

## Contribution of Water, Bread, and Vegetables (Raw and Cooked) to Dietary Intake of Inorganic Arsenic in a Rural Village of Northern Chile

OSCAR PABLO DÍAZ,<sup>†</sup> IRMA LEYTON,<sup>†</sup> OCIEL MUÑOZ,<sup>‡</sup> NELSON NÚÑEZ,<sup>§</sup>  
 VICENTA DEVESA,<sup>||</sup> MARIA ANGELES SÚÑER,<sup>||</sup> DINORAZ VÉLEZ,<sup>||</sup> AND  
 ROSA MONTORO\*<sup>||</sup>

Facultad de Química y Biología and Centro de Estudios en Ciencia y Tecnología de Alimentos,  
 Universidad de Santiago de Chile, Libertador Bernardo O'Higgins 3363, Santiago de Chile,  
 33074 Correo 33, Chile, Programa Agrícola Convenio Codelco Chile-Gobierno II Región, Duplex 167,  
 Chuquicamata, Chile, and Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC),  
 Apdo. Correos 73, 46100 Burjassot, Valencia, Spain

Total and inorganic As contents of cooked vegetables obtained from an arsenic endemic area of Chile were analyzed. Inorganic As intake from those foods, bread, and water was estimated. The study was performed in two different periods, in which the water used by the population for drinking and cooking purposes contained 0.572 (first period) or 0.041  $\mu\text{g mL}^{-1}$  (second period). In the first period, the FAO/WHO reference intake was exceeded by all of the persons interviewed. In the second period, the reference intake was exceeded by all of the persons interviewed ages 13–15. The foods studied contributed 4% (first period) or 25% (second period) of the inorganic As intake. The results show the contribution of food to inorganic As intake and the risk to which those ages 15 or younger are exposed.

**KEYWORDS:** Arsenic; inorganic arsenic; vegetables; cooking; Chile; intake

### INTRODUCTION

Inorganic arsenic [As(III) + As(V)] comprises the most toxic chemical species of As present in foods and drinking water. It has been classified by the International Agency for Research on Cancer (IARC) as a carcinogen to humans (1). Arsenic is distributed so extensively in the world that low levels of it are commonly found in air, soil, and water. However, causes of natural or human origin have produced very high levels of As in certain regions of Argentina (2, 3), Bangladesh (4, 5), Chile (6), China (7, 8), India (9, 10), Mexico (11, 12), Taiwan (13, 14), and Vietnam (15), leading to the appearance in the population of pathologies associated with chronic exposure to inorganic As (AsI). In Bangladesh alone, it is considered that between 35 and 77 million inhabitants are at risk of drinking As-contaminated water (16).

Epidemiological studies carried out in arsenic endemic areas have considered drinking water to be the main source of As for humans (6, 10, 17). However, some studies have shown levels of urinary excretion of AsI higher than those expected as a

consequence of the concentration of this toxin in drinking water (9, 18–20). As a result, it has been considered that foods are responsible for an important part of As intake, and studies on total arsenic (AsT) in food obtained from arsenic endemic areas have increased in recent years (21–25). The contribution of food to the As intake of the population of an arsenic endemic area was initially estimated by Vahter et al. (18), in the Argentine province of Salta, as 30% of the AsT intake. Recent research has obtained results of the same order, with food contributing over 20% of AsT intake in areas of Mexico (26) and India (23, 25). All of this shows that failure to consider the contribution of food to intake of AsI could introduce a substantial bias into the estimation of risks for the population of arsenic endemic areas.

Studies on AsI contents in foods from these areas are scarce (22, 27). Correct estimation of As intake should consider not only the As content of the raw product but also the content of the contaminant in the product in the form (raw or cooked) in which it is consumed by the population. Studies have shown changes in As concentration after cooking of seafood (28) and vegetables (29, 30). In arsenic endemic areas, the high As content in water used for cooking purposes is a further source of contamination, mainly as AsI. This is indicated by the high AsT contents, as much as 1.58  $\mu\text{g g}^{-1}$  wet weight (ww), found in the studies on AsT contents in cooked foods from these contaminated areas (2, 18, 23, 25, 26, 31). These authors suggest

\* To whom correspondence should be addressed. Tel: (+34)963 900 022. Fax: (+34)963 636 301. E-mail: rmontoro@iata.csic.es.

<sup>†</sup> Facultad de Química y Biología, Universidad de Santiago de Chile.

<sup>‡</sup> Centro de Estudios en Ciencia y Tecnología de Alimentos, Universidad de Santiago de Chile.

<sup>§</sup> Programa Agrícola Convenio Codelco Chile-Gobierno II Región.

<sup>||</sup> Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC).

that As-contaminated water used in food preparation is the origin of the high AsT contents detected in cooked food. Nevertheless, there are no prior references to evaluate the influence of cooking on AsI contents.

The present work concentrates on the Chile's Second Region, an area of 126 049 km<sup>2</sup>, situated in the north of the country, between 20°5' and 26°5', where there are high As contents in the environment, associated with quaternary volcanic and present day geothermal activity in the Andes Cordillera (32). The area is extremely arid (it contains the Atacama Desert, one of the most arid in the world), with a dramatic scarcity of water. The only permanent surface water is the 440 km long river Loa, with a mean concentration of As of 1.4 mg kg<sup>-1</sup> (32), which in the various sections of the river is between 2 and 25 times the maximum permitted by Chilean regulations for irrigation water (33). This water has been used for drinking for many years. Between 1958 and 1970, the inhabitants of the regional capital, Antofagasta, consumed water with As contents exceeding 1.8 µg mL<sup>-1</sup> (34). The main cities in the Second Region now have water treatment plants, but most of the small towns do not have these systems, and consequently, the population consumes water with As contents greater than the maximum permitted by Chilean legislation (0.050 µg mL<sup>-1</sup>). This situation has had adverse effects on health (6, 35). Moreover, agricultural activity, with high As contamination in soil, air, or irrigation water, may have a negative effect on the food safety of vegetables grown in the area, in which concentrations of 1.88 µg g<sup>-1</sup> ww of AsT have been detected in corn (21) and 0.613 µg g<sup>-1</sup> ww of AsI in spinach (22).

The aim of the present study was to evaluate the contribution of foods of vegetable origin as consumed by the population (raw or cooked) to the AsI intake of the inhabitants of Chiu Chiu, a rural village in Chile's Second Region exposed to arsenic for many centuries.

## MATERIALS AND METHODS

**Study Area.** The study presented in this paper focuses on the agricultural village of Chiu Chiu, with 1061 inhabitants. It is situated 35 km east of Calama, the city with the second highest population in the region and on the banks of the river Loa before it meets the river Salado. Agriculture is the inhabitants' main economic activity. In the small holdings used for cultivation, concentrations of 50–70 mg kg<sup>-1</sup> of As have been detected in soils (0–20 cm) and 0.07–0.28 mg L<sup>-1</sup> in water used for irrigation (22).

**Food Questionnaire.** A 24 h dietary recall questionnaire was administered to homes in the township of Chiu Chiu. It asked for information about the type and quantity of water and foods ingested the previous day, and how the foods were prepared for their consumption, raw or cooked. The design of the questionnaire was approved by the Regional Ministerial Health Secretariat for Chile's Second Region, and it was administered by professionals from the Health Service in Calama (Second Region) and from the Chile Second Region Government Codelco Agreement Agricultural Program.

The number of interviewees was set at 50, 25 male and 25 female, belonging to the following age groups: ≤15, 20–30, 31–45, 46–56, and ≥73. The interviewees selected belonged to various work categories: students (11), housewives (12), unemployed (3), retired (4), and employed (20). Of those interviewed, 84% declared that they had been living in the village for over 5 years.

**Food Samples.** Almost all of the vegetables and cereals that were declared to have been consumed in the 24 h dietary recall questionnaires were analyzed. For most of these foods, apart from potatoes and tomatoes, local production was sufficient to meet the needs of Chiu Chiu. The land where the products were grown belonged to the communities of Chiu Chiu and Lasana. The raw products were collected from various agricultural small holdings using a random collection procedure in two different periods (first sample collection, April 1999;

second sample collection, December 1999). The samples were transferred to the laboratory in suitable conservation conditions and washed with distilled water to remove remains of soil. All of the samples were analyzed raw, with results that were published by Muñoz et al. (22). Some of these samples were cooked in accordance with the cooking customs of the population.

**Cooked Food Samples.** The cooking treatments applied to the vegetables and cereals were those indicated in the 24 h dietary recall questionnaires: boiling, purée, stew, pie, or omelet. In the preparation of these dishes, the quantity of water and the conditions of the heat treatment were the only differentiating factors between the treatments. In no cases were additional ingredients employed. In the cooking process applied to all samples, we controlled the time (25–45 min) and the quantity of water added (250 mL for garlic and carrot; 500 mL for the other items). To prepare the products in the form of a stew (chard, cauliflower) or omelet (chard, asparagus), basically the vegetables were boiled. To make a pie, chard was boiled and then baked.

To evaluate the effect of the As contents in the water used for cooking on the contents in the cooked food, we used distilled water and the water that the inhabitants of Chiu Chiu used for drinking and cooking in two different periods. Once cooked, the foods were frozen at –20 °C and then freeze-dried. The lyophilized samples were ground in a domestic apparatus, and the resulting powder was vacuum packed and kept in the freezer at 4 °C until analysis.

**Instruments.** The quantification of AsT and AsI was performed with an atomic absorption spectrometer (AAS), Perkin-Elmer (PE) model 3300, equipped with an autosampler (PE AS-90) and a hydride generation flow injection system (PE FIAS-400). Other equipment used included a Genesis SQ 25 Super ES lyophilizer (Virtis, Gardinier, U.S.A.), a PL 5125 sand bath (Raypa Scharlau S. L., Barcelona, Spain), a K1253 muffle furnace equipped with a Eurotherm Controls 902 control program (Heraeus S. A., Madrid, Spain), a KS 125 Basic mechanical shaker (IKA Labortechnik, Merck, Barcelona, Spain), and an Eppendorf 5810 centrifuge (Merck).

**Reagents.** Deionized water (18.2 MΩ cm), obtained with a Milli-Q water system (Millipore Inc., Millipore Ibérica, Madrid, Spain), was used for the preparation of reagents and standards. All chemicals were pro analysis quality or better. All glassware was treated with 10% (v/v) HNO<sub>3</sub> for 24 h and then rinsed three times with deionized water before being used. Calibration standard solutions of As(III) were prepared from a reduced commercial standard solution (1000 mg L<sup>-1</sup>) of As(V) (Merck). As reducing solution, a mixture containing 5% (w/v) KI and 5% (w/v) ascorbic acid was employed.

**AsT Determination (22).** Lyophilized sample (0.25 g) was treated with 1 mL of ashing aid suspension (20% w/v MgNO<sub>3</sub> + 2% w/v MgO) and 5 mL of 50% v/v HNO<sub>3</sub>. The mixture was evaporated on a sand bath until total dryness and placed in a muffle furnace. The temperature was increased progressively, and the maximum temperature 425 ± 25 °C was maintained for 12 h. The calcination process was repeated until white ash was obtained. The white ash was dissolved in 5 mL of 6 mol L<sup>-1</sup> HCl and 5 mL of reducing solution (5% w/v KI + 5% w/v ascorbic acid). After 30 min, the resulting solution was diluted to volume with 6 mol L<sup>-1</sup> HCl and filtered through Whatman No. 1 filter paper into a volumetric flask. The instrumental conditions used for As determination by flow injection hydride generation AAS (FI-HG-AAS) were the following: loop sample, 0.5 mL; reducing agent, 0.2% (w/v) NaBH<sub>4</sub> in 0.05% (w/v) NaOH, 5 mL min<sup>-1</sup> flow rate; HCl solution 10% (v/v), 10 mL min<sup>-1</sup> flow rate; carrier gas argon, 100 mL min<sup>-1</sup> flow rate; wavelength, 193.7 nm; spectral band pass, 0.7 nm; electrodeless discharge lamp system 2; lamp current setting, 400 mA; and cell temperature, 900 °C.

Two reference materials certified for AsT contents (National Institute of Standards and Technology) were employed for QA/QC of analytical data: tomato leaves (SRM 1573) and rice flour (SRM 1568a). The analytical characteristics of the method are as follows: detection limit = 0.006 µg g<sup>-1</sup> wet weight (ww); precision = 2%; accuracy for tomato leaves (SRM 1573) found value = 0.28 ± 0.02 µg g<sup>-1</sup> dw, certified value = 0.27 ± 0.05 µg g<sup>-1</sup> dry weight (dw); accuracy for rice flour (SRM 1568a) found value = 0.29 ± 0.03 µg g<sup>-1</sup> dw, certified value = 0.29 ± 0.03 µg g<sup>-1</sup> dw.

**AsI Determination (22).** The lyophilized sample (0.50 g) was weighed into a 50 mL screw-top centrifuge tube, 4.1 mL of water was added, and the sample was agitated until it was completely moistened. Then, 18.4 mL of concentrated HCl was added, and the sample was agitated again for 1 h and then left to stand for 12–15 h (overnight). The reducing agent (1 mL of 1.5% w/v hydrazine sulfate solution and 2 mL of HBr) was added, and the sample was agitated for 30 s. Then, 10 mL of  $\text{CHCl}_3$  was added and the sample was agitated for 10 min. The phases were separated by centrifuging at 2000 rpm for 5 min. The chloroform phase was separated by aspiration and poured into another tube. The extraction process was repeated two more times. The chloroform phases were combined and centrifuged again. The remnants of the acid phase were completely eliminated by aspiration (acid phase remnants in the chloroform phase cause substantial overestimates of AsI). Possible remnants of solid material in the chloroform phase were eliminated by passing it through Whatman GD/X syringe filters with a 25 mm PTFE membrane (Merck Farma y Química S. A., Barcelona, Spain).

The AsI in the chloroform phase was back-extracted by agitating for 10 min with 10 mL of 1 mol  $\text{L}^{-1}$  HCl. The phases were separated by centrifuging at 2000 rpm, and the aqueous phase was then aspirated and poured into a beaker. This stage was repeated once again, and the back extraction phases obtained were combined. The determination of AsI in the back extraction phase was performed by means of the following procedure: 2.5 mL of ashing aid suspension and 10 mL of concentrated  $\text{HNO}_3$  were added to the combined back extraction phases, after which the solution was evaporated to dryness and then treated in the same way as for AsT (dry ashing mineralization and quantification by FI-HG-AAS).

The analytical characteristics of the method are as follows: detection limit = 0.003  $\mu\text{g g}^{-1}$  ww; precision = 4%; recovery As(III) = 99% and As(V) = 96%. There are no food certified reference materials available for AsI content. The quality criterion adopted, therefore, was the overlapping between the ranges of AsI found in rice flour sample and those reported in a previous study carried out by our laboratory (24) ( $0.110 \pm 0.03 \mu\text{g g}^{-1}$  dw). This value was close to the only existing previous reference for AsI in rice flour ( $0.092 \mu\text{g g}^{-1}$  dw) obtained by a trifluoroacetic acid extraction–ion chromatography–inductively coupled plasma mass spectrometry (36).

## RESULTS AND DISCUSSION

For many years, the inhabitants of de Chiu Chiu consumed water with As contents fluctuating between 0.600 and 0.800  $\mu\text{g mL}^{-1}$  (37). The present work shows the results for food samples collected in two different periods with large differences in the As content in the water to which the inhabitants of Chiu Chiu had access (drinking and cooking purposes). The first samples were collected in April 1999, and the mean AsI content in the water collected from various homes in the village was 0.572  $\mu\text{g mL}^{-1}$ . This concentration was over 10 times greater than the level permitted by Chilean legislation (0.050  $\mu\text{g mL}^{-1}$ ). In August 1999, as a result of the preliminary results obtained in this work, the administrative authority of the Second Region in Chile decided to use water tankers to provide Chiu Chiu with drinking water that conformed to Chilean regulations. Consequently, in the samples collected in the second period (December 1999), the AsI content quantified in the water used by the population for drinking and cooking was much lower (0.041  $\mu\text{g mL}^{-1}$ ).

The vegetables and cereals analyzed in this work were cooked in accordance with the cooking treatments customary in the area, using distilled water and water used by the population in the two sample collection periods. The AsT and AsI that they contained were analyzed. The determination of AsT has no toxicological interest, but its inclusion in this study makes it possible (i) to compare the contamination of the foods analyzed with the data available in the literature, which almost all analyze

**Table 1.** Concentration of Total Arsenic and Inorganic Arsenic in Raw and Cooked Horticultural Foods from the First Sampling Period<sup>a</sup>

horticultural product	preparation		total arsenic	inorganic arsenic
asparagus	raw		NA	NA
	cooked	contaminated water (boiling)	0.16	0.13
beetroot	raw		0.129	0.121
	cooked	distilled water (boiling and peeling)	0.08	0.08
	cooked	contaminated water (boiling and peeling)	0.07	0.06
carrot	raw		0.078	0.060
	cooked	distilled water (peeling and boiling)	0.05	0.03
	cooked	contaminated water (peeling and boiling)	0.07	0.05
chard	raw		0.247	0.190
	cooked	distilled water (boiling)	0.070	0.060
	cooked	contaminated water (boiling)	0.210	0.190
garlic	raw		0.317	0.267
	cooked	distilled water (boiling)	0.13	0.05
	cooked	contaminated water (boiling)	0.37	0.28
maize	raw		0.015	0.010
	cooked	distilled water (boiling)	0.03	0.03
	cooked	contaminated water (boiling)	1.58	1.42
potatoes	raw		0.102	0.090
	cooked	distilled water (peeling and boiling)	NA	NA
	cooked	contaminated water (peeling and boiling)	0.11	0.09
lettuce	raw		0.068	0.061
tomato	raw		0.008	0.008
bread	raw		0.162	0.141

<sup>a</sup> Results expressed in  $\mu\text{g As g}^{-1}$  wet weight (ww). Inorganic arsenic in contaminated water: 0.572  $\mu\text{g As mL}^{-1}$ . Each of the values shown in the table is the mean of three replicates. Contents in raw products taken from ref 22. NA, not analyzed.

AsT and not AsI, and (ii) to evaluate the percentage of AsI with respect to AsT present in these foods. The concentrations of AsT and AsI found in edible portions of the cooked samples are shown in **Table 1** (first sample period) and **Table 2** (second sample period). The tables also show the contents obtained by Muñoz et al. (22) in the same products raw.

**AsT and AsI in Cooked Food.** The AsT concentrations varied between 0.004  $\mu\text{g As mL}^{-1}$ , detected in potatoes and pumpkin boiled with distilled water, and 1.58  $\mu\text{g g}^{-1}$  ww, found in grains of maize boiled in water containing 0.572  $\mu\text{g As mL}^{-1}$ . The As contributed by the water was the cause of this high concentration, as the same maize boiled in distilled water had a much lower AsT concentration (0.03  $\mu\text{g g}^{-1}$  ww). The published references concerning AsT contents in cooked food in arsenic endemic areas show contents similar to those found in the samples obtained from Chile, and it is possible also that the water used in cooking was the cause of the high contents found (**Table 3**).

The concentrations of AsI varied between 0.003  $\mu\text{g As mL}^{-1}$  (pumpkin boiled with distilled water) and 1.58  $\mu\text{g g}^{-1}$  ww (maize boiled in water containing 0.572  $\mu\text{g As mL}^{-1}$ ). Foods with contents greater than 0.100  $\mu\text{g g}^{-1}$  ww were detected in both collection periods (**Tables 1** and **2**): boiled chard, chard pie, boiled garlic, boiled asparagus, asparagus omelet, and boiled spinach. In the literature, there are reports of AsI contents in raw vegetables, both in arsenic endemic areas (22, 27, 38) and in nonarsenic endemic areas (36, 39, 40). However, the only previous reference that we know for AsI contents in cooked vegetables is the study carried out by Schoof et al. (39) in products in the U.S.A., with a maximum concentration of 0.004  $\mu\text{g g}^{-1}$  ww in microwaved peas. The lack of data concerning AsI in cooked samples from arsenic endemic areas shows the novelty of the results reported in the present work.

In the samples analyzed in this study, AsI represents 38 to almost 100% of the AsT existing in the samples, and only in four of them does AsI represent less than 50% of the AsT. These

**Table 2.** Concentration of Total Arsenic and Inorganic Arsenic in Raw and Cooked Horticultural Foods from the Second Sampling Period<sup>a</sup>

horticultural product	preparation		total arsenic	inorganic arsenic
asparagus	raw		0.081	0.065
	cooked	distilled water (boiling)	0.029	0.014
	cooked	contaminated water (boiling) (omelet)	0.051 0.396	0.054 0.325
beans	raw		0.022	0.023
	cooked	distilled water (boiling)	0.007	0.006
	cooked	contaminated water (boiling)	0.057	0.048
beetroot	raw		0.168	0.160
	cooked	distilled water (boiling)	0.050	0.042
	cooked	contaminated water (boiling)	0.058	0.060
cauliflower	raw		0.014	0.010
	cooked	distilled water (boiling)	0.007	0.004
	cooked	contaminated water (boiling) (stew)	0.077 0.041	0.060 0.029
carrot	raw		0.138	0.128
	cooked	distilled water (peeling and boiling)	0.038	0.037
	cooked	contaminated water (peeling and boiling)	0.066	0.065
chard	raw		0.266	0.187
	cooked	distilled water (boiling)	0.099	0.061
	cooked	contaminated water (boiling) (omelet) (stew) (pie)	0.107	0.050
			0.129	0.079
			0.119	0.091
		0.184	0.136	
garlic	raw		0.030	0.030
	cooked	distilled water (boiling)	0.028	0.027
	cooked	contaminated water (boiling)	0.127	0.115
maize	raw		0.152	0.110
	cooked	distilled water (boiling)	0.089	0.070
	cooked	contaminated water (boiling) (boiled grains)	0.117	0.107
			0.084	0.032
onion	raw		0.067	0.075
	cooked	distilled water (boiling)	0.074	0.072
	cooked	contaminated water (boiling)	0.078	0.083
potatoes	raw		0.021	0.024
	cooked	distilled water (peeling and boiling)	0.004	0.004
	cooked	contaminated water (peeling and boiling) (purée) (frying)	0.011	0.012
			0.043	0.039
		0.030	0.018	
pumpkin	raw		0.004	0.003
	cooked	distilled water (boiling)	0.004	0.003
	cooked	contaminated water (boiling)	0.045	0.050
spinach	raw		0.121	0.087
	cooked	distilled water (boiling)	0.050	0.038
	cooked	contaminated water (boiling)	0.276	0.227
lettuce	raw		0.448	0.394
cabbage	raw		0.016	0.013
tomato	raw		0.011	0.009
bread	raw		0.074	0.053

<sup>a</sup>Results expressed in  $\mu\text{g As g}^{-1}$  wet weight (ww). Inorganic arsenic in contaminated water:  $0.041 \mu\text{g As mL}^{-1}$ . Each of the values shown in the table is the mean of three replicates. Contents in raw products taken from ref 22.

**Table 3.** Concentration of Total Arsenic in Water and Cooked Foods in Arsenic Endemic Areas

country	water ( $\mu\text{g As mL}^{-1}$ )	foods ( $\mu\text{g As g}^{-1}$ )	ref
Argentine Andes	0.200	0.427 ww (soup) 0.418 ww (maize porridge)	2, 18
Lagunera Region (Mexico)	0.412	1.58 ww (pinto beans) 1.26 ww (sauce) 1.08 ww (meat)	26
Bangladesh	0.223–0.372	0.228 and 0.377 ww (rice)	37
West Bengal (India)	0.1	0.881 dw (rice)	23

percentages are similar to those found by Schoof et al. (39) in cooked vegetables from nonendemic areas. They are normal percentages in products of vegetable origin (vegetables, cereals, fruit) and much higher than those found in seafood, in which AsI rarely exceeds 10% of the AsT (41). Hitherto, research

carried out on As and its chemical species in foods has concentrated on seafood products. As far as AsI is concerned, the AsI contents in cooked seafood products ( $<0.300 \mu\text{g g}^{-1}$  ww) (28) are similar to or less than those found in cooked vegetables from Chile (Table 1). This indicates the need to extend research on AsI to food products other than those of marine origin.

The results presented in Tables 1 and 2 also show that the AsT and AsI contents in raw food can alter in various ways during cooking treatments, with a consequent effect on the intake of these two contaminants. Cooking treatments such as boiling and frying can alter AsT content by (i) concentration of As through loss of water, volatiles, and, to a lesser extent, certain macronutrients (carbohydrates, lipids, and proteins) and (ii) loss of As through solubilization. Operations prior to cooking, such as peeling of horticultural products, can also lead to a substantial decrease in the contents of AsT and AsI. In the samples analyzed in this study (Tables 1 and 2), cooking with distilled water predominantly produced a decrease in AsT and AsI with respect to the raw product. When the water used for cooking contains As, cooking generally increases the concentration of As, except in the case of samples that have previously been peeled (beetroot, carrot, potatoes). In the samples of garlic, cooked with water containing  $0.572 \mu\text{g As mL}^{-1}$  or with water containing  $0.041 \mu\text{g As mL}^{-1}$ , there was an increase in AsI with respect to garlic cooked with distilled water. The reason could be the presence in garlic of compounds with sulfur moieties such as allicin (thio-2-propene-1-sulfonic acid S-allyl esters), alliin (S-allyl-L-cysteine sulfoxide), and cysteine capable of binding As (42). Indeed, Roy Choudhury et al. (42) attribute the fact that the dietary administration of a crude aqueous extract of garlic to mice in vivo significantly reduces the clastogenic effects of sodium arsenite due to the presence of sulfur-containing moieties in crushed garlic.

The remaining vegetables in which cooking led to an increase in AsI content did not have substantial contents of sulfur groups. The mechanisms for capturing AsI might be related to the incorporation of water into food during cooking. Thus, for maize (Table 1), the increase in AsI might be due to the high starch content in this cereal ( $\approx 87\%$ ), which incorporates a large quantity of water during its gelatinization in the boiling process. Similarly, the hemicellulose present in beans might retain water during boiling, increasing AsI levels after cooking (Table 2). In other samples such as chard (Table 1) and asparagus, spinach, pumpkin, and cauliflower (Table 2), vegetables that retain a considerable quantity of water during boiling, there was also an increase in the AsI contents.

**Estimation of Intake and Evaluation of Risks.** From the data provided by the 24 h recall questionnaire administered to the inhabitants of Chiu Chiu (quantity of water, mL, and food ingested, grams ww) and the AsT and AsI concentrations found in the water, raw and/or cooked food analyzed (Tables 1 and 2), we calculated the intakes of AsT and AsI for each interviewee. These intakes, expressed as  $\mu\text{g/day}$ , are shown in Table 4.

Epidemiological studies concerning human health effects after oral exposure to AsI have made it possible to establish various guideline levels. An intake of AsI at  $10\text{--}50 \mu\text{g AsI/day/kg}$  body weight contributed to vascular problems, which may ultimately lead to necrosis and gangrene of the hands and feet (43). The No Adverse Effect Level (NOAEL) for chronic oral intake has been estimated at  $1 \mu\text{g AsI/day/kg}$  body weight, and the Low Adverse Effect Level (LOAEL) has been estimated at  $10\text{--}100 \mu\text{g AsI/day/kg}$  body weight (44). However, the parameter most

**Table 4.** Daily Dietary Arsenic Intake from Water and Foods in the Village of Chiu Chiu, Second Region, Chile<sup>a</sup>

group	source	first sampling period (water, 0.572 $\mu\text{g As mL}^{-1}$ )				second sampling period (water, 0.041 $\mu\text{g As mL}^{-1}$ )			
		total As ( $\mu\text{g As/day}$ )		inorganic As ( $\mu\text{g As/day}$ )		total As ( $\mu\text{g As/day}$ )		inorganic As ( $\mu\text{g As/day}$ )	
		mean	range	mean	range	mean	range	mean	range
all interviewees	food	66	4–220	55	3–184	38	4–117	31	4–97
	water and food	1400	486–1683	1389	475–1647	131	60–219	125	57–200
13–15 years old	food	56	14–177	47	12–148	34	11–103	30	10–83
	water and food	1435	1185–1516	1443	1158–1684	132	114–175	125	111–154
>20 years old	food	69	4–220	58	3–184	39	4–117	38	4–97
	water and food	1400	486–1683	1378	475–1647	131	60–219	125	57–200

<sup>a</sup>Daily intake reference values for inorganic arsenic: 96.3  $\mu\text{g}$  inorganic As/day for 13–15 age group and 149.8  $\mu\text{g}$  inorganic As/day for >20 age group.

commonly used for the evaluation of AsI risk assessments is the Provisional Tolerable Weekly Intake (PTWI) established by the FAO/WHO: 15  $\mu\text{g AsI/week/kg}$  body weight (45).

To compare the PTWI with the data obtained for the inhabitants of Chiu Chiu, we used two approximations. First, as the questionnaire administered was a 24 h dietary recall, we expressed the PTWI as tolerable daily intake (TDI = PTWI/7 days), with a value of 2.14  $\mu\text{g AsI/kg}$  body weight. Second, 24% of the 50 interviewees were in the 13–15 age range, and we assigned to them a mean body weight of 45 kg. The remaining 76% of the interviewees were aged 20 or over, and we assigned to them a mean body weight of 70 kg. Assuming these body weights, the reference intakes stated by the FAO/WHO are equivalent to 96.3  $\mu\text{g AsI/day}$  for individuals ages 13–15 and 149.8  $\mu\text{g AsI/day}$  for individuals ages 20 or over (TDI  $\times$  kg body weight). We compared these values with the AsI intakes of the inhabitants of Chiu Chiu.

The As intakes for the totality of those interviewed and two sampling periods (Table 4) show that the daily AsI intake represents between 82 and 99% of the daily AsT intake. This is due to the types of foods analyzed, vegetables and cereals, in which, as indicated earlier, AsI is the major species. Previous studies in other arsenic endemic areas, in which the food groups considered in the intake are similar to those analyzed in Chile, estimated the AsI intake by extrapolation from the AsT intake, assuming that AsI represented all of the AsT found (26) or at least 50% of the AsT (23).

The daily AsI intake (Table 4) showed very different realities for each of the two sampling periods. In the first sampling period, the water used for drinking and cooking purposes contained 0.572  $\mu\text{g As mL}^{-1}$ . In neither of the two age groups considered did the mean AsI intake from foods (47 and 58  $\mu\text{g AsI/day}$ ) attain the reference intake for the group. However, one individual in the 13–15 age group, with 148  $\mu\text{g AsI/day}$ , exceeded the reference intake of the FAO/WHO (96.3  $\mu\text{g AsI/day}$ ). For the group of those ages 20 or over, only one of those interviewed, with an intake of 184  $\mu\text{g AsI/day}$ , exceeded the reference value (149.8  $\mu\text{g AsI/day}$ ). When the contribution provided by the water and foods analyzed was considered, the situation worsened remarkably. The FAO/WHO reference intake was exceeded by all of those interviewed; between 12 and 17 times for individuals ages 13–15 and between 3 and 11 times for the others. Consequently, the toxicological risk to which the population of Chiu Chiu has been exposed has been substantial for many years.

In the second sampling period, the water contained 0.041  $\mu\text{g As mL}^{-1}$ . On this occasion, the daily AsI intake contributed by the foods did not exceed the reference intakes for any of those interviewed and was 44% less than the intake from the foods

in the first sampling period. When the contribution of water and foods was considered, the AsI intake for the second sampling period was between 1.2 and 1.6 times greater than the reference intake for all of the interviewees in the 13–15 age group. For the other interviewees (>20 years old), only three individuals, ages 33, 35, and 45, exceeded the reference intake, and these three interviewees all had a high consumption of lettuce and bread, foods with high AsI contents. It is noteworthy that in the second sampling period, although the population was supplied with water with an AsI content below the limit set in Chile, some members of the population had an intake greater than the limit indicated by the FAO/WHO.

The population of Chiu Chiu has been used in numerous epidemiological studies (methylation patterns, AsI–cancer association, etc.) because of their long-term exposure to very high levels of AsI in drinking water (6, 46). One of these studies indicates the prevalence of skin lesions among men (who have been drinking contaminated water for more than 20 years) and children, similar to that reported with corresponding As drinking water concentrations in both Taiwan and West Bengal, India, populations in which extensive malnutrition has been thought to increase susceptibility (46). It appears, therefore, that neither good nutrition nor many centuries of exposure protect the inhabitants of Chiu Chiu from the effects that AsI has on health. The present work shows the risk situation that affects the population with ages between 13 and 15, despite the reduction in As content in water to levels below 0.050  $\text{g As mL}^{-1}$ . It seems necessary, therefore, to lower the limit permitted in drinking water to 0.010  $\mu\text{g As mL}^{-1}$ , a value recommended by the WHO and already applied in many countries.

With respect to the contribution of food to the overall intake of AsI, the importance varies according to the sampling period considered. Bearing in mind the mean daily intake for all of those interviewed (Table 4), in the first sampling period, food (bread and horticultural products) contributed 4% of the total intake, a value that increased to 25% in the second sampling period. These results are in agreement with those reported by Roychowdhury et al. (23); the significance of the intake of AsI from food increases as the concentration of As in water decreases. This result should be taken into account in nonarsenic endemic areas.

The inclusion of other foods, such as seafood and meat, might have changed the final intake of AsI, possibly increasing it. Of those interviewed, 80% declared that they had consumed meat products, particularly chicken and beef, and to a lesser extent llama, mutton, and pork. As in the case of horticultural products, cooking could increase the AsI contents in meat products; this is shown by the high AsT contents found by Del Razo et al. (26) in cooked meat (mean value = 0.34  $\mu\text{g/g ww}$ ). Consump-

tion of fish is not high, and only 8% of the interviewees declared that they had eaten fish the previous day (trout, horse mackerel, and canned tuna).

Because of the scarcity of previous reports on As intake in arsenic endemic areas, it is only possible to compare the results obtained for Chiu Chiu with those found for Taiwan, Mexico, and India. In Taiwan, Schoof et al. (27) analyzed AsI only in raw yams and rice, estimating a mean AsI intake of 50  $\mu\text{g}/\text{day}$  on the basis of these two products. This intake might be considered high, bearing in mind that it only evaluates the contribution of two products and does not take into account the effect of cooking with water containing As. In the Lagunera Region in Mexico (26), the intake of AsT is similar to the value estimated in Chile. This excellent study, which only analyzes AsT, considers the contribution of water, hot beverages, and cooked food to As intake. In the population exposed, which consumes water with a mean content of 0.410  $\mu\text{g mL}^{-1}$  of As, the intake exceeds the PTWI by between six and eight times. Cooked food contributes between 33 and 44% of the daily AsT intake, a percentage higher than that obtained in Chile and possibly attributable to the fact that sauces and soups were considered in the intake. In India, the recent studies carried out by Roychowdhury et al. (23, 25) in West Bengal also provide data for AsT intake from water and from raw and cooked food. Assuming that at least 50% of AsT in food samples is AsI, the maximum intake obtained from water and foods was 708  $\mu\text{g}/\text{day}$  in adult males, which is 4.7 times greater than the TDI. In this case, the daily intake contributed by foodstuffs (rice, vegetables, and spices) was 189  $\mu\text{g}$ , 27% of the daily AsT intake.

The present study is the first work to estimate AsI intake from water and vegetables in arsenic endemic areas on the basis of AsI contents obtained experimentally rather than on the basis of extrapolations from the AsT present in foodstuffs. The results obtained show the substantial contribution that food makes to As intake in contaminated and uncontaminated areas, indicating, as Del Razo et al. (26) commented, that this contribution must be considered when interpreting dose–response modeling based on the use of the available epidemiological data.

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