JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Organoarsenical Species Contents in Fresh and Processed Seafood Products

M. ANGELES SÚÑER,† VICENTA DEVESA,† MARÍA JESÚS CLEMENTE,† DINORAZ VÉLEZ,† ROSA MONTORO,*,† INÉS URIETA,‡ MERCEDES JALÓN,‡ AND MARY LUZ MACHO‡

Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Apartado 73, 46100 Burjassot (Valencia), Spain, and Dirección de Salud Pública, Departamento de Sanidad, Gobierno Vasco, c/Donostia-San Sebastián 1, 01010 Vitoria-Gasteiz, Spain

A study was carried out to determine organic species of arsenic in the main varieties of seafood consumed in the Basque country (Spain). The concentrations of arsenobetaine (AB), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenocholine (AC), and tetramethylarsonium ion (TMA^+) in 64 samples corresponding to different food items are presented. The study provides information about a possible distribution pattern of organoarsenical species in seafood products. AB was detected in all of the samples [0.3−104.1 $\mu g\ g^{-1}$ dry weight (dw)]. DMA was detected in all of the samples except squid and salted cod (0.027−1.757 $\mu g\ g^{-1}$ dw). MMA was detected only in certain fatty fish (0.004−0.028 $\mu g\ g^{-1}$ dw) and bivalves (0.031−0.047 $\mu g\ g^{-1}$ dw). AC was only present in some samples of lean fish (0.014−0.089 $\mu g\ g^{-1}$ dw), and TMA^+ was detected only in anchovy (0.039−0.169 $\mu g\ g^{-1}$ dw) and crustaceans (0.044−0.966 $\mu g\ g^{-1}$ dw).

KEYWORDS: Arsenic; seafood products; monomethylarsonic acid; dimethylarsinic acid; arsenobetaine; arsenocholine; tetramethylarsonium ion

INTRODUCTION

Arsenic is widely distributed in the environment, and the distribution of arsenic species in living organisms varies with the kind of matrix considered. In vegetables, the species traditionally described are As(III) and As(V) (*1*), together with traces of dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) (*2*). However, recent studies have shown the presence of species with a greater degree of methylation in mushrooms (*3*, *4*), lichens (*4*), and plants (*5*, *6*). In land animals, the existence of arsenobetaine (AB), DMA, and arsenoribosides in samples of earthworms (*7*) and of As(III), As(V), DMA, and traces of AB, MMA, and trimethylarsine oxide (TMAO) in ants (*8*) have been described.

In the aquatic environment, the levels of arsenic are far higher than on land, and therefore, since the beginning of arsenic speciation studies (*9*), attention has concentrated predominantly on marine organisms. Arsenic appears in seafood in the form of inorganic species [As(III) and As(V)] and a large number of organic species: AB, DMA, MMA, TMAO, arsenocholine (AC), tetramethylarsonium ion (TMA^+) (*10*−*12*), arsenosugars (*10*, *13*), and arsenolipids (*10*). It is currently considered that the total amount of arsenic ingested by humans depends on the amount of seafood included in the diet (*14*). However, the high arsenic content detected in seafood products, of the order of micrograms per gram, is not subjected to legislative control in most countries. The reason for this must be sought in speciation and in the different toxicities of the arsenic species that are present in fish. The dose that produces a 50% mortality in a given test population (LD_{50}), indicative of acute toxicity, decreases in the following order: As(III) > As(V) > TMA^+ > MMA > DMA > TMAO > AC > AB (*15*).

Numerous studies carried out since 1973 have shown that the predominant chemical form in seafood products is AB, a species considered to be harmless (*10*). However, there are some types of seafood, such as bivalves and other mollusks, for which AB represents not more than 50% of the total arsenic (*13*, *16*−*19*). This indicates the presence of species other than AB in considerable quantities. The type of seafood product considered may therefore have an effect on the kind of arsenic species found in it. The differences between the various kinds of seafood in terms of the content of organoarsenical species may also be the result of factors that affect the process of synthesis and degradation of arsenic in the aquatic environment.

The species on which the greatest amount of data is available in the literature are AB (*20*) and, to a lesser extent, DMA (*21*−*25*) and MMA (*11*, *21*, *22*, *25*). The amount of data is sparse, however, for TMAO (*10*, *11*, *26*), AC (*27*, *11*), and TMA^+ (*11*, *13*, *28*−*30*). For these species, although various methodologies have been developed to deal with their separation and quantification, the number of seafood samples analyzed so far is small.

* Author to whom correspondence should be addressed [telephone (+34) 963 900 022; fax (+34) 963 636 301; e-mail rmontoro@iata.csic.es].
† Instituto de Agroquímica y Tecnología de Alimentos (CSIC).
‡ Dirección de Salud Pública.

10.1021/jf011026s CCC: $22.00
Published on Web 01/18/2002

The purpose of this work is the quantification of AB, DMA, MMA, AC, and TMA^+ in a wide range of fatty fish, lean fish, shellfish, and preserved fish. These samples form part of the Food Chemical Safety Surveillance Program carried out by the Health Department of the Basque Government (Spain) (*31*). The origin and distribution of the organoarsenical species are discussed.

MATERIALS AND METHODS

Reagents. Deionized water (18 MΩ cm) was used for the preparation of reagents and standards. All chemicals were of pro analysi quality or better. The stock standard solutions of MMA and DMA (1000 mg L^{-1}) were prepared by dissolving appropriate amounts of commercially available salts in water: MMA [$CH_3AsO(ONa)_2 \cdot 6H_2O$] (Carlo Erba, Milan, Italy); DMA [$(CH_3)_2AsNaO_2 \cdot 3H_2O$] (Fluka Chemika Biochemika, Madrid, Spain). Standards supplied by Hot Chemical Co. (Tokyo, Japan) were used to prepare aqueous stock standard solutions of AB [$(CH_3)_3As^+CH_2COO^-$], AC [$(CH_3)_3AsCH_2CH_2OH^+Br^-$], and TMA^+ [$(CH_3)_4AsI$].

Sample Collection and Preparation. Samples were purchased from retail outlets throughout the Basque country and collected at monthly intervals over a half-year period in various locations (*32*). The samples used for this study were selected on the basis of their high consumption in the Basque country. In total, 64 samples of seafood products classified into 11 different categories were analyzed. Eight of them consisted of a single seafood product: meagrim, hake, small hake, anchovy, Atlantic horse mackerel, sardine, squid, and salted cod. The remaining three categories included various similar types of seafood products having low individual contributions to the total diet: bivalves (clam and mussel), crustaceans (scampi, shrimp, and prawn), and canned fish (tuna, albacore, and sardine).

Each sample of raw fish was prepared in such a way that inedible portions were removed. In all the fish samples, the head, tail, and digestive tract were removed. In bivalve samples the shell and in crustaceans, the exoskeleton, were eliminated. The squid were carefully cleaned with distilled water, and the cartilaginous skeleton was removed. The individual specimens were minced and blended to give a homogeneous sample. This was frozen at −20 °C and then freeze-dried (FTS Systems, New York). The lyophilized samples were ground in a domestic apparatus (Moulinex), and the resulting powder was stored in previously decontaminated twist-off flasks and kept at 4 °C until analysis.

Organic Species Determination. We used the method described by Suñer et al. (*33*), which offers optimum analytical characteristics and is applicable to determination of MMA, DMA, AB, TMA^+, and AC. The limit of detection varies between 3.6 ng g^{-1} dw for AB and 0.9 ng g^{-1} dw for DMA. The precision ranges from 1 to 12%, and the recovery is >95% for all species.

The method used for extraction and quantification (*33*) is now described. The lyophilized sample (1.00 g) was extracted three times with methanol/water (20 mL, 1 + 1 v/v). The extracts were evaporated to dryness (T = 44 °C), dissolved again in water (3 mL), and filtered through a Whatman 0.45 μm nylon membrane filter prior to quantification. The arsenic species in the extract were separated by means of a switching column system connecting two chromatograph columns: a Hamilton PRP-X200 (cation-exchange column, Teknokroma, Barcelona, Spain) and a Hamilton PRP-X100 (anion-exchange column, Teknokroma). The switching valve (Rheodyne six-port automated) was initially set at a position whereby the columns were not directly connected, so that they could be conditioned, the PRP-X200 with 10 mmol $L^{-1}(NH_4)H_2PO_4$ at pH 4.5 and the PRP-X100 with 1 mmol $L^{-1}(NH_4)H_2PO_4$ at pH 9.3. After conditioning, this position was maintained and the sample was injected into the PRP-X200 column. One minute after injection of the sample, the valve moved to a position that enabled direct connection between the two columns. This position was maintained until 4 min, so that the species that eluted in the period of 1−4 min in the PRP-X200 column were transferred to the PRP-X100 column. The valve then moved back to the initial position, so that each column could be eluted with different mobile phases [PRP-X200 with gradient 10−40 mmol L^{-1} $(NH_4)H_2PO_4$ at pH 4.5 and PRP-X100 with gradient 1−20 mmol L^{-1} $(NH_4)H_2PO_4$ at pH 9.3], and the species retained in each of them could be separated.

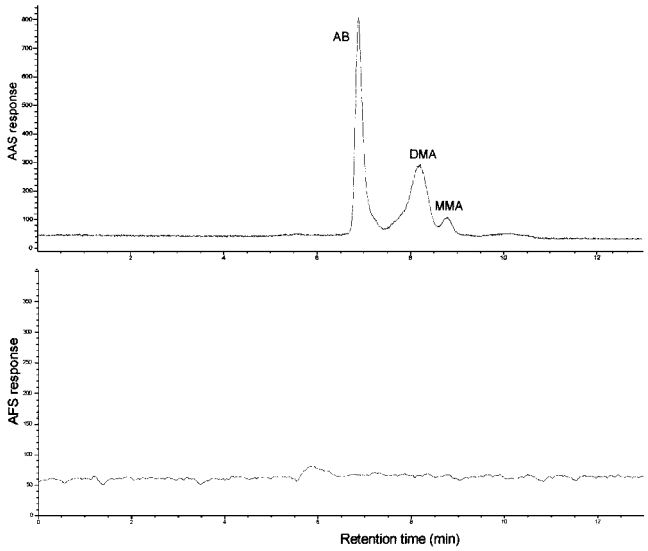

Figure 1. Chromatogram of sardine sample obtained using the column switching system.

After chromatographic separation, the eluates obtained from each column were thermo-oxidized independently using persulfate solution (1.29% w/v in 2.5% NaOH w/v, 1 mL min^{-1} flow rate), and the mixture was passed through a reaction coil (3 m × 0.3 mm i.d.) heated at 155 °C by a bath. Each thermo-oxidized effluent was then cooled in an ice bath and quantified. The thermo-oxidized sample obtained from the PRP-X100 column was mixed with a continuous flow of HCl (3.25 mol L^{-1}, 2.0 mL min^{-1} flow rate) and $NaBH_4$ (1.25% w/v in 0.7% NaOH w/v, 2.0 mL min^{-1} flow rate), using a flow injection system (Perkin-Elmer, PE FIAS-400) to provide hydride generation (HG) in continuous flow mode, and quantified by atomic absorption spectrometer (AAS) Perkin-Elmer model 5000 (PE, Norwalk, CT). The thermo-oxidized sample obtained from the PRP-X200 column was mixed with a continuous flow of HCl (1.5 mol L^{-1}, 6.0 mL min^{-1} flow rate) and $NaBH_4$ (1.5% w/v in 0.7% NaOH w/v, 2.5 mL min^{-1} flow rate) using an HG system (PSA 10.004, PS Analytical). The arsines generated were introduced into the atomic fluorescence spectrometry (AFS) detector, PSA 10.044 Excalibur (PS Analytical).

Assignment of arsenic compounds to the peaks in the chromatograms was performed by matching the retention time to the sample and to the standards or by addition of standards. A calibration curve obtained with a combined standard of arsenic compounds (MMA, DMA, TMA^+, AB, and AC) was used to quantify the resulting chromatograms. Two replicates were analyzed for each sample, and in each batch a BCR-627 sample with certified contents of AB and DMA was analyzed (tuna fish tissue, Institute for Reference Materials and Measurements); the series was analyzed only if there was an overlap between the ranges found and those certified. **Figure 1** shows the chromatogram obtained for a sardine sample.

RESULTS AND DISCUSSION

Table 1 shows the contents of the five organoarsenical species and total arsenic, expressed as arsenic (micrograms per gram dw), in the various seafood products analyzed, the ranges in which the values lie, and the moisture. The samples analyzed include "lean fish" with a fat content of ∼1% (hake, meagrim, and small hake), "fatty fish" with a fat content >1% (anchovy, sardine, and Atlantic horse mackerel), shellfish (bivalves, crustaceans, and squid), and seafood products that had undergone some kind of technological process (salted cod and canned fish).

Arsenobetaine Contents. AB was detected in all of the samples analyzed, and in all of them it was the organoarsenical

Table 1. Organoarsenic and Total Arsenic Contents (Micrograms per Gram of Dry Weight, as As) and Moisture (Percent) in Seafood Samples[a]

	AB	MMA	DMA	TMA^+	AC	total As[b]	moisture
lean fish							
hake	33.2	<LOD	<LOD	<LOD	0.015	32.0	80.6
	7.2	<LOD	<LOD	<LOD	0.014	6.7	83.2
	5.8	<LOD	0.213	<LOD	0.031	6.8	73.5
	3.4	<LOD	0.112	<LOD	<LOD	4.1	80.4
	17.4	<LOD	0.162	<LOD	0.021	17.9	79.1
	3.3	<LOD	0.155	<LOD	0.018	4.7	80.8
range	3.3–33.2		<LOD–0.213		<LOD–0.031		
meagrim	17.7	<LOD	0.079	<LOD	0.043	18.3	77.5
	14.5	<LOD	0.027	<LOD	<LOD	13.4	85.6
	27.7	<LOD	0.098	<LOD	0.076	23.9	78.4
	14.6	<LOD	0.434	<LOD	0.021	19.2	76.1
	3.7	<LOD	0.067	<LOD	0.021	4.5	75.9
	37.8	<LOD	0.177	<LOD	<LOD	33.0	76.1
range	3.7–37.8		0.027–0.434		<LOD–0.076		
small hake	4.9	<LOD	0.101	<LOD	0.078	6.3	81.1
	26.4	<LOD	0.116	<LOD	<LOD	24.3	80.1
	15.1	<LOD	0.205	<LOD	0.089	16.0	79.9
	12.6	<LOD	0.151	<LOD	<LOD	12.5	80.9
	5.8	<LOD	0.076	<LOD	<LOD	5.7	79.5
	7.2	<LOD	0.308	<LOD	0.070	9.9	80.0
range	4.9–26.4		0.076–0.308		<LOD–0.089		
fatty fish							
anchovy	1.7	<LOD	0.064	0.045	<LOD	2.7	77.8
	11.3	<LOD	0.116	0.039	<LOD	12.9	76.7
	8.9	<LOD	0.171	0.041	<LOD	10.6	74.6
	10.7	0.025	0.936	0.089	<LOD	12.5	60.1
	33.7	<LOD	0.106	0.169	<LOD	30.7	77.0
	17.0	<LOD	0.127	0.122	<LOD	19.5	76.9
range	1.7–33.7	<LOD–0.025	0.064–0.936	0.039–0.169			
sardine	6.8	0.022	0.783	<LOD	<LOD	11.0	67.6
	13.4	0.009	1.174	<LOD	<LOD	14.0	68.8
	9.4	0.028	1.181	<LOD	<LOD	12.8	62.2
	1.3	0.004	0.773	<LOD	<LOD	3.9	67.5
	20.2	0.013	1.757	<LOD	<LOD	23.0	67.5
	18.7	0.011	0.634	<LOD	<LOD	18.4	74.2
range	1.3–20.2	0.004–0.028	0.634–1.757				
Atlantic horse mackerel	1.8	0.006	0.482	<LOD	<LOD	3.9	75.1
	1.7	0.007	0.468	<LOD	<LOD	3.8	75.9
	1.6	0.008	0.551	<LOD	<LOD	3.3	77.7
	0.6	0.008	0.535	<LOD	<LOD	2.4	74.6
	4.3	<LOD	0.142	<LOD	<LOD	4.2	77.6
	4.6	0.003	0.397	<LOD	<LOD	5.4	76.7
range	0.6–4.6	<LOD-0.008	0.142–0.551				
shellfish							
squid	2.1	<LOD	<LOD	<LOD	<LOD	4.1	80.0
	4.0	<LOD	<LOD	<LOD	<LOD	6.2	78.4
	24.7	<LOD	<LOD	<LOD	<LOD	27.0	76.1
	0.5	<LOD	<LOD	<LOD	<LOD	2.2	83.1
	1.9	<LOD	<LOD	<LOD	<LOD	3.0	86.7
	0.3	<LOD	<LOD	<LOD	<LOD	0.7	60.4
range	0.3–24.7						
bivalves							
mussel	4.6	0.036	0.121	na	na	11.5	83.2
mussel	5.9	0.032	0.477	na	na	11.4	82.0
mussel	4.5	0.031	0.727	na	na	9.2	83.7
clam	6.7	0.047	1.012	na	na	24.2	84.7
clam	3.9	0.044	0.994	na	na	16.1	86.3
range	3.9–6.7	0.031–0.047	0.121–1.012				
crustaceans							
shrimp	104.1	<LOD	<LOD	0.508	<LOD	102.0	74.3
prawn	21.8	<LOD	<LOD	0.564	<LOD	22.5	76.8
scampi	26.3	<LOD	<LOD	0.966	<LOD	29.7	78.8
prawn	16.4	<LOD	0.026	0.044	<LOD	16.0	78.4
shrimp	34.8	<LOD	0.027	0.087	<LOD	32.9	85.0
range	16.4–104.1		<LOD-0.027	0.044–0.966			

Table 1. (Continued)

	AB	MMA	DMA	TMA^+	AC	total As[b]	moisture
processed fish							
salted cod	2.3	<LOD	<LOD	<LOD	<LOD	2.2	78.4
	1.7	<LOD	<LOD	<LOD	<LOD	1.9	71.0
	1.4	<LOD	<LOD	<LOD	<LOD	1.9	75.8
	2.7	<LOD	<LOD	<LOD	<LOD	2.5	75.4
	2.7	<LOD	<LOD	<LOD	<LOD	3.0	71.3
	0.4	<LOD	<LOD	<LOD	<LOD	1.2	75.5
range	0.4–2.7						
canned fish							
tuna	0.3	<LOD	<LOD	<LOD	<LOD	0.6	50.8
albacore	1.1	<LOD	<LOD	<LOD	<LOD	1.2	51.0
tuna	0.9	<LOD	<LOD	<LOD	<LOD	0.9	55.0
tuna	5.2	<LOD	<LOD	<LOD	<LOD	5.1	52.7
sardine	1.1	<LOD	0.067	<LOD	<LOD	3.0	46.3
albacore	1.0	<LOD	<LOD	<LOD	<LOD	1.2	49.1
range	0.3–5.2		<LOD–0.067				

[a] AB, arsenobetaine; DMA, dimethylarsinic acid; MMA, monomethylarsonic acid; TMA^+, tetramethylarsonium ion; AC, arsenocholine. LOD, limit of detection: AB, 3.6 ng g^{-1}, As dw; MMA, 3.3 ng g^{-1}, As dw; DMA, 0.9 ng g^{-1}, As dw; TMA^+, 2.7 ng g^{-1}, As dw; AC, 2.4 ng g^{-1}, As dw. LOD was calculated by dividing 3 times the standard deviation of the absorbance area readings of nine reagent blanks by the slope of the standard additions curve and taking into account the sample mass and dilution employed in the application of the method. na, not analyzed. [b] Values obtained by Muñoz et al. (*40*).

species with the highest concentration. In the lean fish analyzed the AB concentrations found are in agreement with the great variability in the concentration of AB in the literature for this kind of fish. Although many of these previously reported data (9.8−39.6 μg g^{-1} dw) (*11*, *23*, *24*, *34*, *35*) lie within the range of those found in the present study, there are also reports of concentrations lower (0.38 μg g^{-1} dw) (*36*) or much higher (48.8−187.3 μg g^{-1} dw) (*23*, *35*) than those detected in this study.

In the fatty fish group, the AB concentrations found in the present study are similar to the values found in lean fish. In the literature, data of AB for fatty fish are scarce; the concentrations reported are <10 μg g^{-1} dw and correspond to samples of tuna and mackerel *(11*, *23*, *34*, *37*).

The highest AB concentration in the whole study (104.1 μg g^{-1} dw) was detected in a sample of shellfish (shrimp). For the remaining samples of crustaceans analyzed, the contents were distinctly lower. The bivalves, on the other hand, consisted of a very homogeneous group, with a very narrow range of concentrations. For crustaceans and bivalves, the contents reported by other authors lie within ranges of 1.8−2.0 μg g^{-1} dw for crustaceans (*35*) and 0.8−22.1 μg g^{-1} dw for bivalves (*19*, *24*, *35*). In squid, the AB concentrations obtained in this study were much lower than those of the other shellfish, with the exception of a single sample of squid that had a value of 24.7 μg g^{-1} dw. These values are of the order of those described in the literature for squid (1.13−2.06 μg g^{-1} ww) (*37*).

In the processed fish, the values found in this study lie within the range obtained from the few data reported in the literature (<1.3 μg g^{-1} dw) (*17*, *38*). The low levels of AB in the preserved samples in comparison to the levels in the fresh products might be attributed to a transfer of this arsenic species from the fish to the accompanying liquid or to the salt used for salting the cod. In this respect, previous studies carried out by Vélez et al. (*38*) indicated the existence of AB in the accompanying liquid in canned fish. The weakness of the electrostatic link between AB and fish muscle and the greater osmotic pressure of the external medium favor the passage of AB to the media used for preservation.

Dimethylarsinic Acid Contents. This species of arsenic did not appear in all of the samples analyzed. In lean fish, DMA was found in all of the samples of meagrim and small hake, whereas in the samples of hake DMA was not detected in two of the six samples. All of the samples of fatty fish contained DMA, with the highest concentrations being greater than those found in lean fish. The DMA contents reported in the literature are low for both lean and fatty fish and do not differ greatly from those obtained in the present study [lean fish, 0.12 μg g^{-1} dm (*34*); fatty fish, 0.16−0.5 μg g^{-1} dm (*23*, *25*, *34*)].

In the shellfish group, it was only in the bivalves that DMA was detected in all of the samples analyzed, with contents similar to those reported in the literature (0.06−1.06 μg g^{-1} dw) (*24*, *25*, *39*). In crustaceans only two of the five samples analyzed contained DMA, showing similar levels to those reported previously (*22*, *35*). Finally, DMA was not detected in any of the samples of squid analyzed, nor are there previous references in the literature in this respect.

In the processed fish, DMA appeared only in one sample of canned sardine. In the literature, the data for DMA in preserved fish show very variable concentrations, depending on the kind of product considered [anchovy, 0.603−0.791 μg g^{-1} dm; octopus, 0.029−0.049 μg g^{-1} dw; sardine, 0.477−0.486 μg g^{-1} dw; tuna, 0.038−0.071 μg g^{-1} dw (*25*)].

Monomethylarsonic Acid Contents. MMA was not detected in any of the lean fish or processed fish samples. For the other groups the behavior was irregular. In the fatty fish, all the samples of sardine contained small quantities of MMA; it also appeared in 90% of the samples of Atlantic horse mackerel, but only one sample of anchovy contained this species. In shellfish, MMA was observed only in bivalves, which had the highest concentrations in the whole study.

The data reported in the literature show that sometimes MMA was not detected in lean fish (cod) or in fatty fish (tuna) (*34*), whereas other authors found MMA in a sample of fish but not in bivalves or crustaceans (*22*). Other studies have shown the presence of this species in bivalves in a range of 0.05−0.34 μg g^{-1} dw (*25*, *39*).

Arsenocholine Contents. The presence of arsenocholine was detected only in certain samples of lean fish. In the bivalves, it was not possible to determine AC because of the complexity of the chromatogram obtained, with signs of a matrix effect that could not be eliminated with high dilutions. Data in the literature for this arsenic species are very scarce. Lawrence et al. (*16*) found arsenocholine in two samples of shrimp (3.2−

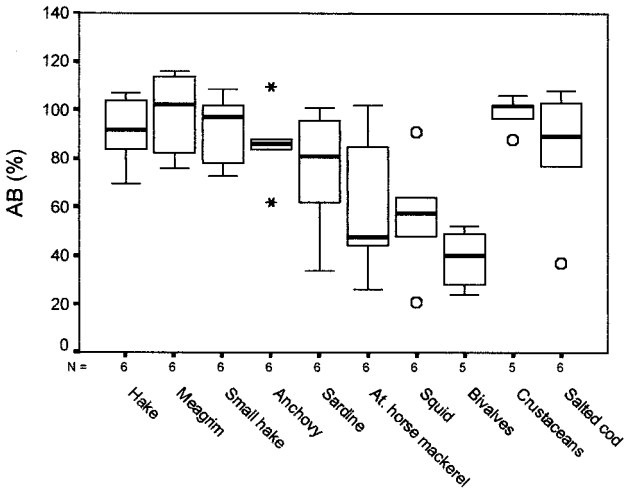

Figure 2. Percentages of total arsenic represented by arsenobetaine (AB) in the types of seafood analyzed. The results of total arsenic were published previously (*40*). (*N* = number of samples analyzed.) Box plot: The box is limited by percentiles 25 and 75 (Turkey hinges), and the inner line corresponds to the median value. The whiskers are the maximum and minimum observed values that are not outliers (o) or extremes (∗).

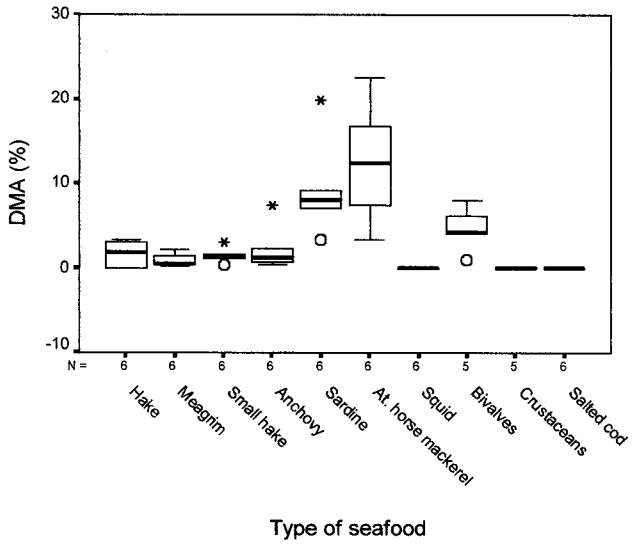

Figure 3. Percentages of total arsenic represented by dimethylarsinic acid (DMA) in the types of seafood analyzed. The results of total arsenic were previously published (*40*). (*N* = number of samples analyzed.) Box plot: The box is limited by percentiles 25 and 75 (Turkey hinges), and the inner line corresponds to the median value. The whiskers are the maximum and minimum observed values that are not outliers (o) or extremes (∗).

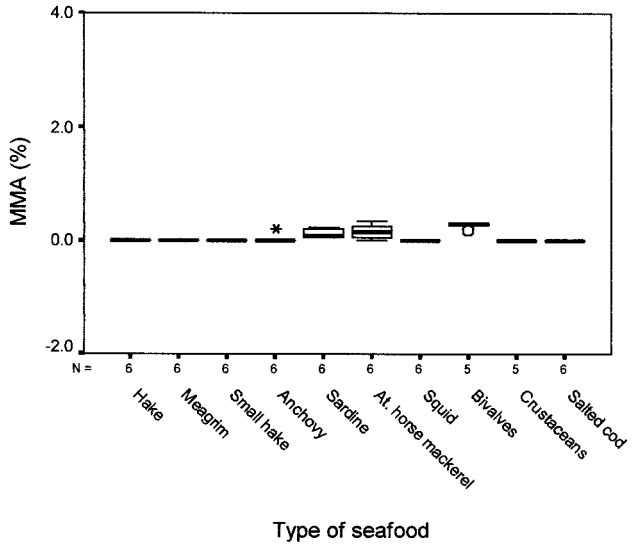

Figure 4. Percentages of total arsenic represented by monomethylarsonic acid (MMA) in the types of seafood analyzed. The results of total arsenic were previously published (*40*). (*N* = number of samples analyzed.) Box plot: The box is limited by percentiles 25 and 75 (Turkey hinges), and the inner line corresponds to the median value. The whiskers are the maximum and minimum observed values that are not outliers (o) or extremes (∗).

5.2 μg g^{-1} ww); Shiomi et al. (*27*) found AC in samples of gastropods (0.1 a 0.7 μg g^{-1} ww), and Larsen et al. (*11*) found AC in a sample of plaice (0.029 μg g^{-1} dw) and in a sample of tuna (0.016 μg g^{-1} dw).

Tetramethylarsonium Contents. This species of arsenic was detected in only 2 of the 11 types of seafood products analyzed, specifically in anchovies and crustaceans. In the samples of bivalves, quantification of TMA^+ was not possible, as in the case of AC, because of the complexity of the chromatogram obtained. In the literature, data on TMA^+ contents are scarce and relate only to samples of bivalves (*19*, *37*, *39*) or crustaceans (*36*, *37*). Consequently, the present study is a pioneering work in relation to TMA^+ contents in fish.

Percentage Distribution of Organoarsenical Species. Data of the concentrations of total arsenic previously reported by Muñoz et al. (*40*) were used to calculate the percentages of arsenic for the organic arsenic species analyzed in the present study. The results obtained are shown graphically in **Figures 2**−**6**. These figures use box plots to show the different percentages of total arsenic represented by a particular species in the various fishes. In this way it is possible to see the dispersion of the results obtained for a particular fish and the differences between one fish and another. **Figure 7** shows the mean of the cumulative percentages of arsenic for the various arsenic species.

In lean fish, arsenobetaine exceeded 70% in all samples (70−116%), whereas DMA represented <3%. AC attained only 1%, and in many of the samples it was not detected. The other species of arsenic, TMA^+ and MMA, were not detected in any of the samples. In the case of lean fish, this sum, including the inorganic arsenic (*40*), accounts for nearly 100% of the total arsenic.

In fatty fish, AB was again the organoarsenical species that represented the highest percentage of total arsenic, although it varied over a wider range (26−110%), and in some samples of Atlantic horse mackerel and sardine it represented <50% of the total arsenic. DMA represented up to a quarter of the total arsenic in fatty fish (0.4−23%). TMA^+ appeared only in anchovies, representing 0.3−1.6% of the total arsenic. MMA appeared in the majority of the samples, although it did not exceed 0.4%. AC was not detected in any of the samples. For fatty fish, the sum of the arsenic provided by the organic species analyzed was different for each type of fish.

For some samples of Atlantic horse mackerel and sardine, the addition of these organic species to the inorganic ones found previously (*40*) did not provide an extraction >60%. This was true for four of the six samples of Atlantic horse mackerel (∼60% of the total arsenic) and for one sample of sardine (59% of the total arsenic) (**Figures 2** and **7**). One possible explanation for this could be the methanol/water extraction method, widely used to extract arsenic species from seafood products (*24*, *18*), with a very good extraction efficiency. The nonquantitative

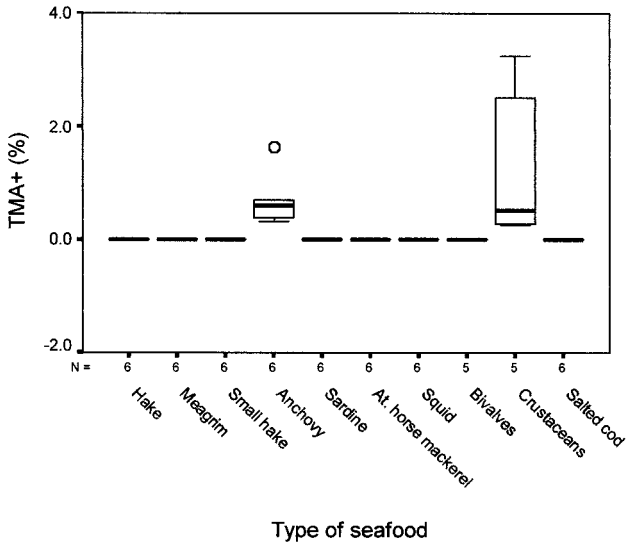

Figure 5. Percentages of total arsenic represented by tetramethylarsonium ion (TMA^+) in the types of seafood analyzed. The results of total arsenic were previously published (*40*). (N = number of samples analyzed.) Box plot: The box is limited by percentiles 25 and 75 (Turkey hinges), and the inner line corresponds to the median value. The whiskers are the maximum and minimum observed values that are not outliers (o) or extremes (∗).

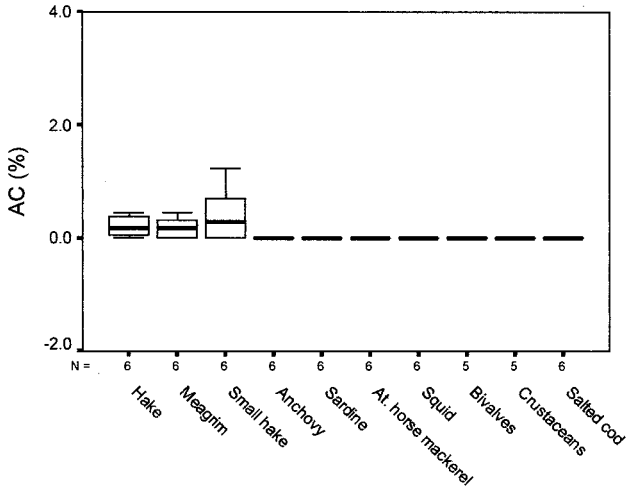

Figure 6. Percentages of total arsenic represented by arsenocholine (AC) in the types of seafood analyzed. The results of total arsenic were previously published (*40*). (N = number of samples analyzed.) Box plot: The box is limited by percentiles 25 and 75 (Turkey hinges), and the inner line corresponds to the median value. The whiskers are the maximum and minimum observed values that are not outliers (o) or extremes (∗).

extractions in some samples in the present study could be attributable to the presence of fat. Fat might reduce the efficiency of the methanol/water extractant by forming a film that prevents humectation of the sample, or there could be arsenolipids in these products that are not extractable with methanol/water (*23*). The greater fat content of fatty fish at certain times of year when they were caught could be responsible for the low extraction observed in some of the samples.

In the shellfish group, each of the seafood products studied had a different pattern of composition. In bivalves there was a predominance of arsenobetaine, although the percentage (24−52%) was far below the values for lean fish and fatty fish. This has also been reported by other authors (*13*, *34*). The percentage

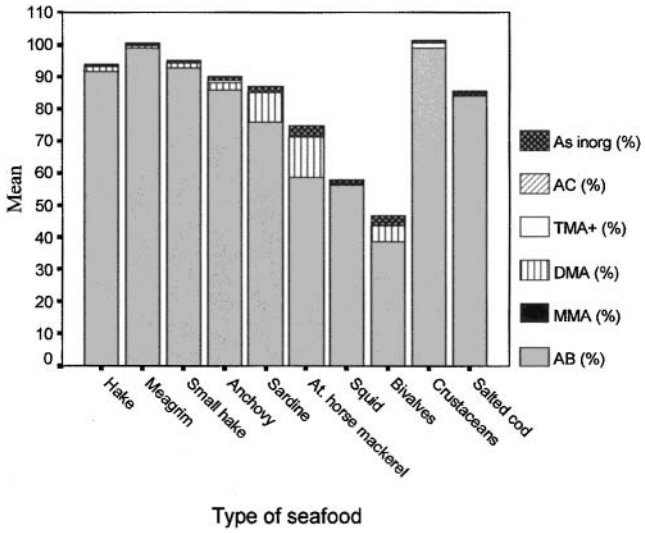

Figure 7. Sum of the mean cumulative percentages of total arsenic represented by the various arsenic species, for each type of seafood analyzed. The results of total arsenic were published previously (*40*).

represented by DMA varied in the range of 1−8%, and the percentage of MMA was extremely low (0.2−0.3%). In all of the bivalve samples, the sum of the concentrations of the organic species quantified plus the inorganic arsenic is less than the arsenic determined in the methanol/water extract and therefore less than the total arsenic in the sample (**Figure 7**). To interpret this result, it must be borne in mind that arsenosugars—which are known to exist in bivalves (*19*, *34*, *37*)—were not quantified. The chromatographic system employed and the nonavailability of commercial standards of arsenosugars made it impossible to analyze them in the present study.

As far as the other shellfish are concerned, for crustaceans, after arsenobetaine (88−106%) the second most important species was TMA^+ (0.3−3%). DMA was detected only in one sample of prawn and one sample of shrimp, in which it represented 0.1% of the total arsenic. In no sample was either MMA or AC observed. For squid, the only cephalopods analyzed in this study, unlike the bivalves and crustaceans, AB was the only species detected. The percentages of this species varied greatly with respect to total arsenic, and for some samples they were very low (21−91%).

In squid, in five of the six samples the sum of all the arsenic species determined, including inorganic arsenic (*40*), represented <65% of the total arsenic. The arsenic present in the extraction residue was analyzed by dry ashing−HG−AAS and was found to contain between 77 and 35% of the total arsenic in the sample. In all of the squid samples, the total arsenic content in the methanol/water extract was close to the arsenic obtained by summing the species quantified by means of HPLC−thermo-oxidation−HG−(AFS or AAS), indicating that no large quantities of arsenic remained unquantified. However, not all of the samples of squid behaved in the same way in response to the extractant.

In all of the samples of salted cod and canned fish AB was detected. In salted cod the percentage of AB varied from 37 to 108% of the total arsenic, whereas in canned fish the percentage depended on the type of canned seafood. DMA was detected only in a sample of canned sardine, at a low percentage (2%).

According to the traditional generalization about AB in seafood products, this species represents >80% in fish and <50% in mollusks (*18*). The results obtained in the present study show that 41% of the samples contained AB at a percentage

<80% of total arsenic. The seafood products involved were not only bivalves; low percentages (21−77%) also appeared in some fatty fish, squid, salted cod, and canned fish. These results contribute to the knowledge about the percentages of AB in seafood products that should be considered in future generalizations.

Origin of Arsenic Species in Seafood. The distribution of arsenic species described above is the natural consequence of the anabolic and catabolic processes that govern the arsenic cycle in the marine environment. Although numerous studies have been devoted to this cycle, it has not yet been fully elucidated. With respect to anabolism, it has been shown that inorganic arsenic, the principal form of arsenic in seawater, is incorporated into the food chain and converted to organic compounds. The anabolism studies that have been carried out provide a valuable contribution, but they have the limitation of having been restricted to specific seafood products or small food chains.

Studies of catabolism have made it possible to discover possible routes for the appearance of arsenic species. Most work has been carried out on AB, revealing that it can be degraded to TMAO, DMA, and MMA, and even inorganic arsenic, for marine microorganisms appear in sediments, marine macroalgae, intestine of marine animals, and suspended substances (*41*). Moreover, the microbial flora that proliferates after the death of the animal and the flora that persists during storage even in refrigerated conditions might be responsible for a process of degradation of arsenic species. An experiment on the transformation of AB by microorganisms present in algae showed the generation of either TMAO or (TMAO + DMA), depending on the different microorganisms that took part in the conversion (*42*). This introduces new alternatives in the degradation possibilities that must be considered. It is possible that modifications in handling conditions (e.g., refrigeration temperature, storage, time between capture and analysis) applied to each batch of seafood after capture may have an effect on the bacterial microflora and consequently on the degradation of arsenic species.

Doubts still exist about the origin of the concentrations of AB—of the order of micrograms per gram—that are detected in some seafood products analyzed. AB may be accumulated from water, as has been shown in laboratory experiments on mussels, rock lobster (*30*), and shrimp (*43*) exposed to it. The efficiency of the accumulation is not the same for all of the organisms mentioned. The presence of AB in estuary waters has been suggested (*44*), but the rapid degradation of this species in natural waters, as reported by Francesconi et al. (*45*), may explain why this source has not been considered as one of the origins of AB present in higher organisms. Food seems to be the principal source of AB. In this connection, in samples of crustaceans Hunter et al. (*43*) showed a preferential assimilation of AB from food spiked with this species in comparison with seawater spiked with AB. Finally, it is possible that compounds of di- and trimethylates ingested with food may be transformed into AB by the particular fish species (*46*, *47*). In the present study, comparison of the AB concentration ranges in the seafood analyzed shows that there is not a clear biomagnification of AB in the food chain. For example, hake, which are seabed predators, and meagrim, which feed on benthic fauna, had AB contents higher than those found in bivalves, which are filter feeders. On the other hand, the form of feeding does not explain what was observed in crustaceans and Atlantic horse mackerel. Despite being herbivores and therefore belonging to a lower level in the food chain, in the crustaceans the AB concentrations were of the same order as those of fish. Atlantic horse mackerel, on the other hand, which feeds on small fish and planktonic crustaceans (*48*), had low AB contents with respect to sardines and anchovies, which feed on plankton.

For the species with a lower degree of methylation, MMA and DMA, various origins have been described. Both can be accumulated from spiked water, although the results are contradictory. For instance, assimilation of MMA by mussels has been shown (*49*), as well as assimilation of DMA by freshwater prawn and shrimp (*50*, *51*). On the other hand, in experiments carried out with blue mussels assimilation of MMA or DMA from water was not observed (*30*). For the metabolic pathways of MMA and DMA, methylation of inorganic arsenic and MMA, respectively, has been described, in samples of freshwater prawn and shrimp (*50*, *51*). Finally, degradation of TMAO (*43*) and AB (*50*) in shrimp has been described as a source of DMA, whereas DMA in freshwater prawn may be partially degraded to MMA (*51*). In the present study, the higher DMA content in fatty fish, bivalves, and crustaceans may therefore have a twofold origin: on the one hand, their greater capability of methylation of species such as MMA assimilated from the water or from the food chain, and, on the other hand, the processes of metabolic degradation, perhaps by enzymatic activity of microbial origin, of species with a higher degree of methylation, such as AB.

The presence of AC in the samples of fish may be due to anaerobic transformation within the fish of trimethylarsonioriboside derivatives found in algae, although this route has been demonstrated only in the laboratory (*52*). On the other hand, mussels exposed to AC had AB as the major arsenic compound and glycerylphosphorylarsenocholine as a minor arsenic compound in their tissues (*30*). This transformation of AC to AB is considered to be a rapid process, which would explain the absence or low levels of AC in seafood (*28*). In the present study, the low percentages of AC found in lean fish and the fact that it was not detected in any other kind of sample may be due to the fact that the mechanism of the formation of AB in lean fish is not as rapid as the corresponding process in fatty fish or in other kinds of seafood.

As far as TMA^+ is concerned, aquarium experiments carried out by Gailer et al. (*30*) showed accumulation by blue mussels of TMA^+ from seawater spiked with this species, although at a lower accumulation efficiency than that of AB and AC. The concentration in gills was double the concentration existing in the whole animal. However, similar experiments have not been carried out in other seafood products. Quantification studies for this arsenic species show higher contents in gonads and digestive glands of blue mussels (*29*) and in gills of clams (*13*, *28*) than in muscle of these organisms. Kaise et al. (*53*) proposed that TMA^+ was generated from AB as a result of degradation due to activity of the microbial flora existing in the gills, although they were not able to demonstrate this by means of degradation tests. If this is the origin of TMA^+, it might be said that in all seafood products that are not gutted immediately after capture the activity of microbial flora could generate TMA^+.

In light of what has been said, interpretation of the results obtained for TMA^+ is not easy. Perhaps the way in which the fish were prepared for the present study may be a factor to be taken into account. In the crustaceans the intestinal tract was not removed, and it is possible that the flora present might act on AB and generate TMA^+ or other species, such as DMA or TMAO. It is more difficult to explain why TMA^+ was found in anchovy but not in any other kind of fish treated in the same conditions. A possible explanation may be the small size of anchovies, which makes it more difficult to gut them completely.

It is possible that part of the digestive tract may have remained in the sample, facilitating the process of microbial degradation. On the other hand, studies performed by Lai et al. (*19*) on samples of scallops detected the existence of TMA^+ in the muscle of these organisms, so that one cannot rule out the possibility that this arsenic species may be accumulated in other tissues of the organism through the activity of the flora in the gills or intestine.

Conclusions. The distribution profile of the organoarsenical species (AB, DMA, MMA, AC, and TMA^+) was characterized in 11 different seafood items. In all of them the predominant species was arsenobetaine, although the arsenic found in this form represented very variable percentages of total arsenic in the various seafood items (21−116%). In lean fish, AB represented >70% of total arsenic. In the samples of fatty fish, squid, and bivalves, the percentages of arsenic in the form of AB lay within the ranges of 26−110, 21−91, and 24−52%, respectively. DMA appeared in most of the seafood items analyzed, in percentages up to 23% of total arsenic (Atlantic horse mackerel). The other arsenic species studied (MMA, TMA^+, and AC) appeared only in certain samples and represented variable percentages of total arsenic, always <4%.

With respect to the efficiency of the extraction, in lean fish the sum of all the arsenic species (including inorganic arsenic) represented almost 100% of total arsenic, which was not the case with the other seafood items. In the case of bivalves, the arsenic determined in the methanol/water extract did not agree with the sum of the arsenic obtained for the various arsenic species analyzed. To interpret this result, it must be borne in mind that arsenosugars—which are known to exist in bivalves—were not quantified. In fatty fish and squid, the arsenic determined in the methanol/water extract agreed with the sum of the quantities of arsenic obtained for the various species of arsenic analyzed. However, the sum of the percentages of arsenic for the various species analyzed, including inorganic arsenic, was less than the total arsenic determined, indicating incomplete extraction of the organic arsenic species with the methanol/water extract. The fact that the fat content of fatty fish is higher than that of lean fish and also that it varies according to the season might explain the variability of the extraction percentages obtained. To complete the distribution profile, it would be interesting to analyze the arsenosugars and the arsenic species in the extraction residue and to test alternative solvent mixtures that might increase the efficiency of the extraction.

ACKNOWLEDGMENT

We are grateful to Sergio Algora, Victoria Benito, and Maite de la Flor for assistance in the performance of analytical work.

LITERATURE CITED

(1) Helgesen, H.; Larsen, E. H. Bioavailability and speciation of arsenic in carrots grown in contaminated soil. *Analyst* **1998**, *123*, 791−796.

(2) Carbonell Barrachina, A. A.; Burló, F.; Valero, D.; López, E.; Martínez Romero, D.; Martínez Sánchez, F. Arsenic toxicity and accumulation in turnip as affected by arsenic chemical speciation. *J. Agric. Food Chem.* **1999**, *47*, 2288−2294.

(3) Byrne, A. R.; Šlejkovec, Z.; Stijve, T.; Fay, L.; Gössler, W.; Gailer, J.; Irgolic, K. J. Arsenobetaine and other arsenic species in mushrooms. *Appl. Organomet. Chem.* **1995**, *9*, 305−313.

(4) Koch, I.; Wang, L.; Reimer, K. J.; Cullen, W. R. Arsenic species in terrestrial fungi and lichens from Yellowknife, NWT, Canada. *Appl. Organomet. Chem.* **2000**, *14*, 245−252.

(5) Mattusch, J.; Wennrich, R.; Schmidt, A. C.; Reisser, W. Determination of arsenic species in water, soils and plants. *Fresenius' J. Anal. Chem.* **2000**, *366*, 200−203.

(6) Kuehnelt, D.; Lintschinger, J.; Goessler, W. Arsenic compounds in terrestrial organisms. IV. Green plants and lichens from an old arsenic smelter site in Australia. *Appl. Organomet. Chem.* **2000**, *14*, 411−420.

(7) Geiszinger, A.; Goessler, W.; Kuehnelt, D.; Francesconi, K.; Kosmus, W. Determination of arsenic compounds in earthworms. *Environ. Sci. Technol.* **1998**, *32*, 2238−2243.

(8) Kuehnelt, D.; Goessler, W.; Schlagenhaufen, C.; Irgolic, K. J. Arsenic compounds in terrestrial organisms III: Arsenic compounds in formica sp. from an old arsenic smelter site. *Appl. Organomet. Chem.* **1997**, *11*, 859−867.

(9) Braman, R. S.; Foreback, C. C. Methylated forms of arsenic in the environment. *Science* **1973**, *182*, 1247−1251.

(10) Phillips, D. J. H. Arsenic in aquatic organisms: a review, emphasizing chemical speciation. *Aquat. Toxicol.* **1990**, *16*, 151−186.

(11) Larsen, E. H.; Pritzl, G.; Hansen, S. H. Arsenic speciation in seafood samples with emphasis on minor constituents: An investigation using high-performance liquid chromatography with detection by inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **1993**, *8*, 1075−1084.

(12) Larsen, E. H. Speciation of dimethylarsinyl-riboside derivatives (arsenosugars) in marine reference materials by HPLC-ICP-MS. *Fresenius' J. Anal. Chem.* **1995**, *351*, 1−7.

(13) Shibata, Y.; Morita, M. Characterization of organic arsenic compounds in bivalves. *Appl. Organomet. Chem.* **1992**, *6*, 343−349.

(14) Ministry of Agriculture, Fisheries and Food. *Food Surveillance Paper 8*; Her Majesty's Stationery Office: London, U.K., 1982.

(15) Shiomi, K. Arsenic in marine organisms: Chemical forms and toxicological aspects. In *Arsenic in the Environment. Part II: Human Health and Ecosystem Effects*; Nriagu, J. O., Ed.; Wiley: New York, 1994; Chapter 12, pp 261−293.

(16) Lawrence, J. F.; Michalik, P.; Tam, G.; Conacher, B. S. Identification of arsenobetaine and arsenocholine in Canadian fish and shellfish by high-performance liquid chromatography with atomic absorption detection and confirmation by fast atom bombardment mass spectrometry. *J. Agric. Food Chem.* **1986**, *34*, 315−319.

(17) Vélez, D.; Ybáñez, N.; Montoro, R. Percentages of total arsenic represented by arsenobetaine levels of manufactured seafood products. *J. Agric. Food Chem.* **1995**, *43*, 1289−1294.

(18) Lagarde, F.; Amran, M. B.; Leroy, M. J. F.; Demesmay, C.; Ollé, M.; Lamotte, A.; Muntau, H.; Michel, P.; Thomas, P.; Caroli, S.; Larsen, E.; Bonner, P.; Rauret, G.; Foulkes, M.; Howard, A.; Griepink, B.; Maier, E. A. Certification of total arsenic, dimethylarsinic acid and arsenobetaine contents in a tuna fish powder (BCR-CRM 627). *Fresenius' J. Anal. Chem.* **1999**, *363*, 18−22.

(19) Lai, V. W.-M.; Cullen, W. R.; Ray, S. Arsenic speciation in scallops. *Mar. Chem.* **1999**, *66*, 81−89.

(20) Vélez, D.; Montoro, R. Arsenic speciation in manufactured seafood products. *J. Food Prot.* **1998**, *61*, 1240−1245.

(21) Howard, A. G.; Arbab Zavar, M. H. Determination of "inorganic" arsenic(III) and arsenic(V), "methylarsenic" and "dimethylarsenic" species by selective hydride evolution atomic-absorption spectroscopy. *Analyst* **1981**, *106*, 213−220.

(22) Kaise, T.; Yamauchi, H.; Hirayama, T.; Fukui, S. Determination of inorganic arsenic and organic arsenic compounds in marine organisms by hydride generation/cold trap/gas chromatography−mass spectrometry. *Appl. Organomet. Chem.* **1988**, *2*, 339−347.

(23) Branch, S.; Ebdon, L.; O'Neill, P. Determination of arsenic species in fish by directly coupled high-performance liquid chromatography-inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **1994**, *9*, 33−37.

(24) Albertí, J.; Rubio, R.; Rauret, G. Extraction method for arsenic speciation in marine organisms. *Fresenius' J. Anal. Chem.* **1995**, *351*, 420−425.

(25) Vélez, D.; Ybáñez, N.; Montoro, R. Monomethylarsonic and dimethylarsinic acid contents in seafood products. *J. Agric. Food Chem.* **1996**, *44*, 859−864.

(26) Edmonds, J. S.; Francesconi, K. A. Trimethylarsine oxide in estuary catfish (*Cnidoglanis macrocephalus*) and school whiting (*Sillago bassensis*) after oral administration of sodium arsenate; and as a natural component of estuary catfish. *Sci. Total Environ.* **1987**, *64*, 317−323.

(27) Shiomi, K.; Orii, M.; Yamanaka, H.; Kikuchi, T. The determination method of arsenic compounds by high performance liquid chromatography with inductively coupled argon plasma emission spectrometry and its application to shellfishes. *Nippon Suisan Gakkaishi* **1987**, *53*, 103−108.

(28) Shiomi, K.; Kakehashi, Y.; Yamanaka, H.; Kikuchi, T. Identification of arsenobetaine and tetramethylarsonium salt in the clam *Meretrix lusoria*. *Appl. Organomet. Chem.* **1987**, *1*, 177−183.

(29) Francesconi, K. A.; Edmonds, J. S.; Hatcher, B. G. Examination of the arsenic constituents of the herbivorous marine gastropod *Tectus pyramis*: Isolation of tetramethylarsonium ion. *Comp. Biochem. Physiol.* **1988**, *90C*, 313−316.

(30) Gailer, J.; Francesconi, K. A.; Edmonds, J. S.; Irgolic, K. J. Metabolism of arsenic compounds by the blue mussel *Mytilus edulis* after accumulation from seawater spiked with arsenic compounds. *Appl. Organomet. Chem*. **1995**, *9*, 341−355.

(31) Jalón, M.; Urieta, I.; Macho, M. L.; Azpiri, M. Metales Pesados y Arsénico. In *Vigilancia de la Contaminación Química de los Alimentos en la Comunidad Autónoma del País Vasco 1990−1995*; Servicio Central de Publicaciones del Gobierno Vasco: Vitoria-Gasteiz, Spain, 1997, pp 29−43.

(32) Urieta, I.; Jalón, M.; Garcia, J.; González de Galdeano, L. Food surveillance in the Basque Country (Spain) I. The design of a total diet study. *Food Addit. Contam.* **1991**, *8*, 371−380.

(33) Suñer, M.; Devesa, V.; Muñoz, O.; Vélez, D.; Montoro, R. Application of column switching in high performance liquid chromatography with on-line thermo-oxidation and detection by HG-AAS and HG-AFS for the analysis of organoarsenical species in seafood samples. *J. Anal. At. Spectrom*. **2001**, *16*, 390−397.

(34) Amran, M. B.; Lagarde, F.; Leroy, M. J. F. Determination of arsenic species in marine organisms by HPLC-ICP-OES and HPLC-HG-QFAAS. *Mikrochim. Acta* **1997**, *127*, 195−202.

(35) Moll, A. E.; Heimburger, R.; Lagarde, F.; Leroy, M. J. F.; Maier, E. Arsenic speciation in marine organisms: from the analytical methodology to the constitution of reference materials. *Fresenius' J. Anal. Chem*. **1996**, *354*, 550−556.

(36) Momplaisir, G. M.; Blais, J. S.; Quinteiro, M.; Marshall, W. D. Determination of arsenobetaine, arsenocholine, and tetramethylarsonium cations in seafoods and human urine by high-performance liquid chromatography-thermochemical hydride generation-atomic absorption spectrometry. *J. Agric. Food Chem.* **1991**, *39*, 1448−1451.

(37) Shibata, Y.; Morita, M.; Fuwa, K. Selenium and arsenic in biology: their chemical forms and biological functions. *Adv. Biophys*. **1992**, *28*, 31−80.

(38) Vélez, D.; Ybáñez, N.; Montoro, R. Migration of arsenobetaine from canned seafood to brine. *J. Agric. Food Chem*. **1997**, *45*, 449−453.

(39) Šlejkovec, Z.; Byrne, A. R.; Smodis, B.; Rossbach, M. Preliminary studies on arsenic species in some environmental samples. *Fresenius' J. Anal. Chem.* **1996**, *354*, 592−595.

(40) Muñoz, O.; Devesa, V.; Suñer, M. A.; Vélez, D.; Montoro, R.; Urieta, I.; Macho, M. L.; Jalón, M. Total and inorganic arsenic in fresh and processed fish products. *J. Agric. Food Chem*. **2000**, *48*, 4369−4376.

(41) Hanaoka, K.; Tagawa, S.; Kaise, T. The fate of organoarsenic compounds in marine ecosystems. *Appl. Organomet. Chem.* **1992**, *6*, 139−146.

(42) Hanaoka, K.; Ueno, K.; Tagawa, S.; Kaise, T. Degradation of arsenobetaine by microorganisms associated with marine macro algae, *Monostroma nitidum* and *Hizikia fusiforme*. *Comp. Biochem. Physiol*. **1989**, *94B*, 379−382.

(43) Hunter, D. A.; Goessler, W.; Francesconi, K. A. Uptake of arsenate, trimethylarsine oxide, and arsenobetaine by the shrimp *Crangon crangon*. *Mar. Biol.* **1998**, *131*, 543−552.

(44) Florencio, M. H.; Duarte, M. F.; Facchetti, S.; Gomez, M. L.; Goessler, W.; Irgolic, K. J.; Van't Klooster, H. A.; Montanarella, L.; Ritsema, R.; Vilas-Boas, L. F.; de Bettencourt, A. M. M. Identification of inorganic, methylated and hydride-refractory arsenic species in estuarine waters. Advances by electrospray, ES-MS, pyrolysis-GC-MS and HPLC-ICP/MS. *Analusis* **1997**, *25*, 226−229.

(45) Francesconi, K. A.; Khokiattiwong, S.; Goessler, W. Uptake and biotransformation processes for arsenic compounds. Presented at the 1st International IUPAC Symposium Trace Elements in Food, Warsaw, Poland, Oct 2000.

(46) Edmonds, J. S.; Francesconi, K. A. The origin of arsenobetaine in marine animals. *Appl. Organomet. Chem.* **1988**, *2*, 297−302.

(47) Francesconi, K. A.; Goessler, W.; Panutrakul, S.; Irgolic, K. J. A novel arsenic containing riboside (arsenosugar) in three species of gastropod. *Sci. Total Environ.* **1998**, *221*, 139−148.

(48) Sikorski, Z. E.; Karnicki, Z. Recursos: disponibilidades. In *Tecnología de los Productos del Mar: Recursos, Composición Nutritiva y Conservación*; Sikorski, Z. E., Ed.; Acribia: Saragossa, Spain, 1994; pp 15−38.

(49) Cullen, W. R.; Pergantis, S. A. Biotransformation of [^{3}H] methylarsonic acid in a static seawater system containing *Mytilus californianus*. *Appl. Organomet. Chem.* **1993**, *7*, 329−334.

(50) Kuroiwa, T.; Ohki, A.; Naka, K.; Maeda, S. Biomethylation and biotransformation of arsenic in a freshwater food chain: Green alga (*Chlorella vulgaris*) → shrimp (*Neocaridina denticulata*) → killifish (*Oryzias latipes*). *Appl. Organomet. Chem.* **1994**, *8*, 325−333.

(51) Kuroiwa, T.; Yoshihiko, I.; Ohki, A.; Naka, K.; Maeda, S. Tolerance, bioaccumulation and biotransformation of arsenic in freshwater prawn (*Macrobrachium rosenbergii*). *Appl. Organomet. Chem.* **1995**, *9*, 517−523.

(52) Shibata, Y.; Morita, M. A novel, trimethylated arseno-sugar isolated from the brown alga *Sargassum thunbergii*. *Agric. Biol. Chem*. **1988**, *52*, 1087−1089.

(53) Kaise, T.; Sakurai, T.; Saitoh, T.; Matsubara, C. Biotransformation of arsenobetaine to trimethylarsine oxide by marine microorganisms in a gill of clam *Meretrix lusoria*. *Chemosphere* **1998**, *37*, 443−449.

Received for review August 1, 2001. Revised manuscript received November 2, 2001. Accepted November 5, 2001. This research was supported by the Comisión Interministerial de Ciencia y Tecnología (CICyT), Project ALI96-0511, for which we are deeply indebted. M.A.S. and V.D. received Spanish Research Personnel Training Grants from the Ministerio de Educación y Cultura and the Generalitat Valenciana (Conselleria de Cultura, Educació i Ciència), respectively.

JF011026S