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Assessing Arsenic Exposure from Consumption of Seafood from Vieques-Puerto Rico: A Pilot Biomonitoring Study Using Different Biomarkers

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Abstract The various toxic effects associated with inorganic arsenic (iAs) warrants that exposure sources be identified. This pilot study evaluated if greater seafood consumption from Vieques-Puerto Rico is associated with increased exposure to iAs. Nail, hair, and urine samples were used as biomarkers of iAs exposure in adult women and men from Vieques classified as high ($n = 31$) and low ($n = 21$) seafood consumers, who reported eating fish and/or shellfish $\geq$1 time per week and once per month or less, respectively. The sum of urinary iAs (As III + As V), monomethylarsonic acid (MA[V]), and dimethylarsinic acid (DMA[V]), denoted as SumAs, fluctuated from 3.3 μg/g Cr (1.2 μg/L) to 42.7 μg/g Cr (42 μg/L) ($n = 52$). Levels of As in nail samples ($n = 49$) varied from 0.04 to 0.82 μg/g dry weight (dw), whereas in hair ($n = 49$) As was only detected in 49 % of the samples with a maximum value of 0.95 μg/g dw. None of the biomarkers of exposure to As exceeded exposure reference values for urine (50 μg/g Cr or 50 μg/L), nails (1 μg/g), or hair (1 μg/g). However, median (10.0 μg/g Cr; 10.6 μg/L) and 95th percentile (31.9 μg/g Cr; 40.4 μg/L) of urinary SumAs were higher in Vieques samples than in the those from the general population of other countries. Among the three biomarkers of exposure, nail samples reflected better the exposure to iAs from seafood consumption with significantly higher average As concentrations in high (0.24 μg/g) than low (0.12 μg/g) seafood consumers. Multivariate results for As in nail samples ($R^2 = 0.55$, $p < 0.0001$) showed a positive association with fish consumption, particularly for men, with levels increasing with years of residency in Vieques.

Arsenic (As) is an ubiquitous element found in the environment in different chemical forms and with various degrees of toxicity. Arsenite (As III) and arsenate (As V), both inorganic As species (iAs), are considered the most toxic environmental and occupational As chemical forms (Agency for Toxic Substances and Disease Registry 2007; Hughes 2006; Navas-Acien et al. 2011). Among the toxic effects associated with iAs are skin disorders, cardiovascular disease, hypertension, peripheral and central nervous system toxicity, reproductive effects, diabetes mellitus, and various types of cancer (e.g., skin, bladder, lung, kidney) (Coronado-González et al. 2007; European Food Safety Authority 2009; Ettinger et al. 2009; Hall et al. 2007; Huang et al. 2009).

Arsenic in the environment occurs from both natural and anthropogenic sources. Drinking groundwater is considered the main natural exposure source to iAs, although recently some food commodities, such as rice grains and rice-based products, are receiving much attention as other important natural dietary sources of iAs (European Food Safety Authority 2009; Joint FAO/WHO Expert Committee on Food Additives 2011; Zhu et al. 2008). Consumption of

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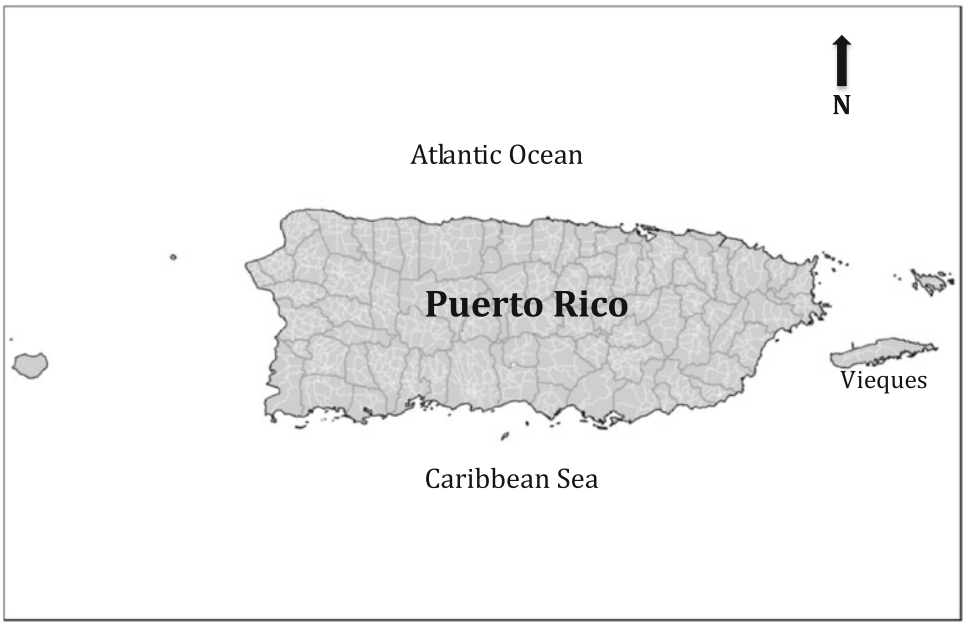

Fig. 1 Location of Vieques, Puerto Rico

fish and other seafood is generally regarded as a minor natural source to iAs because it is considered a major source of nontoxic organic arsenicals, primarily arsenobetaine (AB) (Borak and Hosgood 2007; European Food Safety Authority 2009; Joint FAO/WHO Expert Committee on Food Additives 2011). Anthropogenic activities such as pesticide use in agriculture and wood preservation, coal burning power plants, smelting activities, and military-related activities are known to increase iAs concentrations above natural background levels, resulting in the contamination of soil, water, air, and food (Agency for Toxic Substances and Disease Registry 1997; Cullinane et al. 1988; Hewitt et al. 1995; Hughes 2006; Li et al. 2008; Tsuji et al. 2005; United States Environmental Protection Agency 1992; Wilhelm et al. 2005).

Vieques, an island-municipality of Puerto Rico, is located 9.6 km from the southeast coast of Puerto Rico (Fig. 1). It measures $\sim$145 km^2 and has an estimated population of 9,100 inhabitants (United States Environmental Protection Agency 2009). Vieques does not have major agricultural or industrial activities, and the water supplied to households in Vieques (obtained from mainland Puerto Rico) meets treated—United States Environmental Protection Agency (USEPA) water standards. Since the 1940s, two thirds of Vieques has been used by the United States Navy for naval gunfire support, air-to-ground training, and ammunition storage (United States Environmental Protection Agency 2009). Military practices ceased in May 2003 resulting in the devolution of military lands to local and federal governments, except for certain areas of Vieques (e.g., the Atlantic Fleet Weapons Training Facility) that were included on the USEPA Superfund National Priority List due to the presence of unexploded ordnance and other contaminants (United States Environmental Protection Agency 2009). Therefore, people from Vieques are still concerned about their potential exposure to military-related contaminants from the environment (Agency for Toxic Substances and Disease Registry 2011), especially if their seafood supply is threatened by marine pollution, such as trace metals.

These past military activities could lead to changes in the transport, distribution, and concentrations of trace metals (i.e., iAs) in the coastal marine environment of Vieques, thus enhancing metal bioavailability in marine organisms (Massol-Deyá et al. 2005). For instance, Acevedo-Marín (2004) obtained significantly higher concentrations of Total As, among eight metals studied, in skinless muscle tissues of eight different edible fish species captured in coastal waters of Vieques. Some of the fish samples had As levels above an international criteria value of 2 μg/g wet weight (ww) (Summers et al. 1995). Although As speciation was not performed in fish muscle tissues, when assuming a conservative estimate of 10 % of the Total As concentration as being inorganic (United States Food and Drug Administration 1993), and using the USEPA risk-assessment methodology, levels in edible fish from coastal waters of Vieques were found to represent a human health risk for systemic and cancer effects (Acevedo-Marín 2004). In another study, Agency for Toxic Substances and Disease Registry (2003) reported As concentrations as high as 29.3 μg/g ww in five families of fish species and 48.3 μg/g ww in a shellfish (lobster). Despite the use of two thirds of Vieques for military activities for >60 years, Vieques has an active fishing community. In a previous study performed by Caro et al.

(2000), 80 % of a representative population sample was reported to consume locally caught fish at least once per week. Therefore, there is a concern that levels of As in fish and other aquatic organisms from coastal waters of Vieques represent a significant exposure source to iAs.

To determine if significant human exposure to iAs is occurring, biomonitoring studies are performed using urine, nails, and/or hair as biomarkers of exposure (Hughes 2006; Marchiset-Ferlay et al. 2012). Each biomarker has its own strengths and limitations and measures different time frames of exposure to iAs. For example, urinary arsenic speciation generally allows to differentiate exposure to inorganic from organic (i.e., from seafood) As species and to evaluate the metabolism of iAs by humans (Kile et al. 2009; Navas-Acien et al. 2011; Rivera-Núñez et al. 2012). The sum of urinary iAs (As III + As V) and its methylated metabolites monomethylarsonic acid (MA) and dimethylarsinic acid (DMA) (denoted as SumAs) is used as a biomarker of recent exposure (e.g., days) to iAs (Hughes 2006). Using the proportion or percentage of individual As species in urine with respect to SumAs provides an estimate of the degree of iAs metabolism in the human body (Kile et al. 2009; Steinmaus et al. 2005). However, levels of urinary DMA could also reflect exposure to seafood-derived organic As species (e.g., AB, arsenosugars, and arsenolipids), thus overestimating the exposure and metabolism of iAs (Navas-Acien et al. 2011; Sele et al. 2012; Taleshi et al. 2010). Because of the influence that seafood-derived organic As species have on urinary DMA levels, seafood intake must be considered in exposure studies of iAs (Navas-Acien et al. 2011; Soleo et al. 2008). Contrary to urine, nail and hair testing provides an indication of iAs exposure that occurred months before analysis. Hair and nails reflect exposure times of 2–5 and 6–18 months, respectively (Samanta et al. 2004; Slotnick and Nriagu 2006). A major concern with these two biomarkers of chronic exposure is the adsorption of As from external sources. Both biomarkers require an extensive washing procedure to remove the adsorbed As (Morton et al. 2002; Phan et al. 2011; Slotnick and Nriagu 2006). Despite the limitations of these three biomarkers, they are suitable for measuring chronic low-level As exposure in human populations (Brima et al. 2006; Rivera-Núñez et al. 2012; Saad and Hassanien 2001; Wilhelm et al. 2005). Arsenic in urine, hair, and nails has been associated with various exposure sources, such as drinking water, soil, air, and food intake (including seafood) (Fillol et al. 2010; Mandal et al. 2003; Phan et al. 2011; Rivera-Núñez et al. 2012; Slotnick et al. 2007; Soleo et al. 2008; Tsuji et al. 2005; Wilhelm et al. 2005).

Based on levels of As measured in fish and shellfish from coastal waters of Vieques (Acevedo-Marín 2004; Agency for Toxic Substances and Disease Registry 2003) and on fish-consumption practices of its population (Caro et al. 2000), this study aimed to determine if higher consumption of seafood (fish and shellfish) from Vieques is associated with increased iAs concentrations in urine, nails, and hair from two groups of adult people that were classified as high and low seafood consumers.

Materials and Methods

Participants

Research personnel, with the assistance of a local fisherman, visited houses in July 2006 in different areas of Vieques to identify potential participants, mainly adult men and women sharing the same household. The inclusion criteria for this pilot study were as follows: (1) to be an adult (>21 years of age), (2) residential time in Vieques >5 years, (3) if employed, to have her/his job in Vieques, and (4) to eat seafood (fish and/or shellfish) ≥1 time/week for high seafood consumers, and ≤1 time/month for low seafood consumers. This cut-off point for seafood consumption is based on findings by Wilhelm et al. (2005) who reported significantly higher nail As in individuals consuming fish >1 time/week. After explaining the purpose of the study, those who agreed to participate provided written consent as approved by the University of Puerto Rico (UPR) Medical Sciences Campus (MSC) Human Institutional Review Board. Each participant was provided with a self-administered questionnaire that recorded personal information on seafood (fish and shellfish)—consumption patterns, such as types of seafood consumed, consumption frequency (e.g., times per week), and seafood origin (e.g., if from coastal waters of Vieques) among others. Additional information gathered in the questionnaire included age, gender, weight, height, self-perceived health status, education, years of residency in Vieques, dietary habits (e.g., vitamin and home-grown product intake), occupation, and lifestyles (cigarette smoking and alcohol consumption). The only requested information on drinking water source was whether participants currently drink water from wells. Urine, nail, and hair samples of participants classified as high and low seafood consumers were obtained to determine As concentrations.

Biological Sampling

Participants were instructed to collect their first-voided morning (FVM) urine samples into coded nitric acid-precleaned polyethylene bottles with powder-free gloves provided to avoid contamination. Containers with urine samples were placed in plastic bags and refrigerated. After collection of FVM urine samples, field personnel visited

the house to pick up containers with urine samples, which were then placed in an ice cooler and stored in a freezer at −20 °C.

For the analysis of nails, participants were initially asked to collect toenails clippings; however, due to the difficulty in obtaining enough samples, the sample was changed to fingernails from all ten fingers. A nail cutter was provided with instructions to place the nail samples in a preidentified zip-closure bag and to store them similarly to urine samples. Urine and nail samples were sent to Columbia University (CU) Trace Metals Core Facility Laboratory, New York, USA, for processing and chemical analysis.

Hair samples were taken by research personnel from different locations from the occipital area using stainless-steel blunt tip scissors. Hair samples were placed in a zip-closure bag, placed in a cooler, and transferred to the Department of Environmental Health, UPR–MSC, laboratory to be stored at −20 °C until further analysis.

Sample Processing and Analysis

The analysis of urinary As species at CU consisted of determining the inorganic species As III and As V; the metabolites MA[V] and DMA[V]; and the seafood-derived organic arsenicals AB and arsenocholine (AC) using a Perkin Elmer (PE) inductively coupled plasma-mass spectrometer model ELAN Dynamic Reaction Cell (DRC) II coupled to a PE high-pressure liquid chromatograph series 200 and modified methods used by Reuter et al. (2003) (Chen et al. 2013; Hall et al. 2007). Briefly, 50 µL of 1:10—diluted (with high-performance liquid chromatography [HPLC] mobile phase) urine sample was injected and urinary As species separated using a Hamilton PRP X-100 anion exchange HPLC column (4.1 × 250 mm, 10 µm). The HPLC mobile phase consisted of 10 mM ammonium nitrate + 10 mM ammonium phosphate buffer (pH 9.1) with a flow rate of 1.5 mL/min at ambient temperature (Slavkovich, personal communication, 8 June 2011). Polyatomic interferences were suppressed with the instrument's DRC technology feature using oxygen as a second gas (Wasserman et al. 2011). Urinary As concentrations were adjusted for urinary creatinine (Cr) levels (Hall et al. 2007). Standard solutions were used for instrument calibration. Concentrations of As in standard solutions were chosen to cover the expected As concentrations range in urine samples: 5, 10, and 25 µg/L. For quality control (QC), two different types of samples were used: (1) urine samples with known As concentration were purchased from the Institut de Santé Publique du Québec (Québec, Canada), a laboratory from the ICP-MS Comparison Program for validation analyses; and (2) a set of urine specimens prepared at CU laboratory, each spiked with one of the As species (As III, As V, MA, DMA, AB, and AC) (Gamble et al. 2006; Hall et al. 2007). Total As concentration in these samples were first confirmed by graphite furnace atomic-absorption spectrometry and later run through HPLC–ICP–MS as follows: all QCs were run in the beginning of every working day after initial calibration and then throughout the day after every ten samples (Hall et al. 2007). The separation by HPLC coupled to the ICP–MS–DRC allowed the detection of AC, AB, MA, DMA, As III, and As V without on-line digestion of organic forms (Chen et al. 2013; Gamble et al. 2006). The minimum reporting limit (MRL) was 0.1 µg/L per individual As species (Hall et al. 2007; Slavkovich, personal communication, 2011).

Nail washing and digestion procedures used at CU were performed using methods described by Chen et al. (1999) and Das et al. (1995). To remove external surface contamination, nail samples were placed in a beaker containing 1 % Triton-X-100, sonicated for 30 min, and rinsed several times with deionized water (dw). Acetone was added and the samples sonicated for 10 min, followed by several rinses with dw, and sonicated for an additional 10 min in dw. Nails were dried overnight at 60 °C, weighed, and placed in an acid-washed 15-ml polypropylene tube. A 1 mL of concentrated HNO_3 was added to samples weighing ≤100 mg, and 2.5 mL was added to samples weighing >100 mg. Samples were left to digest for 36 h. After digestion, samples were transferred into 10-mL volumetric flasks (for those weighing ≤100 mg) or 25-mL volumetric flasks (for those weighing >100 mg) and diluted with dw. Arsenic in nails was analyzed using an ICP–MS–DRC method developed from Pruszkowski et al. (1998) with modifications and adjustments based on suggestions from the Perkin Elmer application laboratory (Slavkovich, personal communication, 2011). The final acid concentration for all samples was 10 %, and calibration standards for ICP–MS–DRC work were made in 10 % HNO_3. Digested nail samples were spun for 10 min at 400 rpm to avoid clogging of the ICP–MS–DRC sample introduction system (Slavkovich, personal communication, 2011). QC nail samples were obtained from the Laboratory for ICP–MS Comparison Program (Quebec, Canada) to cover the range of As concentrations and run each day. During the period in which all samples of this study were analyzed, the intraprecision coefficient of variation for nail As was 5.5 % (Slavkovich, personal communication, 2011). The MRL for As in nail was 0.1 µg/L based on the ICP–MS–DRC analysis.

For hair, before the digestion procedure, samples were double-washed, to remove exogenous materials adsorbed to hair with agitation in a mixture of ethyl ether and acetone (v/v, 3 + 1) for 10 min as described by CEM (1991a). After drying at 85 °C for 1 h, samples were treated with a

5 % aqueous ethylene diamine tetraacetic acid solution for 1 h, rinsed with dw, and finally dried at 106 °C during 24 h for determination of dry weight. For the extraction of As, hair samples of 0.4 g, on average, were digested in 5 mL of HNO_3 using a microwave sample preparation unit Model 1000 (CEM) for 40 min with a maximum pressure of 130 PSI (CEM 1991b). Samples were filtered, collected in a 15-mL polyethylene plastic tube, further diluted to 10 mL with dw, and analyzed by GFAAS using a Perkin Elmer atomic absorption spectrophotometer (AAS) AAnalyst 800. A standard reference material, GBW07601—Trace elements in human hair powder (LGC Promochem, Barcelona, Spain), was used as quality control. Blank verification samples were included during AAS analysis to ensure no carryover of As from one sample to the other. The MRL for hair As was 1.0 μg/L based on the AAS analysis.

Statistical Analysis

Summary statistics, frequency distributions, and regression/correlation analyses were performed using STATA™ v. 12.0 (StataCorp, College Station, Texas, USA) for quantitative and qualitative variables. Student *t* test analysis ($p < 0.05$) was used to determine differences in means between the two seafood consumer groups for age, body mass index (BMI), years of residency, and $\log_{10}$—transformed average As concentrations in biological samples. Fisher proportion extended test was applied to categorical variables, such as educational attainment, salary, seafood consumption frequency, consumption of home grown-products, alcohol drinking, and smoking habits. Simple linear regression models of $\log_{10}$—transformed As levels in biological samples as a function of environmental and sociodemographic variables were fitted. Variables with a significance level ≤ 0.1 in the bivariate analysis and with biological plausibility were included in a multiple linear regression model to determine the effect of individual variables on As concentrations in biological samples after controlling for potential confounders. Spearman correlation analyses were applied to determine the relationship of As among the three biomarkers of exposure.

Results

Participant Characteristics

Selected characteristics of high and low seafood consumers ($n = 52$) are listed in Table 1. Based on the information provided on seafood consumption patterns, 31 ($n = 31$) adults (14 women and 17 men) were identified as high seafood consumers, and 21 ($n = 21$) adults (12 women and 9 men) were classified as low seafood consumers. Both groups of seafood consumers showed similarities in terms of years of residence in Vieques, educational attainment, monthly salary, BMI, consumption of home-grown products, alcohol drinking, and smoking habits (Table 1). None of the participants reported to be currently drinking water from wells (data not listed on Table 1). Low seafood consumers were, on average, significantly ($p = 0.005$) younger that high seafood consumers, with women in the low seafood consumer group being on average about 10 years younger ($p = 0.045$).

In terms of seafood consumption, the high seafood consumer group reported to consume both fish and shellfish more frequently than the low seafood consumer group, with fish reported to be substantially more frequently consumed than shellfish (Table 1). The most commonly fish species reported as consumed by high seafood consumers were grunt (*Haemulon* spp.), snapper (*Lutjanus/Ocyurus* spp.), and parrot fish (*Scarus/Sparisoma* spp.), whereas shellfish included lobster (*Panulirus* spp.), octopus (*Octopus* spp.), queen conch (*Strombus* spp.), and the gastropod *Cittarium* spp. (data not listed on Table 1). Seafood intake within 72 h before biological sampling was greater in the high seafood consumer group (Table 1). Women tended to consume less seafood than men, even within the same household. Participants responded in the questionnaire that the seafood species they consumed were from Vieques, except for two high seafood consumers who also reported consuming fish (e.g., tilapia) from other places.

Urinary As Species

Urinary As speciation analysis mainly identified iAs (As III and As V), As pentavalent metabolites (MA and DMA), and the organic arsenical AB. The sum of all of these species were included in the calculation of total urinary As (denoted as Total As), whereas the summation of iAs and its two metabolites MA and DMA are denoted as SumAs (Table 2). Not included in the calculation of Total As were as follows: AC, detected only in two samples at 0.8 and 0.9 μg/g Cr; and an As species suspected to be a thioarsenical (Slavkovich, personal communication, 19 August 2013) detected in 38 % of the urine samples with a maximum concentration of 3.9 μg/g Cr.

Concentrations of urinary iAs were undetected in 44 % ($n = 23$) of the samples, whereas AB and MA were not detected in one urine sample each. Arsenic levels were quantifiable in all nail samples ($n = 49$) analyzed but in only 49 % ($n = 24$) of the hair samples. To facilitate average and proportion calculations, biological samples with As values lower than the MRL were given half the MRL value (Aguilera et al. 2008; Huang et al. 2009).

Table 1 Selected characteristics of participants

Characteristics	High seafood consumers	Low seafood consumers	*p*
Age in years (±SD)			
Female	53.0 ± 12.4 ($n = 14$)	42.9 ± 11.9 ($n = 12$)	0.045
Male	54.5 ± 12.3 ($n = 17$)	45.2 ± 11.7 ($n = 9$)	0.075
Combined	53.8 ± 12.2 ($n = 31$)	43.9 ± 11.5 ($n = 21$)	0.005
Years of residency in Vieques (±SD)	44.6 ± 19.4 ($n = 29$)	42.0 ± 11.4 ($n = 20$)	0.600
Educational attainment	$n = 30$	$n = 21$	
<High school	9	5	0.057
High school	17	7	
>High school	4	9	
Monthly salary ($)	$n = 24$	$n = 17$	
<500	5	1	0.251
500–799	5	2	
800–999	3	2	
1,000–1,299	4	1	
≥1,300	7	11	
BMI (kg/m^2) ± SD			
Female	27.1 ± 5.2 ($n = 12$)	30.0 ± 6.3 ($n = 12$)	0.229
Male	29.0 ± 6.4 ($n = 15$)	32.3 ± 5.8 ($n = 9$)	0.225
Fish consumption	$n = 31$	$n = 21$	
≥once/week	30	0	<0.001
≤once/month	1	21	
Shellfish consumption	$n = 28$	$n = 21$	
≥once/week	12	0	<0.001
≤once/month	16	21	
Seafood intake in the past 72 h[a]	$n = 21$	$n = 18$	
Yes	14	2	
No	7	16	0.001
Consume home–grown plants[b]	$n = 30$	$n = 20$	
Yes	27	17	0.672
No	3	3	
Consume home–grown animals[c]	$n = 30$	$n = 18$	
Yes	9	6	1.000
No	21	12	
Alcohol consumption	$n = 30$	$n = 20$	
Yes	15	6	0.243
No	15	14	
Current smokers	$n = 31$	$n = 19$	
Yes	3	1	1.000
No	28	18	

SD standard deviation

[a] Seafood consists of fish and shellfish

[b] Include fruits, vegetables, and seasoning plants

[c] Include chicken (other birds), rabbit, goat, pigs, and cow

Table 2 lists the arithmetic mean, range, and percentiles of As species in urine, nails, and hair samples of high and low seafood consumers. The urinary SumAs in all 52 samples ranged from 3.3 to 42.7 μg/g Cr, equivalent to 1.2–42.0 μg/L (Table 2). The maximum metabolic index value, as determined by the ratio of MA/DMA, was 0.53, corresponding to a high seafood consumer (Table 2). There were no statistical significant differences between high and low seafood consumers for urinary mean $\log_{10}$—transformed concentrations of SumAs, MA, and DMA, or for the ratio of MA/DMA and their percentages (Table 2). The average percent of iAs was approximately two-fold lower for high seafood consumers than for low seafood consumers ($p < 0.05$), although its average concentration did not exhibit statistical significance difference. Arsenobetaine was the only urinary As species that displayed

Table 2 Arithmetic mean values, range and percentiles of As species in urine, nail, and hair samples

Biomarker	High seafood consumers					Low seafood consumers				
	Mean (SD)	Range	Percentiles			Mean (SD)	Range	Percentiles		
		Minimum–maximum	25th	50th	95th		Minimum–maximum	25th	50th	95th
Urine	$n = 31$					$n = 21$				
SumAs										
μg/g Cr	12.8 (8.5)	3.3–42.7	7.2	9.7	31.9	11.9 (8.4)	3.6–41.0	6.2	10.5	21.3
μg/L	13.5 (10.9)	1.2–42.0	6.3	8.5	40.4	14.7 (10.1)	2.6–41.1	9.6	13.0	29.5
iAs										
μg/g Cr	0.41 (0.54)	0.02–2.2	0.05	0.17	1.7	0.63 (0.57)	0.02–2.1	0.10	0.59	1.8
μg/L	0.46 (0.65)	0.05–2.5	0.05	0.05	1.8	0.79 (0.75)	0.05–2.6	0.05	0.66	2.1
%[a]	3.4 (3.8)*	0.12–17.4	0.7	1.4	9.0	6.1 (5.1)	0.17–17.5	1.7	5.4	17.0
MA										
μg/g Cr	1.5 (0.8)	0.18–4.1	0.97	1.5	3.0	1.2 (0.5)	0.43–2.1	0.73	1.2	2.1
μg/L	1.6 (1.2)	0.05–5.5	0.84	1.4	4.5	1.4 (0.9)	0.29–3.5	0.88	1.2	2.7
%[a]	13.8 (6.4)	4.1–32.9	9.0	12.9	26.4	11.2 (3.7)	5.2–18.8	8.4	11.2	16.5
DMA										
μg/g Cr	10.9 (7.7)	2.0–39.3	5.8	9.0	25.5	10.1 (8.1)	2.9–38.8	5.0	7.0	19.4
μg/L	11.4 (9.8)	0.9–39.5	5.0	7.1	37.2	12.4 (9.0)	2.0–36.8	7.6	11.8	27.9
%[a]	82.9 (7.6)	61.9–93.9	77.5	84.2	93.3	82.7 (8.0)	63.8–94.6	79.3	82.8	93.4
Total As[b]										
μg/g Cr	43.4 (44.0)**	5.3–189.9	17.6	29.6	139	24.8 (46.7)	6.1–220.9	9.4	12.2	125
μg/L	44.0 (54.3)	1.7–265.2	13.2	29.3	199	28.4 (37.2)	4.2–169.4	8.1	14.5	115
AB[b]										
μg/g Cr	30.5 (42.9)**	1.2–185.4	5.2	14.9	117	14.3 (43.9)	0.02–199.6	1.4	3.7	109
μg/L	30.5 (50.6)**	0.5–223.2	3.9	14.7	194	14.4 (34.2)	0.05–153.1	1.4	2.6	98.3
%[c]	54.8 (25.6)**	5.5–97.6	30.8	53.8	88.1	31.1 (24.8)	0.26–90.4	10.8	27.9	81.1
MA/DMA	0.17 (0.10)	0.05–0.53	0.11	0.16	0.36	0.14 (0.06)	0.05–0.29	0.09	0.14	0.24
Nails	$n = 29$					$n = 20$				
μg/g	0.24 (0.16)**	0.07–0.82	0.14	0.19	0.56	0.12 (0.07)	0.04–0.27	0.07	0.09	0.26
Hair	$n = 29$					$n = 20$				
μg/g	0.12 (0.22)	0.008–0.95	0.02	0.04	0.72	0.05 (0.05)	0.006–0.18	0.01	0.02	0.17

SumAs = iAs + MA + DMA; iAs = AsIII + AsV; Total As = iAs + MA + DMA + AB

SD standard deviation

[a] Percent calculated based on SumAs

[b] For low seafood consumers, $n = 20$ after removing an AB outlier

[c] Percent AB calculated based on Total As

* $p < 0.05$

** $p \leq 0.01$

significant differences ($p \leq 0.01$) between these two consumer groups (higher for high seafood consumers), expressed as both average concentration and percentage (Table 2). A high urinary AB concentration (1,373 μg/g Cr) from a participant in the low seafood group (data not included in Table 2) was treated as an outlier and rejected after using the Grubbs test (Taylor 1987). Therefore, it was not included in any of the statistical analyses.

As in Nail and Hair Samples

For nail samples, average As concentrations ranged from 0.07 to 0.82 μg/g in the high seafood consumer group and from 0.04 to 0.27 μg/g in the low seafood consumer group. High seafood consumers obtained statistically significant ($p \leq 0.01$) higher As concentrations in nails (0.24 μg/g) than low seafood consumers (0.12 μg/g), contrasting the

Table 3 Associations of As concentrations in urine (μg/g Cr) and nails (μg/g) with selected sociodemographic and consumption characteristics using simple linear regression analysis

Independent variables	Urine											Nails		
	n[a]	SumAs		iAs		MA		DMA		AB				
		β[b]	R^2 (%) (p)	β[b]	R^2 (%) (p)	β[b]	R^2 (%) (p)	β[b]	R^2 (%) (p)	β[b]	R^2 (%) (p)	n	β[b]	R^2 (%) (p)
Age	52	0.002	1.21 (0.44)	–0.003	0.35 (0.68)	0.003	3.92 (0.16)	0.002	0.88 (0.51)	0.015	6.35 (0.07)	49	0.010	21.88 (**0.001**)
Gender	52	–0.041	0.66 (0.57)	0.007	0.000 (0.96)	–0.028	0.35 (0.68)	–0.046	0.71 (0.55)	–0.138	0.83 (0.52)	49	0.253	19.06 (**0.002**)
Years of residency	49	0.004	7.18 (0.06)	–0.001	0.06 (0.86)	0.005	11.42 (**0.02**)	0.004	6.87 (0.07)	0.004	0.95 (0.51)	47	0.009	25.84 (**0.0003**)
Seafood consumption	52	0.036	0.48 (0.62)	–0.250	4.28 (0.14)	0.104	4.71 (0.12)	0.037	0.44 (0.64)	0.713	21.3 (**0.0007**)	49	0.312	27.94 (**0.0001**)
Fish consumption	52	0.050	0.94 (0.49)	–0.257	4.58 (0.13)	0.119	6.17 (0.08)	0.050	0.82 (0.52)	0.765	24.9 (**0.0002**)	49	0.265	20.36 (**0.001**)
Shellfish consumption	49	–0.081	2.03 (0.33)	–0.109	0.64 (0.59)	–0.052	0.99 (0.50)	–0.086	1.94 (0.34)	0.135	0.59 (0.603)	46	0.204	8.93 (**0.04**)
Seafood intake in the past 72 h	39	–0.042	0.57 (0.65)	–0.347	7.93 (0.08)	–0.028	0.32 (0.73)	–0.042	0.51 (0.67)	0.670	16.37 (**0.012**)	39	–0.013	0.06 (0.89)
Consumption of home–grown plants	50	0.064	0.65 (0.58)	–0.321	3.13 (0.22)	0.007	0.01 (0.95)	0.099	1.34 (0.42)	0.708	9.59 (**0.030**)	47	0.111	1.59 (0.40)

Bolded p values shown in parentheses are <0.05. As levels in urine and nails were $\log_{10}$–transformed

[a] For AB, n is one less subject sample for each of the independent variables after removing an AB outlier

[b] β (slope) is beta estimate

results obtained with urinary SumAs (Table 2). For hair samples, no significant difference was observed between the two consumer groups, although higher As levels were detected in high seafood consumers with a maximum value of 0.95 μg/g.

Regression Analyses for As in Urine and Nails

Regression analyses were only performed for urinary As and nail samples. Because a large proportion of hair samples (>50 %) had undetected levels of As, it was not included in the analysis. Table 3 shows simple linear regression analyses for those sociodemographic (e.g., age, gender) and dietary (e.g., shellfish consumption) variables that were, at minimum, marginally associated ($p \leq 0.1$) with at least one of the urinary As species or with As in nails. The regression coefficients (β estimates) listed in Table 3 represent changes in the expected value of the main outcome (e.g., $\log_{10}$—transformed As concentrations in urine and nail samples) per unit of change in the independent variable. A statistically significant ($p < 0.05$) positive association was found for MA concentrations with the variable "years of residency" (Table 3). In addition, positive significant associations were found for AB levels with the variables "seafood consumption," "fish consumption" "seafood intake in the past 72 h," and "consumption of home-grown plants." These variables predicted each nearly 21, 25, 16, and 10 % of the variability in the urinary levels of AB (Table 3). The highest significant association ($p = 0.0002$) was obtained between AB urine levels and "fish consumption", where the change in the expected value of $\log_{10}$—transformed AB concentration was 0.765 units greater in people that consumed fish ≥1 time/week than in people who ate fish one or fewer times per month [$\beta = 0.765$; 95 % confidence interval (CI) (0.383, 1.15)] (Table 3). Marginally significant ($0.05 < p < 0.1$) positive associations were found between "age" and AB, between "fish consumption" and MA, and between "years of residency" and both SumAs and DMA (Table 3). Marginally significant ($p = 0.08$) negative associations were observed between "seafood intake in the past 72 h" and iAs concentrations (Table 3). No significant associations ($p > 0.05$) were found between gender and any of the urinary As species. Six variables predicted As concentrations in nails with statistically significant ($p < 0.05$) positive associations by simple linear regression (Table 3). The variables "seafood consumption" and "years of residency" were the

most significantly associated with As in nails, with *p*-values of 0.0001 and 0.0003, respectively. These two variables each predicted >25 % of the variability of As concentrations in nails (Table 3).

Because the simple regression model showed that the SumAs and each of the urinary As species related to exposure to iAs were predicted by only one variable at a marginal level (Table 3), a multivariate model was fitted only for concentrations of As in nails. As listed in Table 4, "gender," "years of residency," and "fish consumption" were statistically significant positive predictors of As in nails, whereas consumption of shellfish did not show significance. The β-estimate listed in Table 4 represents the adjusted changes in the expected value of the $\log_{10}$-transformed As concentration in nails per unit of change of these five independent (predictor) variables. For instance, males would have 0.142 units higher As levels in nails than females after controlling for the other variables in the model [β = 0.142; 95 % CI (0.003, 0.280)]. Our multivariate model explained 55 % of the variation in As nail levels.

Spearman Correlation Between As in Urine and Nails

Among the urinary As species, iAs showed positive significant correlation with MA and with SumAs, but it was inversely correlated with AB (rho = −0.570, $p < 0.0001$) (Table 5). The urinary metabolites MA and DMA were highly correlated (rho = 0.675; $p < 0.0001$) with each other and with the SumAs (rho >0.7; $p < 0.0001$). No statistical relationship ($p > 0.05$) was observed between AB and any of the other urinary As species (Table 5). Arsenic in nail samples exhibited significant weak correlations with DMA (rho = 0.291, $p = 0.042$) and SumAs (rho = 0.282, $p = 0.049$).

Table 4 Associations of As concentrations in nails with selected characteristics using multiple regression analysis ($n = 44$)

Characteristic	β-estimate	SE	*p*
Age	−0.0010934	0.0037825	0.774
Gender	0.1416745	0.0684005	**0.045**
Years of residency	0.0077831	0.0027603	**0.008**
Fish consumption	0.2596128	0.0788767	**0.002**
Shellfish consumption	0.0050259	0.0868478	0.954

Bold *p* values are <0.05

SE standard error

As levels in nails were $\log_{10}$—transformed. The entire model explained 55 % ($p > F = <0.0001$) of the variation in nail As levels

Table 5 Spearman's correlation "rho" of As species in urine (μg/g Cr) and nail (μg/g) samples

	Urine				
	iAs	MA	DMA	SumAs	AB
Urine					
***n* = 52**					
MA	0.440	——			
	(**0.001**)				
DMA	0.265	0.675	——		
	(0.057)	(< **0.0001**)			
SumAs	0.348	0.748	0.987	——	
	(**0.011**)	(< **0.0001**)	(< **0.0001**)		
AB[a]	–0.570	0.200	0.250	0.227	——
	(<**0.0001**)	(0.159)	(0.076)	(0.109)	
Asnail					
***n* = 49**	−0.049	0.234	0.291	0.282	0.276[b]
	(0.738)	(0.106)	(**0.042**)	(**0.049**)	(0.058)

p values are shown in parentheses; bolded *p* values are < 0.05

[a] For urine, *AB* $n = 51$ after removing an outlier

[b] For Asnail *versus* AB, $n = 48$

Discussion

People in Vieques are still concerned about their health because of the United States Navy-related environmental contamination and potential exposure to toxic trace metals from dietary sources, including seafood (Acevedo-Marín 2004; Agency for Toxic Substances and Disease Registry 2011; Caro et al. 2000; Díaz and Massol-Deyá 2003; Massol-Deyá et al. 2005). Therefore, this study aimed to determine if increased consumption of seafood (fish and shellfish) from Vieques is associated with increased iAs exposure by measuring its concentration in three biomarkers (urine, nails, and hair) from two groups of adult people who were classified as high and low seafood consumers.

Despite the differences observed in seafood consumption patterns between high and low seafood consumers, there were no significant differences between groups for average concentrations of urinary SumAs. To estimate recent exposure to iAs (as measured in urine), the most common method used is the sum of the concentrations of urinary As III, As V, and the metabolites MA and DMA (referred to in this study as SumAs) (Brima et al. 2006; Steinmaus et al. 2009). Applying this method, none of the urine samples in this study surpassed exposure reference values of 50 μg/g Cr or 50 μg/L of SumAs (Agency for Toxic Substances and Disease Registry 1997; Coronado-González et al. 2007; Tsuji et al. 2005). However, levels of urinary SumAs measured in this study were found to be

higher than those measured in the general population of other countries. For instance, the 50th and 95th percentiles for the SumAs in Vieques samples were higher than those in samples from the United States general population (Caldwell et al. 2009); the French population (Saoudi et al. 2012); Germany (Becker et al. 2003); and Andalusia, Spain (Aguilera et al. 2008) (Table 6). In addition, the 50th and 90th percentiles for the SumAs in Vieques samples exceeded those reported for American Indian communities from Oklahoma, Arizona, and North and South Dakota (Navas-Acien et al. 2009) (Table 6). The comparison with these studies suggests that Vieques participants were exposed to concentrations of iAs, as measured in urine samples, higher than background levels to which the general and the indigenous populations of the United States and other European countries are exposed.

The percent distribution of urinary iAs, MA, and DMA of the two seafood-consumer groups from Vieques also contrasts to the percent distribution reported for various population groups, which is 10–30 % for iAs, 10–20 % for MA, and 60–70 % for DMA (Navas-Acien et al. 2009; Vahter 2000). For instance, >50 % of the participants in this study had %DMA values >80 %, with a maximum value of 94.6 % (Table 2). This indicates that most of them had an effective pattern of iAs methylation. In addition, the methylating capacity and the average level of urinary SumAs did not show statistically significant associations with gender or age. These results differ with other studies reporting that women are better at methylating iAs and that they exhibit lower urinary SumAs than men (Fillol et al. 2010; Lindberg et al. 2008; Tseng 2009; Vahter et al. 2007). Moreover, previous studies have reported that excretion of methylated species (e.g., DMA) increases with age, a trend not observed in our study (Hopenhayn-Rich et al. 1996; Vahter 2000).

Table 6 Comparison of percentiles for the urinary As in Vieques with other populations

Location	*n*	Median	95th percentile
Vieques	52	10.0 μg/g (10.6 μg/L)	31.9 μg/g (40.4 μg/L)
USA[a]	2557	6.0 μg/L	18.9 μg/L
France[b]	1500	3.53 μg/g (4.03 μg/L)	8.9 μg/g (10.68 μg/L)
Germany[c]	4730	3.0 μg/g	15.2 μg/g
	4,741	4.1 μg/L	18.9 μg/L
Spain[d] (Andalusia)	861	1.50 μg/g (1.55 μg/L)	4.70 μg/g (5.09 μg/L)
Native Americans (USA)[e]	60	7.2 μg/g	16.9 μg/g[f]

[a] Caldwell et al. (2009)

[b] Saoudi et al. (2012)

[c] Becker et al. (2003)

[d] Aguilera et al. (2008)

[e] Navas-Acien et al. (2009)

[f] 90th percentile (90th percentile in Vieques = 21.3 μg/g Cr)

The median ratio between MA and DMA in both consumer groups (0.16 and 0.14) were comparable with findings by other studies (Hopenhayn-Rich et al. 1996; Meza et al. 2004; Soleo et al. 2008), although one participant obtained a ratio of 0.53 in the high seafood consumption group. This ratio is used to assess the methylation capacity of individuals as a biomarker of susceptibility to iAs toxicity (Hopenhayn-Rich et al. 1996; Huang et al. 2009; Meza et al. 2004; Soleo et al. 2008), where greater MA/DMA ratio represents greater risk of iAs toxicity.

Compared with results from other studies performed on the general population, one could attribute the observed higher SumAs concentrations and methylation capacity in this study to the presence of DMA from seafood. Previous studies have shown significant correlations between concentrations of DMA, AB, and seafood intake (Navas-Acien et al. 2011; Rivera-Núñez et al. 2012; Soleo et al. 2008). When estimating exposure to iAs using the SumAs, recently published studies agreed that this method could result in overestimation of iAs exposure and methylation capacity because AB and others seafood-derived organic chemicals, such as arsenosugars and arsenolipids, could influence concentrations of urinary DMA (Navas-Acien et al. 2011; Rivera-Núñez et al. 2012; Sele et al. 2012; Taleshi et al. 2010). Normally, to decrease the contribution of organic As from seafood to total urinary As, participants are required to refrain from ingesting seafood for at least 3 days before urine collection (Fillol et al. 2010). Because we wanted to determine the contribution of seafood to iAs intake, our participants were not required to abstain from seafood intake.

In this study, AB was the only urinary As species for which average concentrations showed significant difference ($p \leq 0.01$) between these two seafood-consumer groups (higher in high seafood consumers) (Table 2). The fact that AB was present in most urine samples (98 %) of low seafood consumers could be related to incomplete participant recollection of seafood consumption in the self-administered questionnaire and/or due to additional nonseafood sources. Other studies have reported levels of urinary AB in subjects with little or no seafood consumption attributed to sources other than seafood (Brima et al. 2006; Navas-Acien et al. 2011; Rivera-Núñez et al. 2012), which may explain the positive association between AB and "consumption of home-grown plants" listed in Table 3.

In contrast, average DMA levels did not exhibit significant differences between the two seafood-consumer groups, and its concentration was not predicted by seafood dietary

variables (e.g., consumption of shellfish and fish). This is contrary to other reported studies that showed an association between DMA concentrations and seafood intake (Fillol et al. 2010; Molin et al. 2012; Navas-Acien et al. 2011; Rivera-Núñez et al. 2012; Soleo et al. 2008). In addition, Spearman correlations showed that DMA had significantly higher correlations with MA and SumAs than with AB. This suggests that seafood consumption did not influence significantly the urinary concentrations of DMA in our participants as it was observed in the previously mentioned studies. In controlled seafood (e.g., fish, shellfish, and seaweed) consumption studies with humans, contradictory results had been obtained with respect to levels of urinary DMA after seafood intake. Although Choi et al. (2010) observed significant increases in urinary concentrations of DMA in volunteers whose urine sample was analyzed before and after seafood intake, Hsueh et al. (2002) reported no differences. It appears that DMA derived from seafood is influenced by the type of seafood consumed. In a recent published seafood-controlled dietary study with human volunteers, Molin et al. (2012) obtained different percentages of urinary DMA excretion that depended on the aquatic organisms consumed; highest for the blue mussel (*Mytilus edulis*) consumer group and lowest for the cod (*Gadus morhua*) consumer group. These aquatic organisms, as well as other known seafood DMA sources such as oysters, clams, and seaweeds (Borak and Hosgood 2007), are not staple food items of our participants based on data gathered from the self-administered questionnaire. Recently, the European Food Safety Authority (2009) stressed the importance of conducting As speciation in food items, including seafood, for better dietary-risk characterization. Therefore, research is needed about the As species that accumulate in tropical edible aquatic species, particularly in local seafood consumed in Puerto Rico, including Vieques, to have better risk estimates of dietary iAs from seafood consumption. Although iAs in edible seafood (fish and shellfish) have been found to commonly occur as a minor fraction (<1 %) of the Total As, these studies focused on aquatic organisms from temperate waters (Ruttens et al. 2012; Julshamn et al. 2012).

The fact that SumAs concentrations in this study were higher than in other populations in the United States and other countries, and that no significant differences were observed between high and low seafood consumers in any of the As species, suggests a possible common source(s) of recent exposure to As (other than seafood consumption) that contributes to levels above background concentrations. Other exposure sources to As not contemplated in this study included dietary sources, such as rice. Imported rice consumption is a main staple food item for Puerto Rico, including Vieques. Rice is known to contribute significantly to dietary iAs intake, including DMA (European Food Safety Authority 2009; Zhu et al. 2008). Soil contamination resulting from local anthropogenic sources, such as past military activities in Vieques, could also be considered another potential source to iAs as previously reflected in the accumulation of other metals in edible cultivated plants from Vieques (Díaz and Massol-Deyá 2003).

Although urinary As is used as a biomarker of recent exposure (on the order of days), As in nail and hair samples represent long-term (chronic) exposure (on the order of months) (Agency for Toxic Substances and Disease Registry 2007; Hughes 2006). Multivariate results for As in nail samples showed that increased consumption of fish was associated with higher levels of iAs in nails, particularly for men, and that levels increase with years of residency in Vieques. This finding agrees with previously published studies where significantly higher As concentrations in nails from fingers and toes were associated with seafood intake (Rivera-Núñez et al. 2012; Slotnick et al. 2007; Wilhelm et al. 2005) and that men accumulated significantly more As in nails than women (Wilhelm et al. 2005). In this study, men consumed more seafood than women. The observed positive significant correlation between nail As concentrations with SumAs and DMA has been previously reported in other studies (Rivera-Núñez et al. 2012). However, As concentrations in nails from this study were similar to populations exposed to low environmental levels of As (e.g., from drinking water) in the United States, where As levels in nails fluctuated from <0.01 to 0.81 μg/g, and with a normal human level considered to be ≤1 μg As/g in nails (Agency for Toxic Substances and Disease Registry 2007; Karagas et al. 2000).

The lower detections of iAs in hair samples were not the result of low recovery of iAs from hair sample because the average recovery of 0.198 μg As/g dw from the GBW07601—Trace elements in human hair standard reference material was 78.7 ± 1.5 % ($n = 10$). It is known that iAs has high affinity for keratin-rich tissues, such as nails and hair (Brima et al. 2006; Hughes 2006). The fact that iAs was more frequently detected in nail than in hair samples may be explained by the greater content of cysteine residues in the keratin matrix of nails compared with hair (Mandal et al. 2003). Brima et al. (2006) reported three-fold greater concentrations of As in fingernails than in hair samples attributed to differences in cysteine-content and growth rate (e.g., slower in nails).

This study has some limitations that should be addressed. First, being a pilot study, sample size was small, a possible reason for the lack of association between the SumAs, DMA, and seafood consumption. Second, participants were not randomly selected, which may not be representative of all adults living in Vieques. Third, the self-administered questionnaire did not capture all relevant information from all participants: drinking water and other

dietary sources of As (i.e., rice) were not included. Fourth, not all participants furnished all of the requested information in the self-administered questionnaire (e.g., 32 % of the high seafood group did not provide information about seafood intake in the last 72 h before urine collection). Despite these limitations, this study provides useful information on biomarker validation regarding iAs exposure through diet consumption, specifically seafood, in a population with unique sociodemographic characteristics.

Conclusion

This is first study in Puerto Rico reporting As levels in different biomarkers of exposure (urine, nail, and hair), thus contributing to the existing knowledge on the widespread exposure to As occurring around the world. When evaluating the association of iAs levels in the biomarkers of exposure used in this study with seafood consumption, a statistically significant association ($p < 0.05$) was only obtained for nail samples, especially regarding frequency of fish consumption. Nevertheless, the consumption of fish and other seafood from Vieques does not seem to represent an important pathway of chronic exposure to high levels of iAs if a nail reference As value of 1 μg/g is considered. Although urinary SumAs levels measured in this study were lower than reference exposure values, they were found to be higher than those in the general population of other countries. DMA was not associated with seafood consumption, suggesting that its presence in urine originated from the metabolism of iAs. However, other As dietary sources (e.g., rice), which were not included in the questionnaire, could also be implicated. Therefore, further studies on the relationship between environmental sources (food included) of iAs and As concentrations in biological samples (especially urinary As speciation data) are recommended with special attention to vulnerable populations, such as children as well as women of child-bearing age. This information, lacking in Vieques as well as mainland Puerto Rico, is necessary for better human health protection from chronic exposure to iAs, a public health problem that has been documented worldwide.

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