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Bioaccessibility and Transport by Caco-2 Cells of Organoarsenical Species Present in Seafood

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Organoarsenical standards and raw and cooked seafood (DORM-2, sole, and Greenland halibut) were subjected to in vitro gastrointestinal digestion to estimate arsenic bioaccessibility (maximum soluble concentration in gastrointestinal medium). The in vitro digestion did not modify the chemical form of the organoarsenic species standards. In seafood, bioaccessibility was 67.5−100% for arsenobetaine (AB), 30% for dimethylarsinic acid (DMA), 45% for tetramethylarsonium ion (TETRA), and >50% for trimethylarsine oxide (TMAO). Cooking induced no changes in bioaccessible contents. In addition, transport by Caco-2 cells, an intestinal epithelia model, was evaluated from organoarsenical standards and DORM-2. For standards, transport ranged from 1.7% for AB to 15.5% for TETRA. In DORM-2, transport was observed for only AB (12%), with far higher efficiency than in the case of the standard solution, thus illustrating the interest of using whole foods for studying bioavailability.

KEYWORDS: Bioaccessibility; Caco-2 cells; arsenic; seafood; cooking

INTRODUCTION

In the Western world, fish and fish products are the principal dietary sources of arsenic (*1*, *2*). Arsenic is present in seafood, forming part of a large number of organic molecular species (*3*, *4*)—some of which are considered to be innocuous [arsenobetaine (AB), arsenocholine (AC), trimethylarsine oxide (TMAO) and arsenosugars], whereas others are toxic [dimethylarsinic acid (DMA), monomethylarsonic acid (MA), and tetramethylarsonium ion (TETRA)] (*5*). Inorganic arsenic species, As(III) and As(V) (*6*), have also been detected and are regarded as carcinogens for humans (*7*). Because arsenic species differ widely in their degree of toxicity, speciation analysis is required to afford a more reliable assessment of the health risk associated with the consumption of seafood products.

Generally, seafood is consumed after cooking. During cooking, one should not rule out the possibility that the high temperatures reached may bring about some transformation of the arsenic species present in the initial product. In this aspect, previous works have shown that AB, the main organoarsenical species present in most forms of seafood, is transformed during cooking into TETRA, thus modifying the toxicological risk associated with consumption (*8*, *9*).

After seafood ingestion, gastrointestinal digestion constitutes a first metabolic step in which the organoarsenical species can be metabolized, thus influencing the absorption of arsenic. On the basis of in vitro methods that simulate human gastrointestinal digestion, the evaluation of bioaccessibility (i.e., the maximum soluble concentration of arsenic in simulated gastrointestinal medium) provides an estimation of the maximum fraction of the individual species theoretically available for intestinal absorption (*10*), and this could be a first approach for evaluating the fraction that can reach the blood stream. To date, the only existing data related to arsenic bioaccessibility in foods have been obtained by our group for inorganic arsenic and arsenosugars from seaweeds and rice (*11*−*14*).

It is generally recognized that not all soluble elements are absorbable; consequently, solubility alone is not an adequate marker of bioavailability (*15*). An improvement in the in vitro research of these phenomena has been the incorporation of Caco-2 cell cultures, which facilitate the evaluation of intestinal cell retention and transport processes and offer a more reliable approximation to the in vivo situation in estimating bioavailability at intestinal level (*15*, *16*). The Caco-2 cell line has been widely used for the estimation of mineral bioavailability from foods. In the case of arsenic, an approach to the study of As(III) and As(V) bioavailability with Caco-2 cells from standard solutions has been developed by our group (*17*).

The lack of data on the availability of organoarsenical species in seafood, and the possible modification in risk estimation that may result from taking into account the effect of gastrointestinal digestion, led us to design the present study of the bioaccessibility and transport by Caco-2 cells of organoarsenical species present in seafood and the influence of cooking upon this process.

MATERIALS AND METHODS

Instruments. For arsenic speciation analysis, HPLC−thermooxidation−hydride generation−atomic fluorescence spectrometry was

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employed. The HPLC system (Hewlett-Packard model 1100, Barcelona, Spain) was equipped with a quaternary pump, an on-line degassing system, an automatic injector, and a thermostated column compartment. A switching column valve (Rheodyne six-port automated) was used between two columns: a Hamilton PRP-X200 (cation-exchange column, 10 μm, 250 mm $\times$ 4.1 mm i.d., Teknokroma, Spain) and a Hamilton PRP-X100 (anion-exchange column, 10 μm, 250 mm $\times$ 4.1 mm i.d., Teknokroma). A guard column packed with the same stationary phase (12−20 μm; 25 mm $\times$ 2.3 mm i.d.) preceded the analytical PRP-X200 column. For the thermooxidation step a Julabo model HC heated bath (Merck, Barcelona, Spain) was used. The quantification of arsenic species was performed on a hydride generation (HG) system (PSA 10.004, Analytical, U.K.) coupled to an atomic fluorescence spectrophotometer (AFS) (PSA 10.044 Excalibur PS, Analytical), equipped with a boosted-discharge hollow cathode lamp (BDHCL, Photron, Super Lamp, Victoria, Australia). A Hewlett-Packard model 35900 C digital analogical converter was used to acquire the AFS signal, which was processed by the chromatographic software.

For total arsenic quantification, an atomic absorption spectrometer (AAS) (Perkin-Elmer 3300, Madrid, Spain) equipped with an autosampler (AS-90, Perkin-Elmer) and a flow injection hydride generation (FI-HG) system (FIAS-400, Perkin-Elmer) was employed. An electrothermally heated quartz cell was used.

Other equipment used included a lyophilizer (FTS Systems, New York), a sand bath (PL 5125, Raypa Scharlau S.L., Barcelona, Spain), a muffle furnace (K1253, Heraeus S.A., Madrid, Spain), a mechanical shaker (KS 125, IKA Labortechnik, Merck, Barcelona, Spain), and centrifuges (Eppendorf model 5810, Merck; Heraeus Biofuge Pico centrifuge, Merck; Sorvall RC-50B, Sorvall Instrument, DuPont, France).

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 glassware was treated with 10% (v/v) HNO_3 for 24 h and then rinsed three times with distilled water before being used.

The stock standard solutions of MA [$CH_3AsO(ONa)_2 \cdot 6H_2O$, Carlo Erba, Milan, Italy] and DMA [$(CH_3)_2AsNaO_2 \cdot 3H_2O$, Fluka Chemika Biochemika, Madrid, Spain] were prepared in deionized water. Similarly, standards supplied by Hot Chemical Co. (Tokyo, Japan) were used to prepare stock standard solutions of AB [$(CH_3)_3As^+CH_2COO^-$], AC [$(CH_3)_3AsCH_2CH_2OH^+Br^-$], TMAO [$(CH_3)_3AsO$], and TETRA [$(CH_3)_4AsI$].

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.

Samples. Samples of two fresh fish, sole (*Solea solea*) and Greenland halibut (*Reinhardtius hippoglossoides*), were purchased from local markets in Valencia (Spain). Once the inedible parts (head, tail, and digestive tract) had been removed, the same specimen was analyzed raw and after one cooking treatment, applied without using fat or other additional ingredients. The size of the specimens of seafood made it possible to use the same piece for the determination of the species of arsenic in the raw product and after cooking treatment. Sole was baked in a pan during 5 min, and Greenland halibut was cooked in a microwave oven during 10 min at 700 W. Raw and cooked samples were minced and blended to give a homogeneous sample. This was frozen at −20 °C, lyophilized, ground, and kept at 4 °C until analysis.

Certified reference material DORM-2 (dogfish muscle), obtained from the National Research Council of Canada (CNRC), was also analyzed.

In Vitro Gastrointestinal Digestion. Arsenical standards, samples of raw and cooked seafood (5 g), and samples of DORM-2 (5 g) were digested using a simulated digestion process developed in an earlier study (*11*). Samples were weighed, and cellular grade water (90 mL) was added. The pH was adjusted to 2.0 with 6 mol L^{-1} HCl. After 15 min, the pH value was checked and if necessary readjusted to pH 2.0. Then freshly prepared pepsin solution (1 g of pepsin in 10 mL of 0.1 mol L^{-1} HCl) was added to provide 2 mg of pepsin/g of sample. The sample was made up to 100 g with water and incubated in a shaking water bath (120 strokes min^{-1}) at 37 °C for 2 h. Afterward, the pH value was raised to pH 5.0 by dropwise addition of 1 mol L^{-1} $NaHCO_3$. Then the pancreatin−bile extract mixture (0.2 mg of pancreatin and 1.25 g of bile extract in 50 mL of 0.1 mol L^{-1} $NaHCO_3$) was added to provide 0.5 mg of pancreatin/g of sample and 3 mg of bile extract/g of sample, and the incubation at 37 °C was continued for 2 h. The pH was then adjusted to 7.2 by dropwise addition of 0.5 mol L^{-1} NaOH. Aliquots of 40 g of the complete digests were transferred to polypropylene centrifuge tubes and centrifuged (15000 rpm/30 min/4 °C) to obtain the bioaccessible fraction, in which total arsenic and organoarsenical species were analyzed.

For the arsenic uptake and transport assays with Caco-2 cells, the gastrointestinal digests obtained from DORM-2 were heated (immersion in a water bath for 4 min, 100 °C) to inhibit enzymes used for digestion and then cooled by immersion in an ice bath. Aliquots of the inactivated digests were centrifuged to obtain the bioaccessible fraction. Glucose (5 mM final concentration) and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; BioWhittaker; 50 mM final concentration) were added to the bioaccessible fraction to make it similar to the culture medium and facilitate cell viability, whereas water or NaCl (Merck, Barcelona, Spain) was added to adjust the osmolarity to 310 ± 10 mOsm/kg (freezing point osmometer, Osmomat 030, Berlin, Germany) (*16*).

Cell Culture. The Caco-2 cell line was obtained from the European Collection of Cell Cultures (ECACC 86010202, Salisbury, U.K.). Cultures were maintained and grown as previously described (*13*).

Arsenic Retention and Transport by Caco-2 Cells. This study was undertaken using organoarsenical standard aqueous solutions and DORM-2 bioaccessible fraction. The procedure used has been described elsewhere (*13*). For these assays, Caco-2 cells were seeded onto polycarbonate membrane inserts (Transwell 24 mm diameter and 0.4 μm pore size; Costar Corp.) at a density of 5×10^4 cells cm^{-2}, with 2 mL of minimum essential medium (MEM) in the basal chamber and 1.5 mL of resuspended cells in the apical chamber. The culture medium was changed every 2 days. The Transwell filters were placed into six-well plates dividing an apical (top compartment of insert) from a basolateral compartment. Fifteen to eighteen days after initial seeding, spent culture medium was aspirated from the apical and basolateral chambers, and both cell surfaces of the monolayers were washed three times with phosphate-buffered solution (PBS) [NaCl (140 mM), KCl (2.7 mM), Na_2HPO_4 (6.4 mM), H_2KPO_4 (1.5 mM), all reagents from Merck] at 37 °C. Then 1.5 mL portiond of different concentrations of organoarsenical species diluted in MEM or 1.5 mL of inactivated soluble fraction of DORM-2 were added to the apical chamber, and 2 mL of MEM was added to the basolateral compartment. These cell cultures were incubated at 37 °C, 5% CO_2, and 95% relative humidity for 4 h. After the incubation time, the basal media were recovered by aspiration from the bottom compartment of the inserts, and the organoarsenical species contents were analyzed to evaluate transepithelial transport. Monolayers were detached with a trypsin (2.5 g L^{-1})−EDTA (0.2 g L^{-1}) solution, recovered with 0.5 mL of PBS, and analyzed to evaluate arsenic retention. Arsenic retention (cell monolayer) and transport (basal medium) percentages were calculated with respect to the initial amount of organoarsenical species added within standard solutions or DORM-2 soluble fraction to the Caco-2 cell cultures. Unspiked cells were used throughout each assay.

Total Arsenic Determination. Analysis of raw or cooked seafood, bioaccessible fractions, and cells monolayers was performed by FI-HG-AAS after a dry ashing step (*11*). Throughout the experiment, the quality assurance−quality control of total arsenic measurement was checked by analyzing the certified reference material DORM-2 with each batch of samples.

Organoarsenical Species Determination by HPLC−Thermooxidation−HG-AFS. This analysis was carried out in the fish samples, in the bioaccessible fractions obtained after in vitro digestion, and in the basal medium resulting from the study with Caco-2 cells.

Lyophilized raw and cooked samples (1 g) were extracted three times with 20 mL of a mixture of methanol/water (1+1 v/v). The extracts were combined, evaporated to dryness, dissolved in 2 mL of water, centrifuged, and the supernatant was filtered (Whatman 0.45 μm nylon membrane filter) prior to HPLC injection. Soluble fractions (15

Table 1. Experimental Conditions Employed for the Determination of Organoarsenical Species by HPLC–Thermooxidation–HG-AFS

	HPLC
cationic column	Hamilton PRP-X200; 10 μm polymer base cationic exchange; 250 mm × 4.1 mm i.d.
anionic column	Hamilton PRP-X100; 10 μm polymer base anionic exchange; 250 mm × 4.1 mm i.d.
guard column	Hamilton PRP-X200; 12–20 μm; 25 mm × 2.3 mm i.d.
mobile phase	cationic column: A: 100 mmol L^{-1} [$(NH_4)H_2PO_4$], pH 4.5 B: deionized water gradient program: 0–4 min, 10% A and 90% B 4–11 min, 40% A and 60% B 11–15 min, 10% A and 90% B anionic column: C, 1 mmol L^{-1} [$(NH_4)H_2PO_4$], pH 9.3 D, 20 mmol L^{-1} [$(NH_4)H_2PO_4$], pH 9.3 gradient program: 0–5 min, 100% C 5.5–11 min, 100% D 12–15 min, 100% C
column switching valve	position 1, columns were not directly connected position 2, direct connection between the two columns t = 0–1 min; position 1 t = 1–4 min; position 2 t = 4–20 min; position 1
injection volume	100 μL; injection in PRP-X200
flow rate	1 mL min^{-1}
temperature	10 °C
	Thermooxidation
oxidant	1.29% (m/v) $K_2S_2O_8$ in 2.5% (m/v) NaOH; 1 mL min^{-1} flow rate
reaction coil	3 m × 0.3 mm i.d.
bath temperature	155 °C
	HG-AFS
reducing agent	1.5% (m/v) $NaBH_4$ in 0.7% (m/v) NaOH; 2.5 mL min^{-1} flow rate
HCl solution	1.5 mol L^{-1}, 6.0 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

g) or basal media (2 mL) were lyophilized, redissolved in 2 or 1 mL, respectively, of deionized water, and filtered (0.45 μm Whatmann) prior to HPLC injection.

Samples (100 μL) were injected into the HPLC system, and organoarsenical species were separated using a column switching system between the PRP-X200 cationic exchange column and the PRP-X100 anionic exchange column. The samples was injected onto the PRP-X200 column, and by means of the valve switching program described by Suñer et al. (*18*), it proved to be possible to separate DMA, MA, AB, and As(V) in the PRP-X100 column and TMAO, TETRA, and AC in the PRP-X200 column. In each injection we quantified the eluate from a single chromatographic column, as a result of which two injections of the sample were required to quantify all of the organoarsenical species. The eluate was thermooxidized on-line, cooled in an ice bath, and quantified by HG-AFS using the analytical conditions described in **Table 1**. Arsenic compounds were identified by matching the retention times of the peaks in the sample chromatograms with those obtained from standards. The species were quantified using the calibration curves of the corresponding standards. The limits of detection were as follows: AB, 3.6 ng g^{-1} of dry weight (dw); MA, 3.3 ng g^{-1} of dw; TETRA, 2.7 ng g^{-1} of dw; AC, 2.4 ng g^{-1} of dw; TMAO, 0.9 ng g^{-1} of dw; DMA, 0.9 ng g^{-1} of dw (*18*).

RESULTS AND DISCUSSION

Total Arsenic and Organoarsenical Species in Seafood. The total arsenic and organoarsenical species found in DORM-2 and in raw and cooked fish samples are shown in **Table 2**. In DORM-2, total arsenic content (17.5 ± 0.8 μg g^{-1} of dw) was in good agreement with the certificate value (18.0 ± 1.1 μg g^{-1} of dw). AB, DMA, MA, TMAO, and TETRA were detected. The contents of AB (16.3 ± 0.5 μg g^{-1} of dw) and TETRA (0.26 ± 0.01 μg g^{-1} of dw) were in agreement with the certificate values (AB, 16.4 ± 1.1 μg g^{-1} of dw; TETRA, 0.248 ± 0.054 μg g^{-1} of dw). For the rest of the arsenical species the recorded contents were similar to those described by other authors (*4*, *19*, *20*). The existence of dimethylarsinoylacetic acid, trimethylarsoniopropionate, and two unknown compounds detected in DORM-2 by Sloth et al. (*20*) was not observed in our analyses.

In raw sole, the total arsenic quantified (24.8 μg g^{-1} of dw) was included within the broad range (5−60 μg g^{-1} of wet weight) reported for different species of sole (Lemmon sole, Common sole, and Sand sole) (*21*). The efficiency of extraction, calculated as the sum of arsenical species content with respect to the total arsenic content, was high (89%). AB was the main species, representing 87% of the total arsenic. MA and TMAO were also detected. The high MA content (0.103 μg g^{-1} of dw) exceeded that reported by Suñer et al. (*4*) in a large number of seafood products (0.004−0.047 μg g^{-1} of dw). After cooking, no variations in total arsenic content were observed, although changes were noted in the contents of MA, TETRA, and AC. In effect, the MA content doubled—a result not previously reported in the literature. This observation is toxicologically relevant, because MA in the course of its metabolism generates DMA(III)—a genotoxic species according to the studies made in different cell lines (*22*). TETRA and AC, which were not detected in the raw product, reached similar concentrations after cooking. The generation of TETRA could have been a consequence of the thermal degradation of AB, as evidenced by Devesa et al. (*8*) in sole after frying, grilling, and baking (90−130 °C)—there being no antecedents regarding the generation of AC from other arsenical species. The increase in concentration of MA, TETRA, and AC could also have been a consequence of more efficient methanol/water extraction at room temperature in application to the cooked product.

In raw Greenland halibut, total arsenic was lower than in sole (4.8 μg g^{-1} of dw). AB constituted 63% of the total arsenic, and the sum of the other species detected (MA, TMAO, and TETRA) represented only 4% of the total arsenic. After cooking, the total arsenic content showed no important modifications, and no salient variations in the organic species were observed.

Bioaccessibility after in Vitro Digestion. Aqueous solutions of AB, DMA, MA, TMAO, TETRA, and AC standards were subjected to in vitro gastrointestinal digestion (**Table 3**). The contents in bioaccessible fractions were between 88% for TETRA and 114% for DMA of the initially added amount of each species. No unknown species were detected, thus evidencing that no degradation of these species occurred due to the simulated gastrointestinal conditions.

The in vitro gastrointestinal digestion was also applied to DORM-2, sole, and Greenland halibut. The total arsenic and organoarsenical bioaccessible contents (μg g^{-1} of dry weight,

Table 2. Organoarsenical Species, Total Arsenic Contents, and Extraction Efficiency in DORM-2, Sole, and Greenland Halibut (Micrograms per Gram of Dry Weight, as Arsenic)[a]

sample		AB	DMA	MA	TMAO	TETRA	AC	sum of species	total As	extraction efficiency (%)
DORM-2[b]		16.3 ± 0.5	0.31 ± 0.06	0.02 ± 0.003	nd[c]	0.26 ± 0.01	nd	16.7 ± 0.5	17.5 ± 0.8	95
Sole	raw	21.6 ± 2.0	nd	0.103 ± 0.015	0.367 ± 0.043	nd	nd	22.1 ± 2.0	24.8 ± 1.4	89
	cooked	18.6 ± 1.0	nd	0.217 ± 0.007	0.361 ± 0.022	0.13 ± 0.002	0.12 ± 0.01	19.4 ± 1.0	23.0 ± 1.6	84
Greenland halibut	raw	3.0 ± 0.2	nd	0.057 ± 0.001	0.098 ± 0.006	0.038 ± 0.003	nd	3.2 ± 0.2	4.80 ± 0.04	67
	cooked	2.5 ± 0.02	nd	0.048 ± 0.001	0.088 ± 0.003	0.031 ± 0.004	nd	2.7 ± 0.02	4.40 ± 0.07	77

[a] Results expressed as the mean ± standard deviation of three independent replicates. [b] Certified values for DORM-2 (μg g^{-1} of dry weight): total As, 18.0 ± 1.1; AB, 16.4 ± 1.1; TETRA, 0.248 ± 0.054. [c] Not detected.

Table 3. Bioaccessible Contents of Organoarsenical Species after in Vitro Gastrointestinal Digestion of Standard Solutions[a]

species	initial content (μg mL^{-1}, as arsenic)	bioaccessible content (μg mL^{-1}, as arsenic)
AB	19.0 ± 0.1	18.7 ± 0.5
DMA	16.6 ± 1.3	19.0 ± 0.9
MA	14.9 ± 0.5	15.7 ± 0.7
TMAO	16.5 ± 0.2	14.8 ± 1.2
TETRA	15.1 ± 0.2	13.3 ± 0.3
AC	14.9 ± 1.2	13.9 ± 0.3

[a] Results expressed as the mean ± standard deviation of three independent replicates.

as arsenic) are shown in **Table 4**. The bioaccessibility of these contents, for raw and cooked seafood, was defined as the proportion of total or organic arsenic species in seafood available for absorption, and was calculated as

$$\text{bioaccessibility} = \frac{\text{AsT or organic As in bioaccessible fraction}}{\text{AsT or organic As in seafood}} \times 100$$

For the raw samples, total arsenic bioaccessibility was 97.7% for DORM-2, 98.3% for sole, and 79.2% for Greenland halibut. The cooking process did not affect the bioaccessibility of total arsenic in sole (98.3 vs 102.1%). However, in Greenland halibut an increase in total arsenic bioaccessibility (79.2 vs 100%) was observed. The high total arsenic bioaccessibility in raw and cooked samples evidenced the capacity of the digestive enzymes used in the simulated gastrointestinal process to release the arsenic from the food matrix.

With regard to the bioaccessibility of the arsenical species, in DORM-2 the species detected in the bioaccessible fraction (**Table 4**) coincided with those quantified in the original sample (**Table 2**). Of note is the fact that for AB, DMA, and TETRA, the bioaccessible contents were lower than in the raw sample. However, for MA, the bioaccessible content was 8 times higher (0.16 μg g^{-1} of dw) than that quantified in the methanol/water extract (0.02 μg g^{-1} of dw). The study of the effect of gastrointestinal digestion in arsenic standards (**Table 3**) indicated no generation of MA from another species of greater methylation grade. With the purpose of examining whether sample behavior differs from that seen in the standards, DORM-2 was also digested after being spiked with an AB content (16.3 ± 0.5 μg g^{-1} of dw) similar to the certified one. The results indicate that after the addition of AB, no increase is seen in the solubilized MA content, and the recovery rate for added AB was 96.4 ± 7.0%. This finding seems to indicate that the appearance of the high concentration of MA in the bioaccessible fraction was not due to AB degradation under the simulated gastrointestinal conditions. This could indicate that the in vitro digestion process releases a greater amount of MA from the sample than methanol/water extraction at room temperature.

In raw and cooked sole, only AB and MA were detected in the bioaccessible fraction (**Table 4**). TMAO and TETRA quantified in the sample (**Table 2**) were not detected after in vitro digestion. The bioaccessibility of AB was very high (raw 107%; cooked 115%). The MA content of the bioaccessible fraction exceeded that of the original sample and was 6 times greater for raw sole and 2 times greater for cooked sole.

In Greenland halibut (raw and cooked), only AB and TMAO were detected, the bioaccessibility being 100% for AB and >50% for TMAO. The species MA and TETRA detected in the raw or cooked samples (**Table 2**) were not detected after the in vitro digestion procedure (**Table 4**).

Following gastrointestinal digestion, the cooking of seafood did not modify the bioaccessible content of total As and organic arsenic. The increase in MA content in the bioaccessible fraction (**Table 4**) versus the content found with methanol/water extraction (**Table 2**), observed in DORM-2 and sole, could indicate that the methanol−water extraction mixture, used by most investigators for the extraction of arsenical species from fish, may not be quantitative for MA. Studies by our group (*18*) have found that the addition of MA prior to extraction with methanol−water yields quantitative recoveries, although it is known that tests of this kind—which are necessary for methodological validations—are not fully reliable, because the interactions between standard and matrix need not be of the same nature as those found in the product. Because the purpose of the quantification of arsenical species in seafood is to establish a toxicological evaluation, the use of a characterization process emulating human exposure would be the most adequate approach. The results observed for MA after in vitro digestion effectively advise this.

Retention and Transport by Caco-2 Cells from Standard Solutions and DORM-2. Caco-2 cells were exposed to organoarsenical standards of MA, DMA, AB, AC, TMAO, and TETRA (prepared in MEM at final concentrations of 1 μg mL^{-1}) and to a bioaccessible fraction of DORM-2. The arsenic contents in the cell monolayer (arsenic retention) and in the basal medium (arsenic transport) were evaluated. The methodology employed for arsenic speciation does not allow the quantification of low contents of arsenic species present in cell monolayers, as a result of which we evaluated the total arsenic content retained in the cell monolayers. Arsenic speciation in the basal medium is possible.

For standard solutions, only on the addition of AB was total arsenic detected in the cell monolayers—the content in question representing only 1% of that added. For the rest of the standards, the total arsenic content in the cell monolayer was lower than the limit of quantification of the methodology used (0.64 ng mL^{-1}). In the evaluation of transport, the species detected in both the apical and basal media corresponded to the species

Table 4. Bioaccessible Contents of Total Arsenic and Organoarsenical Species (Micrograms per Gram of Dry Weight, as Arsenic) from DORM-2, Sole, and Greenland Halibut[a]

sample		AB	DMA	MA	TMAO	TETRA	∑ species	total As
Dorm-2	raw	11.0 ± 0.1	0.10 ± 0.002	0.16 ± 0.01	nd[b]	0.11 ± 0.002	11.8 ± 0.1	17.1 ± 1.8
sole	raw	23.1 ± 1.2	nd	0.63 ± 0.01	nd	nd	24.4 ± 0.2	24.4 ± 2.4
	cooked	21.3 ± 1.7	nd	0.51 ± 0.04	nd	nd	21.9 ± 1.8	23.5 ± 0.6
Greenland halibut	raw	3.0 ± 0.1	nd	nd	0.07 ± 0.01	nd	3.1 ± 0.1	3.8 ± 0.3
	cooked	3.1 ± 0.1	nd	nd	0.05 ± 0.01	nd	3.4 ± 0.1	4.4 ± 0.4

[a] Values are presented as mean ± standard deviation of three independent replicates. [b] Not detected.

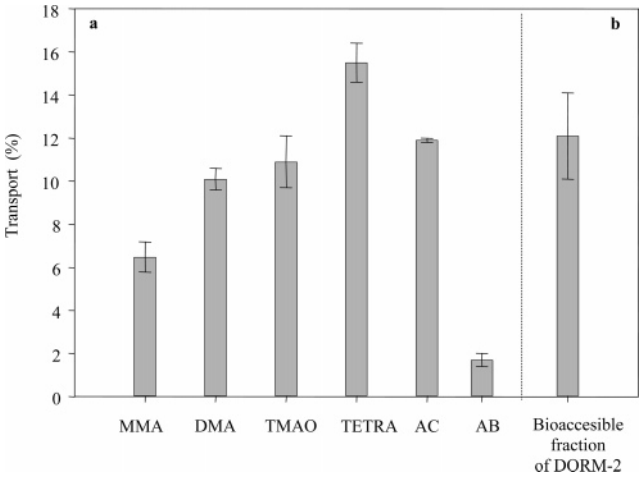

Figure 1. Transport percentages of organoarsenical species by Caco-2 cell culture incubated for 4 h: (**a**) organoarsenical standard solutions (1 μg mL^{-1}) prepared in MEM; (**b**) bioaccessible fractions of DORM-2. Data are presented as mean ± standard deviation of three independent replicates.

added initially, thus showing the absence of species conversion by the Caco-2 cell line. The transport percentages (**Figure 1**) [(amount of arsenic species in basal medium)/(initial content of arsenic species added to cell culture) × 100] ranged between 1.7% for AB and 15.5% for TETRA. With the exception of AB, the transport percentage increased with methylation on the arsenic atom. To our knowledge, no study has examined the involvement of organoarsenical species receptors, carriers, or functional enzymes in the transport of these species across Caco-2 cell membranes. Such studies could contribute to explain this finding.

Following cell culture exposure to the bioaccessible fraction of DORM-2, in which AB, MA, DMA, and TETRA are found, transport was detected only for the major species, AB, on the order of 12% (see **Figure 1**). It should be commented that AB transport was more effective from the bioaccessible fraction of DORM-2 than from AB standard (12 vs 1.7%). This indicates that transport differs between standard solutions and foods—thus reflecting the importance of evaluating arsenic bioavailability in foods, with a view to assessing risk.

Conclusions. Although the presence of different organoarsenical species in seafood is well-known, to our knowledge this is the first report related to arsenic bioavailability in raw and cooked seafood. Following gastrointestinal digestion, the cooking of seafood does not modify the bioaccessible content of total arsenic and organic arsenic, arsenic bioaccesibility from cooked seafood remaining high (100%). The bioaccessible contents of MA in DORM-2 and sole, higher than the MA contents found in the methanol−water extract of the samples, indicate that a more realistic estimation of the toxicological risk involved in the consumption of seafood would require consideration not only of the arsenic species content in the methanol−water extract but also of the gastrointestinal digestion process.

Despite the limitations of in vitro methods, the system combining in vitro digestion/Caco-2 cells offers a good approximation to the in vivo situation and affords information complementary to the results of arsenic metabolism in humans after the ingestion of food.

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