JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 8829−8833 **8829**

Bioavailability of Inorganic Arsenic in Cooked Rice: Practical Aspects for Human Health Risk Assessments

JOSÉ MOISÉS LAPARRA,[†] DINORAZ VÉLEZ,*[,†] REYES BARBERÁ,[‡] ROSAURA FARRÉ,[‡] AND ROSA MONTORO[†]

Institute of Agrochemistry and Food Technology (CSIC), Apdo. 73, and Nutrition and Food Chemistry, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, 46100, Burjassot, Valencia, Spain

Arsenic is present in rice grain mainly as inorganic arsenic. Little is known about the effect of cooking on inorganic arsenic content in rice and its bioavailability. This study evaluated total arsenic and inorganic arsenic in rice cooked with arsenic-contaminated water, the bioaccessibility of As(III) and As(V) after simulated gastrointestinal digestion, and the extent of arsenic retention and transport by Caco-2 cells used as a model of intestinal epithelia. After cooking, inorganic arsenic contents increase significantly. After simulated gastrointestinal digestion, the bioaccessibility of inorganic arsenic reached 63−99%; As(V) was the main species found. In Caco-2 cells, arsenic retention, transport, and total uptake (retention + transport) varied between 0.6 and 6.4, 3.3 and 11.4, and 3.9 and 17.8%, respectively. These results show that in arsenic endemic areas with subsistence rice diets, the contribution of inorganic arsenic from cooked rice should be considered in assessments of arsenic health risk.

KEYWORDS: Inorganic arsenic; rice; cooking; Caco-2; bioavailability

INTRODUCTION

Inorganic arsenic, a term that includes the most toxic species so far detected in foods, As(III) and As(V), has been classified as a human carcinogen by the International Agency for Research on Cancer (*1*). Various areas in Asia and Latin America are currently suffering severe health effects due to arsenic exposure (melanosis, depigmentation, hyperkeratosis, and malignant neoplasms) (*2*, *3*). Previous studies have shown dietary intake from drinking waters and foods to be the most important source of inorganic arsenic for humans. In Asian arsenic endemic areas, a large proportion of the population lives on subsistence diets of rice—a cereal containing a relatively high amount of As as compared with other agricultural products, largely in the form of inorganic arsenic (42−100%) (*4*, *5*). The presence of As in rice is considered yet another disaster for Southeast Asia (*6*).

The toxicity and resulting threat to human health of inorganic arsenic is of course related to its concentration in the food. However, for a better understanding of the implications of rice consumption for the assessment of arsenic-related health risks, the effect of cooking on inorganic arsenic contents and its bioavailability (i.e., the fraction of absorbed arsenic that reaches the systemic circulation) is an aspect to be taken into account. There have been few studies of the effects of rice cooking upon As contents (*4*, *7*), and nothing is known about the bioaccessibility (maximum soluble content in simulated gastrointestinal media) of inorganic arsenic from rice and the absorption of the bioaccessible fraction through the intestinal epithelia.

Because heavy metals must be in soluble form to allow absorption (*8*), one of the main limiting factors in oral bioavailability is the release of inorganic arsenic from ingested food within the gastrointestinal tract. However, it is generally recognized that not all soluble elements are absorbable; consequently, solubility alone is not an adequate marker of bioavailability (*9*). Caco-2 cell monolayers constitute a well-established intestinal epithelial model (*10*). The incorporation of Caco-2 cells grown on solid or microporous supports in in vitro digestion models, allowing mineral uptake and/or transport to be estimated, improves the systems used for bioavailability studies (*10*). The low cost, ease of use, and widespread acceptance of the Caco-2 cell line make this model a valuable tool and an attractive alternative to animal studies for use in conjunction with human trials (*9*).

The aim of the present study was to contribute to a better evaluation of the possible health risks derived from the consumption of rice based on evaluation of the bioavailability of inorganic arsenic in cooked rice. Evaluations were made of the changes in total As and inorganic arsenic contents as a result of cooking, the bioaccessibility of As(III) and As(V), and subsequent retention and transport of bioaccessible total arsenic by Caco-2 cells.

* To whom correspondence should be addressed. Tel: (+34)963 900 022. Fax: (+34)963 636 301. E-mail: deni@iata.csic.es.
[†] Institute of Agrochemistry and Food Technology (CSIC).
[‡] University of Valencia.

10.1021/jf051365b CCC: $30.25
Published on Web 09/30/2005

MATERIALS AND METHODS

Instruments. For As speciation analysis, a high-performance liquid chromatography (HPLC) system (Hewlett-Packard model 1100, Barcelona, Spain) equipped with a quaternary pump, an on-line degassing system, an automatic injector, and a thermostated column compartment was used. Separations were performed on a Hamilton PRP-X100 anion-exchange column (10 μm, 250 mm $\times$ 4.1 mm i.d.; Teknokroma, Barcelona, Spain). A guard column packed with the same material (12−20 μm; 25 mm $\times$ 2.3 mm i.d.) preceded the analytical column. For the thermooxidation step, a Julabo model HC heated bath (Merck, Barcelona, Spain) was used. The quantification of As was performed on a hydride generation system (PSA 10.004, Analytical, Kent, United Kingdom) using an atomic fluorescence spectrometer system (AFS) (PSA 10.044 Excalibur PS, Analytical) equipped with a boosted-discharge hollow cathode lamp (BDHCL, Photron, Super Lamp, Australia). The arsines generated were conveyed to the AFS detector by means of a semipermeable membrane dryer tube (Perma Pure). A Hewlett-Packard model 35900 C digital analogical converter was used to acquire the AFS signal, which was processed by the chromatographic software.

Arsenic determination was performed with an atomic absorption spectrometer (model 3300, Perkin-Elmer, Madrid, Spain) equipped with an autosampler (AS-90, Perkin-Elmer), a flow injection hydride generation system (FIAS-400, Perkin-Elmer), and an electrothermally heated quartz cell.

Other equipment used included a lyophilizer (FTS Systems, New York), a sand bath (PL 5125, Raypa, Scharlau, Barcelona, Spain), a muffle furnace equipped with a control program (K1253, Heraeus, Madrid, Spain), mechanical shakers KS 125 (IKA Labortechnik, Merck; Rotabit, Selecta, Barcelona, Spain), and centrifuges (Eppendorf 5810 Merck; Heraeus Biofuge Pico centrifuge, Merck; Sorvall RC-50B, Sorvall Instrument, DuPont).

Reagents. Deionized water (18.2 MΩ cm) was used for the preparation of reagents and standards. All material was treated with 10% v/v HNO_3 for 24 h and then rinsed three times with deionized water before being used.

Enzymes and bile salts were purchased from Sigma Chemical Co. (St. Louis, MO): pepsin (Porcine: catalog no. P-7000), pancreatin (Porcine; catalog no. P-1750), and bile extract (Porcine; catalog no. B-8631). Water of cellular grade (B. Braun Medical, S. A., Barcelona, Spain) was used throughout the in vitro digestion assay.

A commercial standard solution of H_3AsO_4 (1000 mg L^{-1}, Merck) was used to prepare standards of As(V). For As(III), the stock standard solution (1000 mg L^{-1}) was prepared by dissolving 1.320 g of As_2O_3 (Riedel de Haën, Hanover, Germany) in 25 mL of 20% (w/v) KOH, neutralizing with 20% (v/v) H_2SO_4, and diluting to 1 L with 1% (v/v) H_2SO_4.

Samples. Twelve samples of several types of rice from different manufacturers were acquired in food stores in the city of Valencia, Spain. All samples were analyzed in raw and cooked state. Samples of raw rice were kept at 4 °C until analysis. The rice was cooked using deionized water or deionized water spiked with different levels of As(V) (0.2−1.0 μg mL^{-1}). For cooking purposes, the rice (131 g) was added to boiling water (500 mL) and taken to dryness. The samples were then lyophilized, crushed to a fine powder in a mill, and stored at 4 °C until analysis.

In Vitro Gastrointestinal Digestion. Lyophilized samples of raw and cooked rice, equivalent to 10 g of raw or cooked rice, were digested using a simulated digestion process developed in a previous work (*11*). After the gastric step (0.02 g pepsin/g rice) and the intestinal step (0.5 mg of pancreatin/g rice and 3 mg of bile extract/g rice), aliquots of 40 g were transferred from the total digest (100 g) to polypropylene centrifuge tubes and centrifuged at 26891*g* for 30 min at 4 °C to separate the soluble (bioaccessible) fraction and the precipitate. Total As and arsenic species were analyzed in soluble fractions.

For the arsenic uptake assays with Caco-2 cells, the gastrointestinal digests from the solubility assay were heated for 4 min at 100 °C to inhibit sample proteases and then cooled by immersion in an ice bath. Aliquots of 40 g of the inactivated digests were transferred to polypropylene centrifuge tubes and centrifuged at 26891*g* for 30 min at 4 °C. Glucose (5 mM final concentration) and HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 50 mM final concentration; BioWhittaker, Verviers, Belgium) were added to the supernatant fraction to facilitate cell viability, while water or NaCl was added to adjust the osmolarity to 310 ± 10 mOsm/kg (freezing point osmometer, Osmomat 030, Berlin, Germany).

Cell Culture. The Caco-2 cell line was obtained from the European Collection of Cell Cultures (ECACC 86010202, Salisbury, United Kingdom) and was maintained in minimum essential medium (MEM; Gibco BRL Life Technologies, Paisley, Scotland) at pH 7.4. The MEM was supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Gibco), 1% (v/v) nonessential amino acids (Gibco), 1% (v/v) L-glutamine (BioWhittaker), 1% (v/v) sodium pyruvate (BioWhittaker), 0.22% (w/v) $NaHCO_3$ (Merck), 1% (v/v) HEPES, 1% (v/v) antibiotic solution (penicillin and streptomycin solution, 10000 units/mL of each) (BioWhittaker), and 0.1% (v/v) fungizone (Gibco). Cellular grade water was used for the cell culture. Cells were incubated in a humidified 5% CO_2−95% air mixture at 37 °C. The culture medium was changed every 2 days. The cells at 70% confluency were harvested by using trypsin-EDTA solution (2.5 g L^{-1} trypsin, Sigma; 0.2 g L^{-1} EDTA, Sigma, Barcelona, Spain). After they were detached from the flasks, the cells were resuspended in MEM.

Arsenic Uptake (Retention and Transport) by Caco-2 Cells. The Caco-2 cells were seeded onto polycarbonate membrane filters, Transwell inserts of 24 mm diameter and 0.4 μm pore size (Costar Corp., United States), at a density of 5×10^4 cells cm^{-2}. The Transwell inserts were placed into six well plates dividing an apical from a basal compartment. MEM (1.5 in apical and 2 mL in basolateral side) was changed every 48 h.

Retention and transport experiments were studied with cells grown on filters at 15−18 days after seeding. At this moment, the culture medium was aspirated from the apical and basolateral chambers, and cell monolayers were washed three times with phosphate-buffered solution (PBS) [NaCl, 140 mmol L^{-1}; KCl, 2.7 mmol L^{-1}; Na_2HPO_4, 6.4 mmol L^{-1}; H_2KPO_4, 1.5 m mol L^{-1}; all reagents from Merck]. Afterward, 1.5 mL of inactivated soluble fraction was added to the apical chamber, and fresh MEM (2 mL) was added to basal side. Cell cultures were incubated for 4 h at 37 °C, 5% CO_2, and 95% relative humidity.

At the end of that period, both apical and basal media of the inserts were recovered by aspiration, and total arsenic was analyzed in order to evaluate transepithelial transport. Both cell surfaces of the monolayers were washed three times with PBS, detached with a trypsin-EDTA solution (2.5 g L^{-1} to 0.2 g L^{-1}), and recovered with 0.5 mL of PBS, and the total arsenic was analyzed in order to evaluate arsenic retention. Arsenic retention and transport percentages were calculated with respect to the initial quantity of As added to the Caco-2 cell cultures. Control cells were used through every assay.

The integrity of the monolayer was monitored by measuring the transepithelial electrical resistance value (TEER) (Millicell electrical resistance system, Millicell-ERS; Millipore Iberia, Madrid, Spain), and only cultures with TEER values > 250 Ω cm^{-2} were used in the assays.

Total Arsenic Determination. Analysis was performed by flow injection−hydride generation−atomic absorption spectrometry (FI-HG-AAS) after a dry ashing step (*11*). Triplicate samples of raw rice and lyophilized cooked rice (0.5 g), bioaccessible fraction (2 g), cell monolayers, and basal media were analyzed. Throughout the experiments, quality assurance/quality control of measurement was checked by analyzing the certified reference material rice flour (SRM1568a; National Institute of Standards and Technology, NIST, Gaithersburg, MD) with each batch of sample.

Inorganic Arsenic Determination. Acid digestion, solvent extraction, and FI-HG-AAS were employed for inorganic arsenic determination (*11*). This methodology also quantified the arsenical species monomethylarsonic acid (MMA), although the resulting overestimation may be considered negligible due to the absence (*12*) or low level of MMA contents (2−15 ng/g) (*13*, *14*) described in rice. Triplicate samples of lyophilized raw and cooked rice (1 g) were analyzed. There were no food reference materials with certified inorganic arsenic content; consequently, the quality criterion adopted was the overlapping between ranges of inorganic arsenic found in rice flour 1568a analyzed

Table 1. Instrumental and Analytical Conditions for HPLC-Thermooxidation-HG-AFS

	HPLC
column	Hamilton PRP-X100
guard column	Hamilton PRP-X100; 12–20 μm; 25 mm × 2.3 mm id
mobile phase	A: 5 mmol l^{-1} [$(NH_4)H_2PO_4$], pH 5.75 B: 100 mmol l^{-1} [$(NH_4)H_2PO_4$], pH 5.75
gradient program	0–4 min: 100% A 4.1–10 min: 50% A and 50% B 10.1–15 min: 100% A
injection volume	100 μL
flow rate	1 mL min^{-1}
temperature	25 °C
	Thermooxidation
oxidant	1.29% (w/v) $K_2S_2O_8$ in 2.5% (w/v) NaOH; 1 mL min^{-1} flow rate
reaction coil	3 m × 0.3 mm id
bath temperature	155 °C
	Hydride Generation-AFS
reducing agent	1.5% (w/v) $NaBH_4$ in 0.7% (w/v) NaOH; 2.5 mL min^{-1} flow rate
HCl solution	1.5 mol L^{-1}; 6 mL min^{-1} flow rate
carrier gas	argon; 300 mL min^{-1} flow rate
dryer gas	air; 2.5 L min^{-1} flow rate
hydrogen flow rate	60 mL min^{-1}
resonance wavelength	193.7 nm
primary current	27.5 mA
boost current	35.0 mA

with each batch of samples and that reported in a previous study (0.110 ± 0.003 μg g^{-1} dw) (*12*, *15*).

Determination of As(III) and As(V) by HPLC-Thermooxidation-HG-AFS. For arsenic speciation, 15 g of soluble fractions was lyophilized, redissolved in 2 mL of deionized water, and filtered (0.45 μm Whatman) prior to HPLC-thermooxidation-HG-AFS quantification. The instrumental and analytical conditions used were described in **Table 1**. Arsenic compounds were identified by matching the retention times of the peaks in the sample chromatograms with those obtained from As(III) and As(V) standards and were quantified using aqueous curves of the corresponding standards.

Statistical Analysis. A one-factor analysis of variance and the Tukey test were applied to determine differences in the uptake and transport assay. A significance level of $p < 0.05$ was adopted for all comparisons. Statgraphics Plus version 4.0 (Statistical Graphics) was used for the statistical analysis (*16*).

RESULTS AND DISCUSSION

Effect of Cooking on Rice Arsenic Contents. Total arsenic and inorganic arsenic in raw and cooked rice were analyzed (**Table 2**). Deionized water and deionized water spiked with 0.5 μg mL^{-1} of As(V) were employed for cooking purposes.

In raw rice, total arsenic ranged from 0.29 to 0.41 μg g^{-1} dry weight (dw), and inorganic arsenic ranged from 0.1 to 0.20 μg g^{-1} dw. The percentage of inorganic arsenic with respect to total arsenic is greater in whole grain (45−67%) than in white grain (26−34%). These contents are included within the inorganic arsenic interval reported by other authors for raw rice (0.02−0.56 μg g^{-1} dw), implying percentages of 11−100% of total arsenic (*4*, *5*, *12*−*14*, *17*).

Cooking of the samples with deionized water produces no important modifications in the total arsenic and inorganic arsenic contents (**Table 2**). However, cooking with contaminated water produces a 5−17-fold increase in the inorganic arsenic content of raw rice. The effect of contamination of the water used for cooking upon the inorganic arsenic of rice, and consequently upon the toxicity of the product, is of great relevance for Asian

Table 2. Effect of Cooking on Rice Arsenic Content: Total Arsenic and Inorganic Arsenic Contents (μg g^{-1}, Dry Weight) in Raw and Cooked Rice (Samples A–D)[a]

samples			total As	inorganic As
		white rice		
A	raw		0.41 ± 0.01	0.14 ± 0.01
	cooked	deionized water	0.44 ± 0.04	0.14 ± 0.01
		water with 0.5 μg mL^{-1} As(V)	2.82 ± 0.04	2.02 ± 0.07
B	raw		0.38 ± 0.04	0.10 ± 0.01
	cooked	deionized water	0.30 ± 0.03	0.13 ± 0.01
		water with 0.5 μg mL^{-1} As(V)	2.54 ± 0.04	1.74 ± 0.06
		whole grain		
C	raw		0.29 ± 0.01	0.13 ± 0.01
	cooked	deionized water	0.28 ± 0.02	0.17 ± 0.01
		water with 0.5 μg mL^{-1} As(V)	2.25 ± 0.14	1.81 ± 0.09
D	raw		0.30 ± 0.02	0.20 ± 0.02
	cooked	deionized water	0.25 ± 0.02	0.14 ± 0.01
		water with 0.5 μg mL^{-1} As(V)	1.41 ± 0.11	1.06 ± 0.03

[a] The results are expressed as means ± standard deviation of three independent replicates.

arsenic endemic areas. At present, the presence of As in raw rice is considered a new disaster for Southeast Asia (*6*), with documented levels of up to 1.83 μg g^{-1} in raw rice in western Bangladesh (*18*). Contamination is expected to worsen with cooking, since the As contents in drinking and cooking water are often alarming (up to 3.7 μg mL^{-1}) (*2*) and considerably exceed the maximum values allowed by the World Health Organization (WHO) (10 μg l^{-1}) (*19*). This underscores the importance of assessing the toxic agent (inorganic arsenic) in the product in the form in which it is actually consumed by the population.

Inorganic Arsenic Bioavailability and Estimation of the Potential Toxicological Risk. To carry out this study, the water used for cooking the rice was spiked with concentrations of As(V), which include the concentration range reported in drinking water in Asian arsenic endemic areas. **Table 3** shows the As(V) contents in water employed for cooking, total arsenic in raw rice, total arsenic and inorganic arsenic in cooked rice, and total arsenic, As(III), and As(V) in the bioaccessible fraction of cooked rice.

The contents of total arsenic in raw rice (0.05−0.53 μg g^{-1} dw) increase considerably after cooking (0.88−4.21 μg g^{-1} dw), as a consequence of the presence of As(V) in the cooking water. The increase in total arsenic in foods as a result of the cooking water used has been described in arsenic endemic areas (*15*, *20*−*22*). The total arsenic content of the bioaccessible fraction (1.06−3.93 μg g^{-1} dw) evidences the high bioaccessibility of arsenic from cooked rice (>90%).

The inorganic arsenic content after cooking (0.81−3.73 μg g^{-1} dw) represents over 80% of total arsenic. Speciation analysis of inorganic arsenic in the bioaccessible fraction has shown that As(V) is the main chemical form found in all samples—as would be expected, since it is the species added to cooking water. A variable relationship between As(V) and As(III) was found (2.8−19.2). Bioaccessible inorganic arsenic [estimated as As(III) + As(V)] varies from 0.8 to 3.1 μg g^{-1} dw, which implies that a big proportion of inorganic arsenic present in the samples (63−100%) could be available for intestinal absorption.

One aspect that must be pointed out is that although bioaccessibility can be related to maximum oral bioavailability,

Table 3. Bioaccessibility: Total Arsenic Contents in Raw Rice, Total As and Inorganic Arsenic Contents in Cooked Rice, and Total As, As(III), and As(V) in the Bioaccessible Fraction of Cooked Rice.[a] All Results Expressed as μg g^{-1}, Dry Weight.

		raw	cooked		bioaccessible cooked rice		
sample[b]	As(V) in water[c] (μg mL^{-1})	total As sample	total As sample	inorganic As sample	total As bioaccessible	As(III) bioaccessible	As(V) bioaccessible
E	0.4	0.53 ± 0.003	1.96 ± 0.01	1.66 ± 0.002	1.78 ± 0.04	0.221 ± 0.017	1.046 ± 0.055
F	0.6	0.05 ± 0.001	2.28 ± 0.11	2.36 ± 0.08	2.37 ± 0.15	0.074 ± 0.009	1.422 ± 0.334
G	0.6	0.13 ± 0.008	2.29 ± 0.05	1.87 ± 0.10	2.36 ± 0.20	0.122 ± 0.012	1.733 ± 0.103
H	0.7	0.25 ± 0.008	3.05 ± 0.03	3.13 ± 0.17	3.09 ± 0.08	0.330 ± 0.064	1.877 ± 0.094
I	0.9	0.13 ± 0.001	3.66 ± 0.77	3.34 ± 0.08	3.93 ± 0.07	ND	2.514 ± 0.036
J	1.0	0.09 ± 0.002	4.21 ± 0.09	3.73 ± 0.04	3.83 ± 0.04	0.221 ± 0.027	2.911 ± 0.202
K	0.2	0.25 ± 0.02	0.88 ± 0.03	0.81 ± 0.007	1.06 ± 0.04	0.222 ± 0.004	0.622 ± 0.010
L	0.4	0.17 ± 0.004	1.51 ± 0.15	1.49 ± 0.02	1.26 ± 0.15	0.196 ± 0.005	1.18 ± 0.02

[a] The results are expressed as means ± standard deviation of three independent replicates; ND, not detected. [b] Sample type: E, red wholegrain; F, basmati white; G, I, K, round white; H, large white; J, bomba white; and L, large Thai. [c] As(V) contents in water used for cooking.

Table 4. Bioavailability: Total Arsenic Retention, Transport, and Total Uptake by Caco-2 Cells from Cooked Rice[a]

	total As soluble	cell monolayer	basal medium	uptake (%)[d]		total uptake
sample[b]	added (ng)[c]	(ng) (retention)	(ng) (transport)	retention	transport	(%)[e]
F	97.0 ± 6.9 a	1.9 ± 0.6 a,b	6.2 ± 0.8 a	2.0 ± 0.7 a	6.3 ± 0.8 a,c	8.3 ± 1.3 a
G	98.4 ± 7.4 a	6.3 ± 0.9 d	11.2 ± 0.9 b	6.4 ± 0.9 b	11.4 ± 0.9 b	17.8 ± 1.1 b
E	133.7 ± 3.0 b	1.0 ± 0.04 a	12.0 ± 1.4 b	0.7 ± 0.03 c	8.9 ± 1.1 b,c	9.7 ± 1.1 a
H	128.3 ± 3.2 b	2.4 ± 0.3 b,c	11.3 ± 2.4 b,c	1.9 ± 0.2 a	8.4 ± 1.9 b,c	10.7 ± 2.0 a
I	153.8 ± 2.4 c	3.3 ± 0.7 c	5.3 ± 2.5 a	2.2 ± 0.4 a	3.5 ± 1.6 d	5.6 ± 1.4 c
J	159.3 ± 2.1 c	0.9 ± 0.4 a	5.3 ± 1.4 a	0.6 ± 0.2 c	3.3 ± 0.9 d	3.9 ± 0.5 c

[a] The results are expressed as means ± standard deviation of three independent replicates. Different superscript letters indicate significant ($p < 0.05$) differences in the same column. Moisture: sample E, 51.6%; sample F, 73.4%; sample G, 71.9%; sample H, 72.3%; sample I, 74.2%; and sample J, 72.1%. Values required in order to calculate, from the total arsenic uptake values, the consumption of cooked rice needed to reach the tolerable daily intake established by the FAO/WHO. [b] Sample type: E, red wholegrain; F, basmati white; G, I, round white; H, large white; and J, bomba white. [c] Total As content in the aliquot (1.5 mL) of bioaccessible fraction added to cell cultures. [d] Percentages of retention or transport calculated with respect to the amount added. [e] Total cellular uptake evaluated as [(retention + transport)/total arsenic content added to cell culture] × 100.

it is recognized that the subsequent processes of absorption in the intestinal epithelia could reduce the fraction of inorganic arsenic that reaches the bloodstream. An improvement in this in vitro system has been achieved by incorporating Caco-2 cell cultures, which allow the evaluation of intestinal cell retention and transport processes. Total arsenic in bioaccessible fraction added to Caco-2 cells, in cell monolayer (retention), basal medium (transport), and percentages of total uptake (retention + transport) are shown in **Table 4**.

In the cell monolayer, the total arsenic content varies from 0.9 to 6.3 ng. It must be pointed out that the low As content in cell monolayers and basal medium does not allow arsenic speciation with HPLC-FI-HG-AFS; as a result, only total arsenic quantification is possible. When considering the transport, the total arsenic contents in basal media vary between 5.3 and 12.0 ng. Similar arsenic additions to culture (samples E−H and I−J) imply amounts of transported arsenic (ng) of the same order (see basal medium)—with the exception of samples F and G.

On expressing retention, transport, and total uptake as percentages calculated with respect to the amount added, it is seen that arsenic retention (0.6−6.4%), transport (3.3−11.4%), and total cellular uptake (3.9−17.8%) cover a wide range, possibly due to the different nature of the rice analyzed. The lack of statistical correlation between the soluble total arsenic added to the Caco-2 cells and the total uptake observed indicates that although arsenic may be bioaccessible after gastrointestinal digestion, other soluble components of the rice may cause differences in the extent of absorption.

To date, the assessment of human health risk due to exposure to inorganic arsenic from food typically has been established based on the initial inorganic arsenic content. However, considering total uptake (%) as a measure of As relative bioavailability affords a better estimation of risk associated with the consumption of contaminated rice. Taking the lowest (3.9%) and highest (17.8%) total arsenic uptake values obtained in the rice analyzed (see **Table 4**), the consumption of 5.7 and 1.2 kg of cooked rice/day, respectively, would be required to reach the tolerable daily intake (TDI) established by the FAO/WHO for inorganic arsenic (150 μg inorganic arsenic/day for a person weighing 70 kg) (*23*). In Asian arsenic endemic areas where the population depends heavily on rice for caloric intake, an average adult male consumption of 1.5 kg cooked rice/day has been reported (*7*). Consequently, the population might reach the TDI with a single food.

This paper reinforces the concern about the impact that As-contaminated rice consumption may have for the health of the population in Asian arsenic endemic areas. Cooking and bioavailability of inorganic arsenic are aspects that must be considered in order to obtain a more reliable estimate of potential human health risks associated with arsenic in foods.

LITERATURE CITED

(1) IARC (International Agency for Cancer Research). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs*; International Agency for Cancer Research: Lyon, 1987; Vol. 1−42, Suppl. 7.

(2) Das, D.; Chatterjee, A.; Mandal, B. K.; Samanta, G.; Chakraborti, D.; Chanda, B. Arsenic in groundwater in six districts of West Bengal, India: The biggest arsenic calamity in the world. Part II: Arsenic concentration in drinking water, hair, nail, urine, skin-scale an liver tissue (Biopsy) of the affected people. *Analyst* **1995**, *120*, 917−924.

(3) Rossman, T. G. Mechanism of arsenic carcinogenesis: an integrated approach. *Mutat. Res.* **2003**, *533*, 37−65.
(4) Schoof, R. A.; Yost, L. J.; Crecelius, E. A.; Irgolic, K.; Goessler, W.; Guo, H. R.; Greene, H. Dietary arsenic intake in Taiwanese district with elevated arsenic in drinking water. *Hum. Ecol. Risk Assess.* **1998**, *4*, 117−135.
(5) Schoof, R. A.; Yost, L. J.; Eickhoff, J.; Crecelius, E. A.; Cragin, D. W.; Meacher, D. M.; Menzel, D. B. A market basket survey of inorganic arsenic in food. *Food Chem. Toxicol.* **1999**, *37*, 839−846.
(6) Meharg, A. A. Arsenic in rice—Understanding a new disaster for South-East Asia. *Trends Plant Sci.* **2004**, *9*, 415−417.
(7) Bae, M.; Watanabe, C.; Inaoka, T.; Sekiyama, M.; Sudo, N.; Bokul, M. H.; Ohtsuka, R. Arsenic in cooked rice in Bangladesh. *Lancet* **2002**, *360*, 1839−1840.
(8) Ellickson, K. M.; Meeker, R. J.; Gallo, M. A.; Buckley, B. T.; Lioy, P. J. Oral bioavailability of lead and arsenic from a NIST standard reference soil material. *Arch. Environ. Contam. Toxicol.* **2001**, *40*, 128−135.
(9) van Campen, D. R.; Glahn, R. P. Micronutrient bioavailability techniques: Accuracy, problems and limitations. *Field Crops Res.* **1999**, *60*, 93−113.
(10) Ekmekcioglu, C. A physiological approach for preparing and conducting intestinal bioavailability studies using experimental systems. *Food Chem.* **2002**, *76*, 225−230.
(11) Laparra, J. M.; Vélez, D.; Montoro, R.; Barberá, R.; Farré, R. Estimation of arsenic bioaccessibility in edible seaweed by an in vitro digestion method. *J. Agric. Food Chem.* **2003**, *51*, 6080−6085.
(12) Heitkemper, D. T.; Vela, N. P.; Stewart, K. R.; Westphal, C. S. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **2001**, *16*, 299−306.
(13) D'Amato, M.; Forte, G.; Caroli, S. Identification and quantification of major species of arsenic in rice. *J. AOAC Int.* **2004**, *87*, 238−243.
(14) Sanz, E.; Muñoz-Olivas, R.; Cámara, C. A rapid and novel alternative to conventional sample treatment for arsenic speciation in rice using enzymatic ultrasonic probe. *Anal. Chim. Acta* **2005**, *535*, 227−235.
(15) Díaz, O. P.; Leyton, I.; Muñoz, O.; Núñez, N.; Devesa, V.; Súñer, M. A.; Vélez, D.; Montoro, R. Contribution of water, bread and vegetables (raw and cooked) to dietary intake of inorganic arsenic in a rural village of Northern Chile. *J. Agric. Food Chem.* **2004**, *52*, 1773−1779.
(16) Box, G. E. P.; Hunter, N. G.; Hunter, J. S. In *Statistics for Experimenters. An Introduction to Design, Data Analysis and Model Building*; John Wiley & Sons: New York, 1978.
(17) Lamont, W. H. Concentration of inorganic arsenic in samples of white rice from the United Sattes. *J. Food Compos. Anal.* **2003**, *16*, 687−695.
(18) Meharg, A. A.; Rahman, M. Arsenic contamination of Bangladesh paddy field soils: Implications for rice contribution to arsenic consumption. *Environ. Sci. Technol.* **2003**, *37*, 229−324.
(19) WHO. In *Guidelines for Drinking-Water Quality, Vol. 1. Recommendations*; WHO: Geneva, 1993.
(20) Vahter, M.; Concha, G.; Nermell, B.; Nilsson, R.; Dulout, F.; Natarajan, A. T. A unique metabolism of inorganic arsenic in native Andean women. *Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. Sect.* **1995**, *293*, 455−462.
(21) Del Razo, L. M.; Garcia-Vargas, G. G.; Garcia-Salcedo, J.; Sanmiguel, M. F.; Rivera, M.; Hernandez, M. C.; Cebrian, M. E. Arsenic levels in cooked food and assessment of adult dietary intake of arsenic in the Region Lagunera, Mexico. *Food Chem. Toxicol.* **2002**, *40*, 1423−1431.
(22) Roychowdhury, T.; Uchino, T.; Tokunaga, H.; Ando, M. Survey of arsenic in food composites from an arsenic-affected area of West Bengal, India. *Food Chem. Toxicol.* **2002**, *40*, 1611−1621.
(23) WHO. *Evaluation of Certain Food Additives and Contaminants, 33rd Report of the Joint FAO/WHO Expert Committee on Food Additives*; Technical Report Series 776; WHO: Geneva, 1989.

Received for review June 9, 2005. Revised manuscript received August 25, 2005. Accepted August 26, 2005.

JF051365B