intoxication (42). However, the selenium concentrations in water were low, and the concentrations in plasma were similar to those reported for other countries (43).

Another interesting finding in the present study was the increasing percentage of urinary DMA, and the corresponding decreasing percentage of inorganic As, with increasing  $U\text{-As}_{\text{met}}$  in the children, but not in the women. However, our previous studies showed a higher percentage of DMA and a lower percentage of inorganic As in the urine of the women in S. A. Cobres, as compared to women with fairly low exposure in other Andean villages ( $5\text{--}15 \mu\text{g/l}$  in drinking water) (11). This may indicate an induction of the methylation of inorganic As to DMA. In contrast, previous studies on adult subjects have shown a decrease in the percentage of DMA, and a corresponding increase in the percentage of MMA, with increasing  $U\text{-As}_{\text{met}}$  (39). Our results could also be interpreted as a higher  $U\text{-As}_{\text{met}}$  excretion in individuals with a higher methylating capacity, as experimental studies have shown that DMA is more rapidly excreted in the urine than is inorganic As (14). To evaluate if that was the case in the present study, we calculated the  $B\text{-As}/U\text{-As}_{\text{met}}$  ratio assuming that B-As reflects the retention of As and  $U\text{-As}_{\text{met}}$  reflects the excretion. As the  $B\text{-As}/U\text{-As}_{\text{met}}$  ratio decreased significantly with the increasing percentage of DMA in the urine, which was used as a measure of the methylating capacity, it seems clear that the individuals with the most efficient methylation also had the lowest retention of As. However, the higher percentage of DMA observed in children with about  $600 \mu\text{g/l } U\text{-As}_{\text{met}}$ , compared to those with  $200 \mu\text{g/l } U\text{-As}_{\text{met}}$  (60% vs. 44%), could not explain all the differences in  $U\text{-As}_{\text{met}}$  ( $400 \mu\text{g/l}$ ), why the results still indicate an induction of As methylation with increasing exposure level. Further,  $B\text{-As}/U\text{-As}_{\text{met}}$  also decreased with the increasing percentage of DMA in the women in S. A. Cobres, for whom there was an apparent increase in percentage of DMA with increasing  $U\text{-As}_{\text{met}}$ . Obviously, further studies on factors influencing As methylation and excretion in humans are needed.

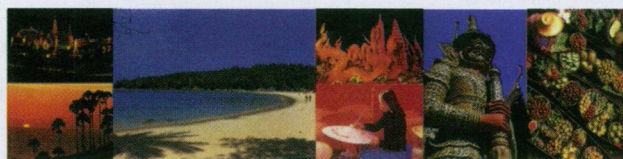


**Figure 3.** Association between the ratio of  $B\text{-As}/U\text{-As}_{\text{met}}$  and the percentage of DMA in the urine of children and women from San Antonio de los Cobres and children from Taco Pozo. Abbreviations: B-As, blood arsenic concentration;  $U\text{-As}_{\text{met}}$ , sum of urinary arsenic metabolites; DMA, dimethylarsinic acid.

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NOVEMBER 29-DECEMBER 4, 1998  
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