Total and Inorganic Arsenic in Fresh and Processed Fish Products

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Total arsenic and inorganic arsenic contents were determined in 153 samples of seafood products consumed in the Basque Country (Spain): fish (white fish and blue fish), mollusks, crustaceans, and preserved fish. White fish presented higher levels of total arsenic and lower levels of inorganic arsenic than the blue fish, indicating possible differences in the metabolization of inorganic arsenic. For total arsenic, 66% of the samples exceeded the maximum permitted level by the strictest international legislation in seafood products [1 μg g^{-1}, wet weight (ww)]. The levels of inorganic arsenic were considerably lower than the maximum authorized in New Zealand (2 μg g^{-1}, ww), the only country with legislation for inorganic arsenic in fish and fish products. It is recommended that legislation based on levels of inorganic arsenic should be established.

Keywords: *Arsenic; inorganic arsenic; seafood products*

INTRODUCTION

Total diet studies carried out recently in various countries (Canada, Spain, UK, USA, and The Netherlands) show that seafood is the most significant source of arsenic in the diet (Dabeka et al., 1993; Jalón et al., 1997; Ysart et al., 1999; Gunderson, 1995; van Dokkum et al., 1989). In seafood products, arsenic can be found in various chemical forms that differ in their degree of toxicity and the pathologies associated with them. The latter include cardiovascular, dermatological, hematological, hepatic, and renal illnesses. In this connection, the International Agency for Research on Cancer (IARC) has assigned arsenic, inhaled or ingested, to Group I human carcinogens, because there is sufficient evidence from epidemiological studies to support a causal association between arsenic exposure and cancer (Tsuda et al., 1992). The principal cancers that arsenic can cause include those of the urinary−genital and respiratory apparatuses, and skin cancer.

The most toxic forms of arsenic are the inorganic ones (As III and As V). The organic forms of arsenic present in fish [arsenobetaine (AB), arsenocholine (AC), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), trimethylarsine oxide (TMAO), and tetramethylarsonium ion (TMA^+)] show a lower toxicity or are considered practically nontoxic (Shiomi, 1994). As III is found in living organisms bound to −SH groups of cytosolic proteins and macromolecular constituents (Styblo et al., 1996), causing inhibition of metabolic enzymes and triggering the acute toxicity attributable to inorganic arsenic (Liebl et al., 1995). The symptoms of acute arsenic poisoning follow a general pattern independent of the forms of inorganic arsenic given, probably as a result of the interconversion of these forms by in vivo oxidation/reduction reactions (Tsuda et al., 1992). Therefore, it is not necessary to distinguish between the two inorganic arsenic species. Consequently, the estimation of human health risks from the intake of seafood products might depend on the intake of inorganic arsenic in these products. This intake must be compared with the toxicological reference values established by the World Health Organization, WHO (1989).

Extensive studies on the customary levels of inorganic arsenic in seafood products has not been possible, largely because of the difficulty involved in developing simple, reliable, and inexpensive systems for the quantification of inorganic arsenic in foods. Moreover, the very few data of inorganic arsenic reported in the literature are usually isolated data, not necessarily representative of the levels of inorganic arsenic existing in the various types of fish.

Since 1990 the Basque (Spain) Government's Health Department has been carrying out a Total Diet Study (Urieta et al., 1991), as part of its Food Chemical Safety Surveillance Program. The main objective of the study is to assess exposure to contaminants and nutrients of concern and to evaluate the risk associated with that exposure. The total arsenic intake in the period 1990−1995 was very high (297 μg day^{-1}) (Jalón et al., 1997), higher than in traditional fish-consuming countries such as Japan (280 μg day^{-1}) (Tsuda et al., 1995). Consequently, a collaboration was set up between the Public Health Directorate of the Basque Government's Health Department and the Institute of Agrochemistry and Food Technology (IATA-CSIC), to undertake studies on inorganic arsenic in seafood products.

This work provides information on total and inorganic arsenic in a wide range of samples of fresh, canned, and salted fish consumed in the Basque Country (Spain). The various groups of fish were compared with respect to their levels of total and inorganic arsenic. The levels found were examined in the light of prevailing legislation. It is recommended that legislation based on levels of inorganic arsenic should be established.

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MATERIALS AND METHODS

Instrumentation. The equipment used included a Perkin-Elmer (PE) 3300 atomic absorption spectrometer (Perkin-Elmer Hispania, S. A., Madrid, Spain) equipped with a flow injection system (PE FIAS-400) coupled with an autosampler (PE AS-90), to provide hydride generation in a flow injection mode; a lyophilizer equipped with a microprocessor controlling the lyophilization process (FTS Systems, New York); a PL 5125 sand bath (Raypa, Scharlau S. L., Barcelona, Spain); a K 1253 muffle furnace equipped with a Eurotherm Controls 902 control program (Heraeus S. A., Madrid, Spain); a KS 125 basic mechanical shaker (IKA Labortechnik, Merck Farma y Química, S. A., Barcelona, Spain) and an Eppendorf 5810 centrifuge (Merck Farma y Química, S. A., Barcelona, Spain).

Reagents. Deionized water (18 MΩ cm) obtained with a Milli-Q water purification system (Millipore Inc., Millipore Ibérica, Madrid, Spain) was used for the preparation of reagents and standards. All chemicals, including standards and solutions, were of *pro analysi* quality or better: hydrochloric acid, $\rho = 1.19$ g ml^{-1} (Merck Farma y Química, Barcelona, Spain); nitric acid, $\rho = 1.38$ g ml^{-1} (Merck); hydrobromic acid, 48% (Fluka, Barcelona, Spain); hydrazine sulfate (Acros, Barcelona, Spain); chloroform (Merck); methanol (Merck); magnesium nitrate (Prolabo, Barcelona, Spain); magnesium oxide (Merck); potassium iodide (Merck); sodium borohydride (Merck).

The stock standard solutions of As III (1000 mg L^{-1}) were prepared by dissolving 1.320 g of arsenic trioxide (Riedel de Haën, Hannover, Germany) in 25 mL of 20% (w/v) KOH solution, neutralizing with 20% (v/v) H_2SO_4, and diluting to 1 L with 1% (v/v) H_2SO_4. The standards of As III were prereduced before use.

The ashing agent used was a mixture of 20% w/v $Mg(NO_3)\cdot 6H_2O$ and 2% w/v MgO. As prereducing solution for the standard and samples before total and inorganic arsenic quantification, a mixture containing 5% w/v KI and 5% w/v ascorbic acid was employed. As reducing solution for hydride generation, sodium tetrahydroborate (III) solution (0.2% w/v), prepared by dissolving $NaBH_4$ powder in 0.05% w/v NaOH solution and filtering through Whatman No. 42 paper, was used. All glassware was treated with 10% v/v HNO_3 for 24 h, and then rinsed three times with Milli-Q water before being used.

Sample Collection. Samples were purchased from retail outlets throughout the Basque Country and collected at regular intervals through the year in different locations (Urieta et al., 1991). The samples used for this study were selected on the basis of their high domestic consumption in the Basque Country. In total, 153 samples of seafood products classified into 13 different food categories were analyzed. Eight of the categories consisted of a single seafood product: *meagrim, hake, small hake, anchovy, Atlantic horse mackerel, sardine, salted cod,* and *squid*. The remaining five encompassed various similar types of seafood products whose individual contribution to the total diet is low: *other white fish* (blue whiting, cod, pouting), *other blue fish* (albacore, salmon, tuna, largehead hairtail, Atlantic pomfret, rubberlip grunt, Atlantic mackerel, bream, trout), *bivalves* (clam, mussel), *crustaceans* (scampi, shrimp, prawn), and *canned products* (tuna, albacore, sardine).

Sample Preparation. The samples were prepared by separating the edible portions from the inedible portions (intestines, scales, heads, and bones). The edible portions were rinsed with distilled water and dried. Each type of seafood was minced and blended to give a homogeneous sample, using an ordinary domestic mixer. Subsequently, the individual samples were frozen at −20 °C and afterward freeze-dried. The lyophilized samples were crushed and homogenized to a fine powder in a domestic mill. The resulting powder was stored in previously decontaminated twist-off flasks and kept at 4 °C until analysis.

Determination of Total Arsenic. The lyophilized sample (0.25 ± 0.01 g) was weighed, and 1 mL of ashing aid suspension and 5 mL of 50% (v/v) HNO_3 were added, and the mixture was evaporated on a sand bath until total dryness. The sample was then dry ashed as described by Ybáñez et al. (1991). The ash from the mineralized samples was dissolved in 5 mL of 50% (v/v) HCl and 5 mL of reducing solution (KI−ascorbic acid). After 30 min, the resulting solution was diluted to volume with 50% (v/v) HCl and filtered through Whatman No. 1 filter-paper into a 25 mL calibrated flask. The instrumental conditions used for arsenic determination by flow injection-hydride generation-atomic absorption spectrometer (FI−HG−AAS) were the following for FI−HG: loop sample, 0.5 mL; reducing agent, 0.2% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH, 5 mL min^{-1} flow rate; HCl solution 10% (v/v), 10 mL min^{-1} flow rate; carrier gas, argon, 100 mL min^{-1} flow rate. Conditions for AAS were: wavelength, 193.7 nm; spectral band-pass 0.7 nm; electrodeless discharge lamp system 2, lamp current setting 400 mA; cell temperature 900 °C.

Determination of Inorganic Arsenic. The method used has been described in a previous paper (Muñoz et al., 1999a). The lyophilized sample (0.50 ± 0.01 g) was weighed into a 50 mL screw-top centrifuge tube, 4.1 mL of water was added, and the sample was agitated until it was completely moistened. Then 18.4 mL of concentrated HCl was added, and the sample was agitated again for 1 h, and then left to stand for 12−15 h (overnight). The reducing agent (1 mL of 1.5% w/v hydrazine sulfate solution and 2 mL of HBr) was added and the sample was agitated for 30 s. Then 10 mL of $CHCl_3$ was added and the sample was agitated for 3 min. The phases were separated by centrifuging at 2000 rpm for 5 min. The chloroform phase was separated by aspiration and poured into another tube. The extraction process was repeated two more times. The chloroform phases were combined and centrifuged again. The remnants of the acid phase were completely eliminated by aspiration (acid phase remnants in the chloroform phase cause substantial overestimates of inorganic arsenic). Possible remnants of organic material in the chloroform phase were eliminated by passing it through Whatman GD/X syringe filters with a 25 mm PTFE membrane (Merck Farma y Química S. A., Barcelona, Spain).

The inorganic As in the chloroform phase was back-extracted by agitating for 3 min with 10 mL of 1 mol L^{-1} HCl. The phases were separated by centrifuging at 2000 rpm, and the aqueous phase was then aspirated and poured into a beaker. This stage was repeated once again and the back-extraction phases obtained were combined. When the back-extraction phase generated emulsions that could not be broken by centrifuging at over 2000 rpm, the emulsion was transferred to the beaker. Ashing aid suspension and HNO_3 were added and the result was heated gently in the sand bath for not more than 30 s. The emulsion was then broken and the chloroform phase formed was removed by aspiration.

The determination of inorganic arsenic in the back-extraction phase was performed by means of the following procedure: 2.5 mL of ashing aid suspension and 10 mL of concentrated HNO_3 were added to the combined back-extraction phases. The result was evaporated and treated in the same way as for total arsenic.

Determination of MMA. The method employed has been described in a previous paper (Vélez et al., 1996). The lyophilized sample was extracted with methanol/water (1:1 v/v) and the extract was collected after centrifugation. This process was repeated three times, and the extracts were evaporated to dryness and redissolved in 3 mL of water. MMA was determined in the water extract by using HPLC−HG−AAS.

RESULTS AND DISCUSSION

Total Arsenic Contents. To interpret the results obtained, the various types of seafood products analyzed were divided into four groups: "white fish," "blue fish," "shellfish," and "preserved fish." The white fish group consisted of fish with a fat content < 1% and demersal and/or benthic habits (meagrim, hake, small hake, and other white fish). The blue fish group contained pelagic fish with a fat content >1% (anchovy, Atlantic horse mackerel, sardine, and other blue fish). The shellfish

Table 1. Levels of Total Arsenic, Inorganic Arsenic, and Humidity in White Fish; Results of Arsenic Expressed in μg g^{-1} (dw)

meagrim ($n = 12$)[a]			hake ($n = 12$)[a]			small hake ($n = 12$)[a]			other white fish ($n = 7$)[a]		
total As	inorganic As	humidity, %	total As	inorganic As[e]	humidity, %	total As	inorganic As	humidity, %	total As	inorganic As	humidity, %
18.30	0.018	77.5	32.04	0.023	80.6	6.28	0.035	81.1	37.84[b]	0.047[b]	80.0
29.94	0.031	79.3	4.51	0.015	81.3	19.57	0.016	78.9	44.89[b]	0.050[b]	80.3
31.68	0.021	79.3	15.04	0.008	81.3	17.50	0.029	79.5	51.34[b]	0.044[b]	80.3
13.42	0.031	85.6	6.71	0.008	83.2	24.29	0.024	80.1	62.91[c]	0.029[c]	81.9
19.24	0.116	76.1	4.10	0.037	80.4	12.54	0.042	80.9	9.49[c]	0.044[c]	78.4
33.04	0.066	76.1	4.67	0.044	80.8	9.93	0.021	80.0	41.65[d]	0.047[d]	81.4
3.08	0.086	74.4	4.71	0.016	80.5	4.05	0.016	80.6	62.97[d]	0.046[d]	78.7
12.18	0.047	80.5	3.72	0.020	81.8	12.58	0.021	80.3			
53.57	0.010	79.5	12.73	0.036	78.0	9.43	0.034	82.0			
23.93	0.028	78.4	6.77	0.054	73.5	15.97	0.032	79.9			
23.26	0.036	73.6	6.22	0.024	78.2	11.88	0.040	76.2			
4.54	0.038	75.9	17.88	0.020	79.1	5.71	0.043	79.5			

Ranges of Levels Found in the Samples Analyzed

meagrim		hake		small hake		other white fish	
total As	inorganic As	total As	inorganic As	total As	inorganic As	total As	inorganic As
3.08−53.57[e]	0.010−0.116[e]	3.72−32.04[e]	0.008−0.054[e]	4.05−24.29[e]	0.016−0.043[e]	9.49−62.97[e]	0.029−0.050[e]

[a] n, Number of samples analyzed. [b] Blue whiting ($n = 3$). [c] Cod ($n = 2$) [d] Pouting ($n = 2$). [e] Limit of quantification for inorganic arsenic, 0.008 μg g^{-1}, dw.

group included the mollusks (bivalves and squid) and crustaceans. The seafood products that had undergone some kind of technological process were grouped as preserved fish (canned fish and salted cod).

Tables 1−4 show the levels of total arsenic [μg g^{-1}, dry weight (dw)], the ranges in which they lie, and the percentages of humidity for four groups: white fish, blue fish, shellfish, and preserved fish. The results are expressed in dry weight to avoid the possibility that different humidity levels in the samples might mask differences between the levels of total and inorganic arsenic.

White Fish. The meagrim and other white fish present the highest levels and the greatest dispersion of data (Table 1). In the case of meagrim, the higher levels may be due to the fact that these benthic fishes, belonging to the order Pleuronectiformes (flatfish), are characterized by feeding predominantly on bivalves, which are filterers and consequently present high levels of arsenic. The hake and small hake, as expected, present similar levels of total arsenic.

The range of levels of total arsenic reported in the literature for white fish ranges from 4.43 to 196.10 μg g^{-1}, dw (Brooke and Evans, 1981; Vlieg et al., 1991; Attar et al., 1992; Leah et al., 1992; Larsen et al., 1993; Branch et al., 1994; López et al., 1994; Ackley et al., 1999; Muñoz et al., 1999a). The highest values correspond to samples of plaice and lemon sole, and are much higher than the highest concentration found in all of the white fish in the present study (62.97 μg g^{-1}, dw) (Table 1). This may be attributed to the fact that the samples of flatfish reported in the literature could have been captured in highly contaminated areas, whereas the ranges found in other samplings were less extensive (20.4−88.8 μg g^{-1}, dw) (Leah et al., 1992), and similar to those found in the present study (3.08−62.97 μg g^{-1}, dw).

Blue Fish. The levels for sardine and anchovy are the highest in the group, and also present a similar distribution (both species belong to the order Clupeiformes) (Table 2). The samples of Atlantic horse mackerel analyzed (2.20−14.57 μg g^{-1}, dw) present lower levels than the samples of anchovy and sardine (Table 2), but are similar to those reported in the literature (0.96−18.75 μg g^{-1}, dw) (Brooke and Evans, 1981; Vlieg et al., 1991; Attar et al., 1992; Larsen et al., 1993; López et al., 1994; Ackley et al., 1999; Muñoz et al., 1999a,b).

The widest range within a group was found in other blue fish, where the sample of rubberlip grunt had a remarkably high level (74.96 μg g^{-1}, dw). As this value is an outlier, the range of total arsenic for the remaining other blue fish analyzed (1.02−20.44 μg g^{-1}, dw) would be of the same order as that found for the other seafood products that make up this group.

Shellfish. The bivalves present the levels of total arsenic distributed in the narrowest range in this group (Table 3). The samples of mussel presumably come from mussel beds, which would explain their uniformity. The levels reported in the literature for bivalves lie in a range of 1.18−24.20 μg g^{-1}, dw (Phillips, 1990; Vlieg et al., 1991; Vyncke et al., 1992; Larsen et al., 1993; López et al., 1994; Schuhmacher and Domingo, 1996; Šlejkovec et al., 1996; Muñoz et al., 1999a,b), similar to the range found in this study (9.15−24.22 μg g^{-1}, dw) (Table 3). The levels reported in the literature for squid vary within a range of 0.89−26.21 μg g^{-1}, dw (Vlieg et al., 1991; López et al., 1994; Vélez et al., 1996; Muñoz et al., 1999 a,b), which overlaps the range found in this study (0.68−34.27 μg g^{-1}, dw) (Table 3).

The crustaceans present the widest range of total arsenic concentrations in the shellfish group and in the whole study (1.24−102.03 μg g^{-1}, dw) (Table 3). The levels reported in the literature for crustaceans also present a very wide range 2.31−149.16 μg g^{-1}, dw) (Brooke and Evans, 1981; Flanjak, 1982; Vlieg et al., 1991; Attar et al., 1992; Larsen et al., 1993; López et al., 1994; Muñoz et al., 1999a,b).

Preserved Fish. The levels of total arsenic for the preserved fish are the lowest in the whole study and also present the least dispersion (Table 4). The canned seafood products correspond to blue fish (tuna, albacore, and sardine), fresh specimens of which were also analyzed (Table 2). The arsenic levels in the fresh

Table 2. Levels of Total Arsenic, Inorganic Arsenic, Monomethylarsonic Acid (MMA), and Humidity in Blue Fish; Results of Arsenic Expressed in μg g^{-1} (dw)

anchovy ($n = 12$)[a]				Atlantic horse mackerel ($n = 12$)[a]		
total As	inorganic As	MMA[c]	humidity, %	total As	inorganic As	humidity, %
2.73	0.042	<0.009	77.8	3.94	0.198	75.1
12.86	0.103	<0.009	76.7	3.75	0.147	75.9
10.55	0.083	<0.009	74.6	3.29	0.084	77.7
12.53	0.197	0.025	60.1	2.37	0.163	74.6
30.73	0.066	<0.009	77.0	4.23	0.059	77.6
19.53	0.189	<0.009	76.9	5.40	0.095	76.7
18.16	0.156	<0.009	72.2	2.20	0.057	75.7
36.87	0.408	<0.009	76.5	2.49	0.097	76.8
16.57	0.137	<0.009	72.8	8.71	0.076	77.5
12.24	0.123	<0.009	73.2	5.79	0.143	77.8
3.72	0.050	<0.009	77.8	14.57	0.035	79.8
4.31	0.065	<0.009	77.1	3.59	0.176	74.7
2.73−36.87[b]	0.042−0.408[b]	<0.009−0.025[b]		2.20−14.57[b]	0.035−0.198[b]	
sardine ($n = 11$)[a]				other blue fish ($n = 17$)[a]		
total As	inorganic As	MMA[c]	humidity, %	total As	inorganic As	humidity, %
10.99	0.221	0.022	67.6	6.29[d]	0.040[d]	70.5
13.97	0.208	0.009	68.8	3.78[e]	0.037[e]	72.8
12.79	0.191	0.028	62.2	1.02[e]	0.031[e]	71.7
3.89	0.176	<0.009	67.5	3.34[f]	0.032[f]	71.4
22.97	0.289	0.013	67.5	5.68[f]	0.142[f]	67.0
18.41	0.172	0.011	74.2	1.06[g]	0.057[g]	72.7
27.62	0.290	0.016	75.8	11.82[h]	0.015[h]	75.0
7.02	0.183	<0.009	72.2	4.62[h]	0.012[h]	75.0
14.32	0.366	0.009	73.2	5.20[h]	0.011[h]	77.1
10.73	0.237	<0.009	67.7	74.96[i]	0.069[i]	78.5
11.92	0.218	0.018	68.9	4.37[j]	0.136[j]	74.7
				20.44[k]	0.082[k]	80.2
				1.02[l]	0.022[l]	74.5
				1.41[l]	0.057[l]	76.9
				1.54[l]	0.036[l]	74.4
				1.60[l]	0.032[l]	73.6
				2.02[l]	0.037[l]	74.6
3.89−27.62[b]	0.172−0.366[b]	<0.009−0.028[b]		1.02−74.96[b]	0.011−0.142[b]	

[a] n, Number of samples analyzed. [b] Ranges of levels found in the samples analyzed. [c] Limit of quantification for MMA, 0.009 μg g^{-1}, dw. [d] Albacore ($n = 1$). [e] Salmon ($n = 2$). [f] Tuna ($n = 2$). [g] Largehead hairtail ($n = 1$). [h] Atlantic pomfret ($n = 3$). [i] Rubberlip grunt ($n = 1$). [j] Atlantic mackerel ($n = 1$). [k] Bream ($n = 1$). [l] Trout ($n = 5$).

Table 3. Levels of Total Arsenic, Inorganic Arsenic, Monomethylarsonic Acid (MMA), and Humidity in Shellfish; Results of Arsenic Expressed in μg g^{-1} (dw)

bivalves ($n = 12$)[a]				squid ($n = 12$)[a]			crustaceans ($n = 11$)[a]		
total As	inorganic As	MMA	humidity, %	total As	inorganic As	humidity, %	total As	inorganic As	humidity, %
24.22[b]	0.651[b]	0.047	84.7	4.07	0.048	80.0	29.72[d]	0.270[d]	78.8
16.12[b]	0.877[b]	0.044	86.3	6.24	0.039	78.4	46.81[d]	0.281[d]	81.1
11.53[c]	0.176[c]	0.036	83.2	27.02	0.038	76.1	102.03[e]	0.208[e]	74.3
11.37[c]	0.213[c]	0.032	82.0	2.16	0.046	83.1	32.85[e]	0.169[e]	85.0
10.12[c]	0.367[c]	0.034	78.6	3.04	0.041	86.7	1.24[f]	0.076[f]	73.4
9.15[c]	0.412[c]	0.031	83.7	0.68	0.022	60.4	7.26[f]	0.199[f]	75.5
12.41[c]	0.284[c]	0.027	81.2	3.99	0.043	74.8	22.48[f]	0.276[f]	76.8
9.42[c]	0.226[c]	0.015	75.4	34.27	0.024	80.1	16.01[f]	0.131[f]	78.4
14.79[c]	0.314[c]	0.035	82.5	11.90	0.046	81.3	34.41[f]	0.120[f]	75.3
17.48[c]	0.385[c]	0.041	82.3	9.08	0.035	76.2	3.26[f]	0.090[f]	74.7
11.83[c]	0.259[c]	0.026	82.9	8.82	0.055	78.8	3.04[f]	0.134[f]	79.1
11.43[c]	0.205[c]	0.024	84.1	11.74	0.026	78.2			
9.15−24.22[g]	0.176−0.877[g]	0.015−0.047[g]		0.68−34.27[g]	0.022−0.055[g]		1.24−102.03[g]	0.076−0.281[g]	

[a] n, Number of samples analyzed. [b] Clam ($n = 2$). [c] Mussel ($n = 10$). [d] Scampi ($n = 2$). [e] Shrimp ($n = 2$). [f] Prawn ($n = 7$). [g] Ranges of levels found in the samples analyzed.

products (sardines and other blue fish) are in general much higher than the processed samples of the same fish. These results agree with those found by Vélez et al. (1996) for anchovy (fresh, 14.93 and 18.80; in vinegar, 5.72 and 6.33) and sardines (fresh, 15.50 and 16.72; canned, 3.27 and 4.85) (results expressed as μg g^{-1}, dw). This difference in levels may be due to loss of part of the soluble arsenic species during processing and storage. In this connection, Vélez et al. (1997) detected arsenic (AB and DMA) in the accompanying liquid in a wide range of fish canned in brine, with the AB found in the accompanying liquid representing 5−67% of the total arsenic in the canned product (fish and brine). The AB in the accompanying liquid can only come from the fish, because none of the ingredients used to make the brine (water and salt) provides this species of arsenic.

The levels of total arsenic for salted cod are the lowest in the whole study, and also lower than the levels in the two samples of fresh cod (other white fish, Table 1). This could be because the salting process to which the cod is subjected causes it to dehydrate, with part of the

Table 4. Levels of Total Arsenic, Inorganic Arsenic, and Humidity in Preserved Fish; Results of Arsenic Expressed in μg g^{-1} (dw)

canned ($n = 11$)[a]			salted cod ($n = 12$)[a]		
total As	inorganic As[f]	humidity, %	total As	inorganic As	humidity, %
0.60[b]	<0.008[b]	50.8	2.22	0.029	78.4
0.89[b]	0.022[b]	55.0	1.92	0.044	71.0
5.09[b]	<0.008[b]	52.7	1.86	0.018	75.8
1.53[b]	0.021[b]	55.3	2.48	0.025	75.4
0.62[b]	0.025[b]	36.6	3.01	0.028	71.3
2.18[b]	0.036[b]	50.1	1.17	0.040	75.5
0.80[b]	0.010[b]	55.0	2.46	0.042	74.8
1.23[c]	0.016[c]	51.0	2.83	0.054	71.8
1.23[c]	0.013[c]	49.1	1.71	0.033	75.2
8.12[d]	0.196[d]	59.7	1.40	0.031	79.8
3.02[d]	0.089[d]	46.3	1.75	0.010	75.9
			1.78	0.055	80.3
0.60−8.12[e]	<0.008−0.196[e]		1.17−3.01[e]	0.010−0.055[e]	

[a] n, Number of samples analyzed. [b] Tuna ($n = 7$). [c] Albacore ($n = 2$). [d] Sardine ($n = 2$). [e] Ranges of levels found in the samples analyzed. [f] Limit of quantification for inorganic arsenic, 0.008 μg g^{-1}, dw.

arsenic species weakly bound to the fish muscle being solubilized in the liquid exuded.

Inorganic Arsenic Contents. The method used to quantify inorganic arsenic also determines 100% of the MMA existing in the seafood product. Previous reports indicated that the mean levels of MMA in seafood products led to a negligible overestimate of inorganic As (Muñoz et al., 1999b). The present study confirmed this assumption in 10 of the 13 categories analyzed, where the levels of MMA were below the quantification limit (LOQ) of the method (LOQ for MMA = 0.009 μg g^{-1}, dw) (Vélez et al., 1996) not affecting the values of inorganic As obtained. However, for anchovy, sardine, and bivalves the MMA levels varied between 0.009 and 0.047 μg g^{-1}, dw. For those samples, the level of inorganic As has been corrected by taking into account the level of MMA (Tables 2, 3). Tables 1 to 4 show the levels of inorganic arsenic expressed as μg g^{-1}, dw, for each of these groups.

White Fish. The levels of inorganic arsenic in the seafood products that make up this group are very low and also show low dispersion (Table 1). There are few data in the literature for inorganic arsenic in white fish, and they vary within a range of <0.05−0.1 μg g^{-1}, dw (Brooke and Evans, 1981; Larsen et al., 1993; López et al., 1994; Muñoz et al., 1999b). This range overlaps the range found in the present study (0.008−0.116 μg g^{-1}, dw) (Table 1).

Blue Fish. The sardines present the highest levels of inorganic arsenic in this group. The values given in the literature for inorganic arsenic in blue fish lie in a range of 0.033−0.300 μg g^{-1}, dw (Brooke and Evans, 1981; Branch et al., 1994; López et al., 1994; Muñoz et al., 1999a,b), which is of the same order as that found in this study (0.011−0.408 μg g^{-1}, dw) (Table 2).

Shellfish. The levels of inorganic arsenic in this group are very different (Table 3). The bivalves (clam and mussel) present the highest levels in the whole study (0.176−0.877 μg g^{-1}, dw). In the literature, the ranges of values for bivalves are in general the highest in seafood products (0.140−1.588 μg g^{-1}, dw) (Larsen et al., 1993; López et al., 1994; Šlejkovec et al., 1996; Muñoz et al., 1999a,b), and are also similar to those found in this study. The squid present the lowest levels of inorganic arsenic in the group (0.022−0.055 μg g^{-1}, dw). There are few data in the literature for levels found in samples of squid [0.035 μg g^{-1}, dw (Muñoz et al., 1999a,b); 0.100 μg g^{-1}, dw (López et al., 1994)], but they

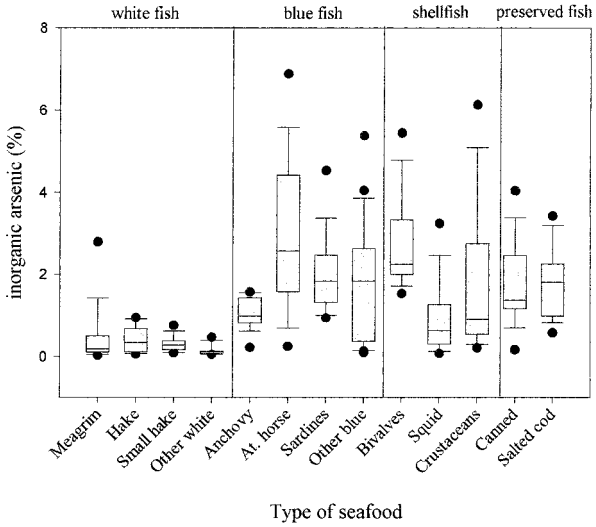

Figure 1. Percentages of inorganic arsenic with respect to total arsenic in the 13 types of seafood products studied. The vertical lines that divide the graph separate the seafood products into the four groups analyzed. The box represents the concentrations in the population found between percentiles 25 and 75. The line dividing the box represents the value of the median. The whiskers below and above the box comprise the concentrations situated between percentiles 10 and 90. The dots represent the outlying data beyond percentiles 10 and 90.

are of the same order as those found in this study. The crustaceans (scampi, shrimp, and prawn) present a level between those of bivalves and squid (Figure 1b). In the literature, levels of inorganic arsenic in crustaceans are generally high, lying in a range of <0.068−1.260 μg g^{-1}, dw (Brooke and Evans, 1981; Flanjak, 1982; Larsen et al., 1993; López et al., 1994; Muñoz et al., 1999a,b).

Preserved Fish. The levels of inorganic arsenic for this group are low, varying within a range of <0.008−0.196 μg g^{-1}, dw and also show low dispersion (Table 4). No data for processed seafood products were found in the literature, so that it was not possible to make a suitable comparison with the values found in this study.

Percentages of Inorganic Arsenic with Respect to Total Arsenic. Figure 1 shows the percentages of inorganic arsenic with respect to total arsenic for the thirteen types of seafood products analyzed. These percentages vary among the different types of seafood and even in the same seafood. The range of percentages

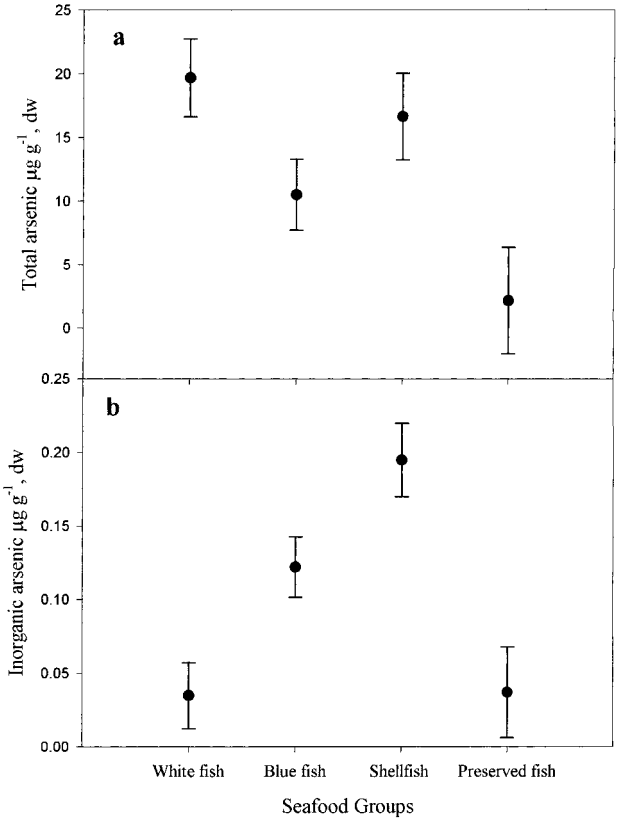

Figure 2. Confidence intervals for the mean concentrations of total arsenic (a) and inorganic arsenic (b) in white fish, blue fish, shellfish, and preserved fish groups. The confidence intervals were obtained by Fisher's least-significant-difference procedure for a significance level of 5%.

is between 0.02 and 6.88%. This range is very similar to that obtained from data in the literature (0.15−11%) (Brooke and Evans, 1981; Flanjak, 1982; López et al., 1994; Muñoz et al., 1999a,b). White fish, as a whole, presents the lowest percentages of inorganic arsenic (0.02−2.79%).

Comparison of Levels of Total and Inorganic Arsenic in the Various Groups of Fish. A one-way ANOVA analysis was performed with four levels: white fish, blue fish, shellfish, and preserved fish. The analysis showed significant differences in the concentrations of total arsenic ($p = 0.002$) and inorganic arsenic ($p < 0.001$). To establish between which groups the differences lie, the confidence intervals for the means of each group were obtained, based on Fisher's least-significant-difference procedure for a significance level of 5%. The intervals are constructed in such a way that if two means are the same, i.e., correspond to a pair of means which have no statistically significant difference, their confidence intervals at 95% will overlap. The results obtained are represented in Figure 2 a and b.

For total arsenic the level in preserved fish is different from the levels in all the others. The white fish and blue fish levels are very different from one another. The shellfish level lies between the levels for white fish and blue fish, with its confidence interval overlapping theirs. The significant differences in preserved fish with respect to the other three groups may be due to alterations in the contents during processing, as suggested earlier.

In the case of inorganic arsenic, the blue fish and shellfish levels are different from one another and also different from the levels of the white fish and preserved fish considered as a whole. No differences are seen between these last two groups. For "shellfish, the differences in inorganic arsenic could be justified on the basis that philogenetically they are very remote from the vertebrates which make up the other three groups, all of which are fish.

In the case of the white fish and blue fish, levels of total arsenic in white fish are higher than those in blue fish, whereas the levels of inorganic arsenic show the opposite. These significant differences between the two groups, in both total and inorganic arsenic, lead us to suggest the existence of different behavior patterns with respect to the efficiency of the metabolism for the conversion of inorganic arsenic. Bearing in mind that the As (V) absorbed is reduced in the blood to As (III) (Vahter et al., 1995), we attributed the difference in metabolism to the ability of arsenite, as established by other researchers (Howard et al., 1995), to replace and/or compete with nitrogen in the metabolic pathways. The levels of nonprotein nitrogen, consisting of ammonium and biogenic amines (analogues of the organoarsenic species), are lower in white fish (200−300 mg per 100 g of sample) than in blue fish (400−800 mg per 100 g of sample) (Contreras, 1994). In our opinion, higher levels of amines present in blue fish could displace the inorganic arsenic from the pathways of detoxification by methylation, causing inorganic arsenic to accumulate in these fishes. This would explain the higher level of inorganic arsenic found in this group. On the other hand, the lower levels of amines present in white fish would enable the inorganic arsenic to be more efficiently methylated and consequently excreted, thereby reducing its concentration in the tissues of those fishes.

Legislation and Health Considerations. At the international level, very few countries have published laws to regulate the maximum concentration of total arsenic in seafood products (British Food Manufacturing Industries Research Association, 1993). The lowest level specified in legislation for fish and fish products is 1 μg g^{-1} wet weight (ww) in Singapore, Malaysia, and Australia. In the present study, the levels of total arsenic vary within a range of 0.30−26.22 μg g^{-1}, ww, with 66% of the samples analyzed presenting levels higher than 1 μg g^{-1}, ww, meaning that they could not be sold in those countries. Because the levels of total arsenic found in this study are representative of the levels present in seafood products, it is to be expected that a large proportion of the products sold would exceed the concentration of 1 μg g^{-1}. Consequently, the validity of such strict legislation in seafood products is questionable, and so is its degree of enforcement. On the other hand, Hong Kong has the most tolerant legislation in the world, with maximum concentrations of 6 μg g^{-1}, ww for fish and fish products and 10 μg g^{-1}, ww, for shellfish and shellfish products. Of the samples analyzed, 14% of the fish products exceeded the level legislated by Hong Kong for those products, whereas only one of the 35 shellfish samples analyzed exceeded 10 μg g^{-1}, ww. With this legislation, most of the samples could be sold, and therefore the situation is very different from the one described previously. This disparity in legislative criteria for maximum permitted levels of total arsenic could cause problems for sales of certain seafood products, without there being proven evidence of a health risk for the consumer. Similarly, the more

permissive legislative systems could allow sales of seafood products with high levels of inorganic As.

The countries that make up the European Union do not restrict the levels of total arsenic in seafood products. This could be attributed to research carried out on fresh seafood products, which has showed that AB contributes at least 80% of the total arsenic in them (Phillips, 1990; Larsen et al., 1993). This finding, together with the negligible toxicity of AB (Sabbioni et al., 1991), may have led to the idea that total arsenic should not be regulated, as ingestion of seafood does not present a health risk for consumers. However, it has recently been shown in fresh and processed seafood products that arsenic from AB represents less than 50% of the total arsenic in some products (Vélez and Montoro, 1998). Consequently, there is a need to quantify the inorganic arsenic and carry out studies to extend the information available about the levels of other potentially dangerous arsenic species (MMA, DMA, and TMA^+).

With respect to inorganic arsenic, there are only two countries in the world that have legislation to regulate the maximum level in seafood (British Food Manufacturing Industries Research Association, 1993). Australian legislation permits 1.0 μg g^{-1}, ww, of inorganic arsenic as the maximum level in seaweed (edible kelp), and the legislation in New Zealand allows 2.0 μg g^{-1}, ww, in fish and fish products. The levels of inorganic arsenic reported in this study are considerably below the only value legislated in fish and fish products (2.0 μg g^{-1}, ww), because, of the 153 samples analyzed in this study, only 2% exceed a concentration of 0.1 μg g^{-1}, ww, of inorganic arsenic, and none of them reach 0.2 μg g^{-1}, ww.

Taking the New Zealand legislation as a basis and assuming a seafood product that contains the maximum permitted level of inorganic arsenic, a daily consumption of approximately 70 g of product (edible portion) would be needed to reach the Provisional Tolerable Weekly Intake − PTWI − (15 μg inorganic arsenic/kg of body weight/week) established by the WHO (1989), which, for a person of 68 kg, would correspond to 146 μg day^{-1}. Although the mean consumption of seafood products varies considerably according to the country considered, a daily intake of 70 g is attained in certain areas such as the Basque Country, where mean daily intake of seafood is 89 g day^{-1} (Urieta et al., 1991). In the worst of cases, represented by clams (a product that is consumed both raw and cooked), which present the highest level of inorganic arsenic in the whole range of samples (0.100 and 0.121 μg g^{-1}, ww), 1300 g day^{-1} of edible portion of raw product (> 5 kg of product with valves), would have to be consumed in order to reach the PTWI for a person weighing 68 kg.

Despite what was stated earlier, there are reasons that indicate the need to legislate on the basis of levels of inorganic arsenic instead of total arsenic. One reason is that seafood products with high levels of inorganic arsenic (clams, bivalves, and crustaceans) could present a risk to consumers with high intake, who might reach intakes exceeding the PTWI. For example in Taiwan a maximum consumption rate of 139 g day^{-1} of oysters per individual can be reached (Han et al., 1998). Nevertheless the intake of inorganic arsenic attributable to the oysters has not yet been established, because inorganic arsenic has been estimated assuming that it accounts for 10% of total arsenic content. On the other hand, it would be advisable to have regulatory measures designed to ensure the harmlessness of products intended to be sold from world areas with high arsenic contents as a result of natural or anthropogenic causes. For such regulation, total arsenic is not a suitable parameter. Moreover, high levels of total arsenic do not always imply high levels of inorganic arsenic, and similarly, low levels of total arsenic do not always imply low levels of inorganic arsenic. Various samples analyzed corroborate this statement, i.e., two samples of crustaceans: shrimp (total arsenic 102.03 μg g^{-1}, dw; inorganic arsenic: 0.208 μg g^{-1}, dw) and prawn (total arsenic: 7.26 μg g^{-1}, dw; inorganic arsenic: 0.199 μg g^{-1}, dw). This implies ratios of inorganic arsenic/total arsenic of 0.002 for shrimp and 0.030 for prawn. Legislation based on total arsenic might simply prevent sales of the shrimp, which has a higher level of total arsenic, whereas the toxicological risk, determined by the level of inorganic arsenic, is the same for the two products.

Consequently, the best way of going about introducing legislation for a contaminant such as arsenic in seafood products would be to follow the model of the New Zealand legislation, regulating the maximum permitted levels for inorganic arsenic, thereby only preventing sales of those products that, as a result of contamination of natural or human origin, may reach levels of inorganic arsenic harmful to human health.

CONCLUSIONS

Because inorganic arsenic comprises the more toxic species, it is the maximum level of inorganic arsenic that should be regulated in legislation for arsenic in seafood products. The introduction of legislation for levels of total arsenic in seafood products does not guarantee the harmlessness of the product.

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