Exposure to inorganic arsenic in drinking water and total urinary arsenic concentration in a Chilean population

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Abstract

The relationship of inorganic arsenic exposure through drinking water and total urinary arsenic excretion in a nonoccupationally exposed population was evaluated in a cross-sectional study in three mayor cities of Chile (Antofagasta, Santiago, and Temuco). A total of 756 individuals in three population strata (elderly, students, and workers) provided first morning void urine specimens the day after exposure and food surveys were administered. Arsenic intake from drinking water was estimated from analysis of tap water samples, plus 24-h dietary recall and food frequency questionnaires. Multilevel analysis was used to evaluate the effects of the age group and city factors adjusted by predictor variables. Arsenic levels in drinking water and urine were significantly higher in Antofagasta compared with the other cities. City-and individual-level factors, 12% and 88%, respectively, accounted for the variability in urinary arsenic concentration. The main predictors of urinary arsenic concentration were total arsenic consumption through water and age. These findings indicate that arsenic concentration in drinking water continues to be the principal contributing factor to exposure to inorganic arsenic in the Chilean population.

Keywords: Inorganic arsenic; Urinary arsenic; Risk assessment; Drinking water; Multilevel analysis

1. Introduction

There is extensive evidence that arsenic (As) exposure in humans can produce acute and chronic effects on several organ systems, including cardiovascular, nervous, hepatic, respiratory, gastrointestinal, and skin. Genotoxic effects have also been described (Basu et al., 2001; Gebel, 2001a; Kannan et al., 2001; Centeno et al., 2002; Abernathy et al., 2003).

In terms of chronic effects, sufficient epidemiologic evidence exists to establish the carcinogenic effects of inorganic As in humans (WHO, 1993; EPA, 1984). Studies in smelters that emit As have detected higher rates of lung cancer in exposed workers (Tello, 1988; Smith et al., 1992; Enterline et al., 1995; Ferreccio et al., 1996, 1998). However, toxicological studies in animals have not shown sufficient evidence that As is carcinogenic by itself; this might suggest a promoting effect on cancer and interaction with other substances and environmental factors (Byrd

et al., 1996; Gebel, 2001b; Bode and Dong, 2002; Centeno et al., 2002).

Chile has high As levels in the soil and air due to both natural and anthropogenic sources, the latter being generated by emissions from multiple copper smelters. This has led to the contamination of drinking water in the country's northern region, a situation emerging as a major public health concern. Between the years 1958 and 1970, the average arsenic content in the drinking water in Antofagasta, Region II (Fig. 1), was 860 μg/L.

Treatment of these waters to remove As began only in 1970, reducing progressively the arsenic content to an average of 40 μg/L in 1995 (Rivara et al., 1997). Arsenic is one of the few substances shown to cause cancer in humans through consumption of drinking water. There have seen a number of extensive reviews of the occurrence and potential health effects of arsenic (ATSDR, 2000; IPCS, 2001; NRC, 2001).

Accurate exposure information is needed for risk assessment decision making. Because inorganic As has a half-life in the body of few days (it has been reported that 45–85% of arsenic ingested in the human body is excreted in urine within 1–3 days), its presence in the urine has been used as biomarker of recent exposure (Crecelius, 1977; Hwang et al., 1997; Calderon et al., 1999; Karagas et al., 2001; Chen et al., 2002). Use of this biomarker has increased the power of recent studies, because exposure misclassification is minimized. However, as reported in some studies, at similar levels of arsenic exposure a range of urinary arsenic has been observed, probably due to a large interindividual variability either in water consumption or in arsenic excretion, or both (Hopenhayn-Rich et al., 1996a, b; Del Razo et al., 1999).

Fig. 1. Map of the cities of Chile studied: Antofagasta, Region II; Santiago, Metropolitan Region XIII; and Temuco, Region IX.

The present study is part of a larger investigation whose general purpose was to evaluate total nonoccupational As exposure in environmental media in three large cities of Chile. The objective of the present study is to assess the relationship between inorganic arsenic exposure through drinking water and total urinary arsenic excretion and its variability by age and geographic area.

2. Material and methods

2.1. Study design and site selection

The study was cross-sectional and took place between 1999 and 2000. Urban areas of Chile were represented by three cities chosen from among eight in the country with large populations (Fig. 1): Antofagasta (north), Santiago (center), and Temuco (south).

2.2. Sample size

The study population in each city was categorized in three strata: elderly, students, and workers (61–85, 13–15, and 22–58 year old, respectively), representing 69% of the population of big cities in the country. The sample size was calculated based on preliminary data on urinary arsenic levels in students (FONDEF, 1992), which indicate that there is a gradient of exposure from higher in the north (Arica-Antofagasta), intermediate in the center (Santiago-Rancagua), and lower in the south (Concepción-Temuco). Thus, the amounts of 70, 40, and 20 μg/L, respectively, with standard deviations of 40, 30, and 12 μg/L were used. Therefore, the coefficient of variation was calculated for each city (57%, 75%, and 60%, respectively), indicating that for a precision of 10 μg/L, the necessary sample size to evaluate arsenic exposure through urinary biomarkers was 64, 100, and 64 people, for Antofagasta, Santiago, and Temuco, respectively. Considering a potential refusal of 10% of eligible participants, the target sample size was set at 756 people in total. The sample contacted was 789 people in total, which included 5% lost due to lack of urine sample and incomplete or unclear survey responses.

2.3. Subject selection

The sampling framework for students and elderly were the school (eight basic grades) and "Elderly Clubs" (Clubes de Ancianos) registered in the municipalities of each city. The sample of workers in Antofagasta and Temuco was obtained from employers affiliated with the mutual security systems and whose activities were not related with the production or use of As. In Santiago, the sample of workers consisted of employees of the different municipalities of Santiago's metropolitan area. Depending of the sampling unit, a multistage design was

used. All these centers were selected in a "systematic sampling with at random start." This means that the first sampling unit is selected at random. The objective was to have at least a 10% (sampling ratio) sample size estimated in each sampling unit (Rubin and Babbie, 1989).

In order to be eligible, participants needed to live in the city for at least 1 year. People were excluded if they had occupational exposure to As, used hair coloring, or had a renal or hepatic disease. Informed consent was obtained from all adult participants, and in the case of students, parental consent was obtained. Data were collected in each city by a trained team according to procedures and field protocols previously established.

2.4. Exposure assessment

Survey instruments were elaborated, with slight modifications between the three age strata, to obtain demographic information (age, sex, socioeconomic status), potential exposure to industrial sources of As, time spent in different environments (indoor, outdoor), contact with soil (in students), and active and passive tobacco smoking. Diet and water consumption were assessed using a standard validated food frequency questionnaire covering approximately food items (Witschi, 1990) and a 24-h dietary recall inventory (Thompson and Byers, 1994). Both dietary surveys were applied by trained nutritionists.

2.5. Sample collection

2.5.1. Collection and pretreatment of urine
Written instructions regarding the hygienic conditions of the collection of samples and a 250-mL plastic container were provided to all participants. The container was previously treated with hydrochloric acid and rinsed with deionized water to avoid possible contamination with As. Subjects were asked to provide the first morning void (FMV) of the day following the survey. Information regarding the consumption of fish and shellfish that could produce high values of As in the specimen was also collected. Each participant was requested to obtain a sample of at least 250 mL of the FMV, which was immediately transported to the laboratory, pretreated with chloridric acid (HCl), frozen, and sent in a cryopreserver container to the laboratory of Antofagasta University for the final analyses.

2.5.2. Water sampling
A total of 35 tap water samples of 250 mL each were obtained in Antofagasta ($n = 10$), Santiago ($n = 15$), and Temuco ($n = 10$), which were taken in the houses, schools, and work places. Water samples were transported to the local laboratory, where they were pretreated with HCl and then sent to the laboratory of the University of Antofagasta.

For assignment of individual arsenic exposure, information on total water consumption and arsenic level of drinking water in the residential area was used. Water samples were taken in each of the sampling units (schools, municipalities, and elderly clubs) and the study participants were georeferenced using a geographical information system (GIS). This way, the arsenic level was assigned to the nearest source of water assessed to each individual in relation to his or her residence. We assume that the variation in the arsenic concentration in this media is smaller because all water sources with which the city is supplied are processed in the same water treatment plant. The daily water intake was estimated from patterns of consumption that the interviewees reported for the last 24 h, which were integrated and expressed in liters per day. When this information was missing, water intake was estimated from the food frequency survey. The total individual daily arsenic exposure through water was calculated by the following formula: As-wi (μg/day) = water intake (L/day)*[As] in drinking water (μg/L).

2.6. Laboratory analyses

2.6.1. Total urinary arsenic concentration (Tu-As: μg/L), arsenic in drinking water (As-dw: μg/L), and urinary creatinine (Uc: g/L)
Arsenic analyses were made in an atomic absorption spectrophotometer, Perkin-Elmer Model 4000, with hydride-generator system MHS-20, an electric oven, and discharge lamp EDL. The detection limit was 10 ppb. When the arsenic concentrations were close to or below the detection limit of the equipment, as in Santiago y Temuco, the arsenic concentration was determined using the addition of standard solutions with a known concentration of arsenic to the analyzed sample. In this way it can be determined by the difference and therefore its absorbance assessed and after a correction is performed in accord with the calibration curve. The quality controls of these determinations were made by means of the use of water and urine standard certificated by National Institute of Standard and Technology (NIST) and Certified Reference Material-Trace Metals in Drinking Water Standards (CRM-TMDW), respectively. Creatinine was determined in accord with the method of Newman and Price (1999).

2.7. Statistical analysis

Exploratory and descriptive analyses were conducted on characteristics of the sample and the arsenic exposure estimates for each population stratum. To examine associations, analysis of variance was used to compare

means and medians between each strata and cities. Multiple comparisons were made using Dunnett's test. These analyses were performed with SPSS V10.05 software (SPSS, 1999). Multilevel analysis was used to evaluate the effects of city and age group on total urinary arsenic concentration adjusted by predictor variables. SAS PROC MIXED (SAS, 1999) was used to fit models to a three-level hierarchical data structure consisting of individuals nested in age strata and age strata nested in cities. We also fit multiple linear regression models to describe the relationships between total urinary arsenic, adjusted for creatinine, and total arsenic consumption from water, adjusting for potential confounding variables (age, gender, BMI, and smoking). The variables used in the final model were identified from an exploratory univariate analysis and consideration of previous studies. The following models were evaluated.

Full multilevel model (mixed effects):

$$(\text{Tu} - \text{As/c})_{ij}k = \beta_0(\text{grand mean}) + \beta_1(\text{As} - \text{wi})i$$
$$+ \beta_2(\text{age})k + \beta_3(\text{gender})k + \beta_4(\text{BMI})k$$
$$+ \beta_5(\text{smoking})k + \varepsilon_k + \varepsilon_{j(k)} + \varepsilon_{i(j(k))},$$

where i, k, and j are individual, strata, and city level, respectively. $(\text{Tu-As/c})_{ijk}$ is the ith individual total urinary arsenic level in the jth stratum of the kth city, As-wi is total arsenic in water intake in ith individual, $\varepsilon_{i(j(k))}$ is the random individual variance within strata nested in cities, $\varepsilon_{j(k)}$ is the random strata variance nested in cities, ε_k is the random city variance, and $\beta_0 - \beta_5$ are fixed effects.

Multiple regression models:

$$(\text{Tu} - \text{As/c}) = \beta_0 + \beta_1(\text{As} - \text{wi}) + \beta_2(\text{age}) + \beta_3(\text{gender})$$
$$+ \beta_4(\text{BMI}) + \beta_5(\text{smoking}) + e,$$

where β_0 is the regression constant, $\beta_1 - \beta_5$ are regression coefficients to be estimated, and e is the error of prediction.

The extent of tobacco smoke exposure was assessed by the smoking index (SI) (cigarettes/day × 365). A smoker was defined as a person with an SI of 800. Both present smokers and former smokers at the time of the analysis were considered as smokers.

3. Results

The sample contacted was 789 people in total, but 33 (5%) were excluded due to lack of urine sample and incomplete or unclear survey responses, leaving a final study population of 756 subjects. The excluded subjects were similar demographically to the total sample. Characteristics of the study population are shown in Table 1.

There were no essential differences between cities in any age group in age, height, weight, BMI, or education among students. Among the elderly, Antofagasta has fewer men and fewer smokers. Among students, Antofagasta also had the lowest proportion of smokers, while Santiago had the highest. The workers group, in general, was similar in all three cities.

Arsenic concentrations and intake levels are shown by city and age strata in Table 2. The concentration in

Table 1
General characteristics of studied subjects stratified by age strata and city

Strata	Elderly[e]			Students[s]			Workers[w]		
City	n	Mean	SD	n	Mean	SD	n	Mean	SD
Antofagasta[1]	67			71			70		
Age (years)		71.3[e]	5.8		13.6[s]	0.8		32.2[w]	7
BMI (kg/m^2)		28.1	4.5		21.5	3.6		25.1	4.6
School level (years)		6.9[e,1]	3.5					13.7[w,1]	2.9
Gender (male %)		16.9[e,1]			50.7			52.7	
Smoking (%)		4.2[e]			11.3[s]			51.4[w]	
Santiago[2]	110			114			111		
Age (years)		71.5[e]	6.7		13.4[s]	0.7		38.5[w,2]	11
BMI (kg/m^2)		28.3	4.4		21.6[s]	3.7		26.5	3.7
School level (years)		5.4[e]	3.7					12.4[w]	4.2
Gender (male %)		32.2[e,2]			57.4			54.7	
Smoking (%)		10.4[e]			24.6[s]			52.8[w]	
Temuco[3]	71			71			71		
Age (years)		71.6[e]	5.0		13.3[s]	0.6		33.3[w]	9.5
BMI (kg/m^2)		29.1	4.8		20.7	2.7		25.3	3.6
School level (years)		5.2[e]	3.7					10.9[w]	3.2
Gender (male %)		49.3[3]			45.8			49.3	
Smoking (%)		12.7[e]			18.1[s]			56.9[w]	

e: elderly; s: students; w: workers; e ≠ s ≠ w: ($P<0.05$); 1: Antofagasta; 2: Santiago; 3: Temuco; 1 ≠ 2 ≠ 3: ($P<0.05$); BMI: body mass index; SD: standard deviation.

Table 2
Water intake, arsenic determination in drinking water and urine stratified by age strata and city

Strata	Elderly[e]					Students[s]					Workers[w]				
City	n	Mean	SD	Median	Range	n	Mean	SD	Median	Range	n	Mean	SD	Median	Range
Antofagasta[1]	67					71					70				
Water intake (L/day)		1.2	0.4	1.15	0.1–2.4		1.1[1]	0.5	1.1	0.1–2.7		0.9	0.6	0.8	0.1–2.7
As-dw (µg/L)		35.8[1]	2	34.6	34–40		36.8[s,1]	1.7	37.4	33–40		36.1[1]	1.4	36.7	33–38
As-wi (µg/day)		41.5[1]	16	40.9	3–88		41.2[1]	16.7	40.8	13–91		32.8[w,1]	21.3	29.6	3.6–101
Tu-As (µg/L)		38.4	26.2	33.7[e,1]	4–161		60	41.8	49.8[1]	6–209		61.4	50.9	47.7[1]	9–363
Tu-As/c (µg/g)		83.9	64.9	66.2[e,1]	3–350		69.7	66.1	50.3[1]	5–466		62.1	77.4	47.4[1]	6–567
Santiago[2]	110					114					111				
Water intake (L/day)		1	0.3	1	0.3–2		0.9	0.5	0.9	0.1–2.3		1.3[w,2]	0.7	1.2	0.2–3.3
As-dw (µg/L)		1.2[e]	0.4	1.1	0.7–2		1.4[s,2]	0.6	1.5	0.6–2		1.3[2]	0.5	1.4	0.6–2.5
As-wi (µg/d)		1.24	0.6	1.08	0.2–3		1.3[2]	1	1.11	0.1–5.3		1.66[w,2]	1	1.3	0.2–6
Tu-As (µg/L)		23.8	21.6	16.3	3.5–153		32	20.6	27.8[s,2]	5–125		23.1	23.2	17.7	2.4–170
Tu-As/c (µg/g)		36.9	35.8	26.6[2]	3–277		26	17.2	22.5[2]	5–101		18.5	18.5	13.9[w,2]	2.6–141
Temuco[3]	71					71					71				
Water intake (L/day)		1.2	0.5	1.1	0.2–2.5		0.8	0.4	0.8	0.2–1.7		0.9	0.4	0.8	0.2–1.7
As-dw (µg/L)		0.7	0.1	0.8	0.6–1.0		0.8[3]	0.1	0.9	0.6–1.4		0.8[3]	0.2	0.9	0.6–1.4
As-wi (µg/d)		0.9[e]	0.5	0.96	0.1–2		0.69[3]	0.3	0.65	0.1–1.7		0.7[3]	0.4	0.63	0.5–3
Tu-As (µg/L)		23.6	26.9	13.7[e]	6–151		20.7	14.5	17.8[3]	4.4–94		26.2	24.7	18.7	3–129
Tu-As/c (µg/g)		30.8	37.4	17.6[e,3]	5–170		17.5	10.6	11.6[3]	5.5–76		18.9	21.8	11.6	2–121

As-dw: arsenic in drinking water; As-wi: arsenic water intake; Tu-As: total urinary arsenic; Tu-As/c: total urinary adjusted by creatinine.
e: elderly; s: students; w: workers; e ≠ s ≠ w: ($P < 0.05$); 1: Antofagasta; 2: Santiago; 3: Temuco; 1 ≠ 2 ≠ 3: ($P < 0.05$).

drinking water and the intake from water were markedly higher (by an order of magnitude) for all strata in Antofagasta compared to Santiago and Temuco. The values in Santiago were modesty higher than those in Temuco. Water intake was generally similar for all cities and strata, with the exception of higher consumption among Santiago workers. Urinary arsenic concentrations were of the order of 2–3 times higher in Antofagasta than in the other cities, both before and after adjustment for creatinine. The adjustment had a notable effect among the elderly and is consequently retained in subsequent analyses. Within cities, adjusted urinary As concentrations were similar for students and workers, and higher for the elderly.

Fig. 2 presents the relationship between Tu-As/c and As-wi by city and age strata; as the figure indicates, there was great individual variability in the arsenic concentration in urine within each stratum and city. A positive relationship was observed between the arsenic excreted in urine and the total arsenic exposure through the water in workers and students of Antofagasta; however, the elderly showed a negative relationship. In contrast, Santiago and Temuco strata did not show a clear relationship.

When a conditional multilevel model with city and age strata as fixed effects and arsenic intake, age, gender, BMI, and smoking as random effects was fit to adjusted urinary arsenic concentration, the random individual effects within stratum and city accounted for 87.6% of the variance. Among individual-level variables, arsenic intake and age were significantly associated with urinary

arsenic, but gender, BMI, and smoking were not (Table 3). The concentration of As in urine increased 0.53 µg/g for each additional microgram per day of arsenic intake. Neither city nor the interaction of city and age strata had any additional effect; these factors explained 11.9% and 0.5% of the total variance, respectively.

Multiple regression analyses were performed for each age stratum within the cities, using the same predictor variables (data not shown). A model for the entire study population explained about 20% of the variability of total urinary arsenic concentration (Tu-As/c); As-wi and age were significant predictor variables. When these models were stratified by city and age strata, the variability was between 1% and 15%, with the highest R^2 in Antofagasta and the lowest in Santiago and Temuco. Age and As-wi were significant predictor variables in Antofagasta, but not in the latter two cities.

4. Discussion

The population of the north of Chile has been exposed to high levels of arsenic in drinking water and air for many years, and has high morbidity and mortality rates from illnesses associated with exposure to this metal. The Region II of Chile presents the highest rates of mortality for cancers of the lung, bladder, and skin, which significantly exceed the national averages (Ferreccio et al., 1998, 2000).

The most common source of As exposure at a population level is the consumption of contaminated
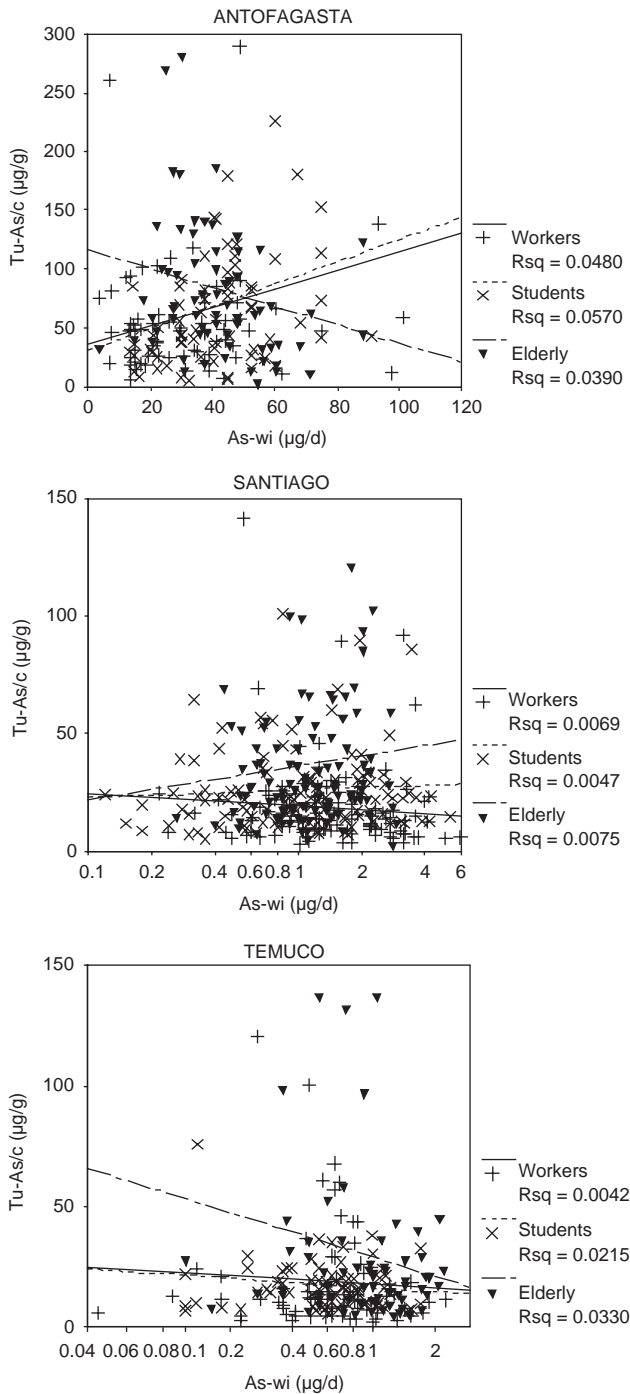
Fig. 2. Distribution of total urinary arsenic adjusting by creatinine (Tu-As/c) and arsenic in water intake (As-wi) stratified by age and city.

water (Gebel, 2000). The main source of As is the presence of arsenic-rich minerals and rocks in the aquifer, and less often contamination by waste materials resulting from mining and smelting activities (Tchounwou and Tchounwou, 1999). In the case of northern Chile, natural geological strata (volcanogenic sediments, basin lakes, thermal springs closed) and anthropogenic (mining) activities would be responsible (Nordstrom,

2002; Buchet and Lison, 2000). Several arsenic chemical species may be found in water, but inorganic forms, particularly the pentavalent species, predominate; trivalent As is found only under anaerobic conditions.

In this study, the median consumption of water in the 3 age strata and cities was generally similar. A great variability was observed within strata, however. This could be explained by cultural patterns and individual and environmental factors. The highest consumption was in the group of the elderly, followed by the students and the workers. Similar water consumption patterns have been reported in diverse studies in general populations and diverse population strata (Kurttio et al., 1998; Wyatt et al., 1998).

As concentrations in the drinking water were relatively homogeneous within each studied area. Of the analyses made in the samples taken in each city only As(V) was determined and the levels assessed in Antofagasta were significantly higher compared with those in Santiago and Temuco. Other studies in this area have found only As(V) in drinking water (Sancha et al., 1992; Hopenhayn-Rich et al., 1996b). None of these concentrations exceeded the national arsenic standard (50 μg/L). However, Antofagasta exceeded on average 3.6 times the value recommended by the WHO in 1993 (10 μg/L). On the other hand, the concentrations found in Santiago and Temuco were significantly lower than this reference value (WHO, 1993; Smith et al., 2002). Similar concentrations were determined for the city of Antofagasta (40 μg/L) in the period 1988–1994 (Rivara et al., 1997; Ferreccio et al., 2000). This means that this population continues being chronically exposed to concentrations that surpass the values recommended by the WHO (1993).

Recent studies have determined that the current standard (50 μg/L) would not be adequately protective against cancer and this concentration would be associated with substantial increased risk of cancer and systemic dermal, reproductive, and metabolic effects (Rahman et al., 1999; Morales et al., 2000). Long-term low-dose exposure to inorganic arsenic also causes cardiovascular effects including arrhythmias, hypertension, heart and brain ischemia, peripheral artery disorders, Raynaud's syndrome, and Blackfoot disease (ATSDR, 1993; Chen et al., 1997; Buchet and Lison, 2000). EPA (1984) investigators estimated that the lifetime risk of skin cancer for an individual who consumed 2 L of water per day at 50 μg/L could be as high as 2 in 1000, which required the EPA to revise the standard for arsenic in the drinking water, proposing a level of 10 μg/L finally in January of 2001 (Abernathy et al., 2003).

Clearly geological factors would largely explain the arsenic concentrations in the drinking water in the area of Antofagasta. The north of Chile is known to have natural high arsenic levels in the water and the soil

Table 3
Multilevel analysis: parameters estimates for total urinary arsenic adjusted by creatinine (Tu-As/c)

G	Coefficient	Estimate	SE	*P*-value
Variance component				
Random city variance	ε_k	245.00	281.60	0.1922
Random age strata variance nested in city	ε_{jk}	10.70	20.33	0.2993
Random individual variance within age strata nested in cities	ε_{ijk}	1805.10	94.20	<0.0001
Fixed effects				
Grand average (Tu-As/c)	β_0	30.62	13.40	0.1495
As-wi (µg/day)	β_1	0.53	0.15	0.0006
Age (years)	β_2	0.27	0.09	0.0026
Gender (male = 1; female = 0)	β_3	−2.59	3.20	0.4182
BMI (kg/m^2)	β_4	−0.14	0.3	0.7161
Smoking (yes = 1; no = 0)	β_5	−2.54	3.70	0.4916

As-wi: arsenic water intake; BMI: body mass index; SE: standard error.

(Rivara et al., 1997). On the other hand, the geographical factor in the case of Santiago and Temuco would have a marginal influence in the present arsenic levels in the water. Therefore, in these cities arsenic concentration may be explained primarily by environmental factors such as anthropogenic contamination of air and food. Sancha and co-workers determined that 85 and 72.7% of the total exposure to arsenic in Antofagasta and Santiago comes from the water, while in Temuco 80.2% would come from food (Sancha et al., 1998). Using information on food intake in Santiago, Garmendia estimated that seafood explained 67% of total arsenic intake through food (Garmendia, 2003).

Urinary arsenic concentration has been used for determination of exposure and for assessment of health risk. Arsenic species are important short-term biomarkers and have been used in many epidemiological studies (Foa et al., 1987; Hakala and Pyy, 1995; Chen et al., 2002). Our analysis of arsenic excreted in the urine verified great variability within each stratum of the studied cities. Previous studies suggest that these differences can be partially explained by individual factors related to arsenic metabolism. In a study carried out in a highly exposed Chilean population, Hopenhayn-Rich and collaborators found that 30% of the variability observed in metabolism of the arsenic was associated with individual attributes such as sex, ethnicity, and the duration of exposure (Hopenhayn-Rich et al., 1996b). Genetic factors may also explain some of this variability. Concha and collaborators argued, based on a study of women exposed to high concentrations of arsenic in drinking water, that the high level of observed variability is explained by genetic polymorphisms in the enzymes that metabolize the arsenic, especially certain enzyme methyltransferases (Concha et al., 2002). Another source of variation among studies might be the type and frequency of the collected urine sample (first morning or 24 h), as well as the type of adjustment used. In this study, the FMV was used and the obtained results were adjusted for creatinine.

A hierarchical multilevel model showed that individual and city factors both had notable effects on the variability in the concentration of total urinary arsenic. Age and arsenic consumed through water were important individual-level predictors. It is probable that at the relatively low levels of arsenic exposure reported in this study, other individual-level factors such a gender, BMI, and smoking have a low impact on the arsenic excreted in urine. Similar results were reported by Kurttio et al. (1998) in Finland; they did not find significant associations between gender or smoking and urinary arsenic excretion after adjusting for age. In contrast, other studies performed in northern Chile found that individual factors such as gender, smoking, ethnicity, and length of exposure had a significant effect on the quantity of urinary arsenic metabolites (Vahter, 2000).

Factors other than drinking water are likely to be the principal source of urinary arsenic in Santiago and Temuco. Regression models stratified by city showed that arsenic intake from water was a not significant predictor of the arsenic level in urine in these cities. Environmental pollution and ingestion through food may explain these findings. In a previous study in Santiago, arsenic intake through food was the only significant predictor of urinary arsenic (Garmendia, 2003). Kurttio et al. (1998) concluded that the contribution of arsenic from food to the total arsenic excreted in urine is most prominent when the level of arsenic in water is low, as it is in Santiago.

The most important limitation of our study is not to have speciated arsenic. Because of technical and financial limitations we could not assess arsenic species in Santiago and Temuco. We assessed some arsenic species in Antofagasta but we decided not to show these data in this paper because one of the objectives was to compare different population strata in big cities of

Chile. Other limitations of this study are related to the methods of estimating arsenic intake. Our estimates of arsenic ingestion from drinking water were based on average arsenic concentrations for neighborhoods, rather than household measurements, and did not account for the consumption of any water other than tap water from the participants' residences, such as bottled water or water consumed as work or school. The effects of this procedure are likely to have been relatively small, however. In Antofagasta, all residential drinking water comes from the same source, so the potential for geographic variability in concentrations is minimized. However, consumption of bottled water would reduce the arsenic intake of Antofagasta residents. Santiago and Temuco have water supplies with diverse origins and the potential for more variable concentrations, but arsenic levels in those cities were extremely low, so neither bottled water nor geographic variation are likely to have had significant effects. Errors in reporting water consumption are also possible; such errors are likely to have been greatest in those cases when it was necessary to estimate the ingestion of water through the food frequency questionnaire, rather than from 24-h recall. Finally, other sources of arsenic, such as food, air, and occupational exposure, were not considered in the estimates.

The type of urine sample used is also a potential source of uncertainty. However, various authors have concluded that there is no difference in the rate of excretion of urinary arsenic in the FMV compared with samples taken over a period of 24 h or more extended periods (Calderon et al., 1999; Hinwood et al., 2002).

In conclusion, these findings indicate that the arsenic concentration in drinking water continues to be the principal factor contributing to exposure to inorganic arsenic in the Chilean population. Although arsenic concentrations in drinking water are low in the center and south areas of Chile, these areas have other exposure sources that should be defined through further research. Despite the relatively low arsenic exposure levels determined in this study, the effects from the chronic exposure of the population of the north of Chile should be evaluated in future studies. Diverse studies have evaluated the metabolism of arsenic in populations exposed to high levels; however, it is also necessary to evaluate arsenic metabolism at low exposures.

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