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Total and speciated urinary arsenic levels in the Spanish population

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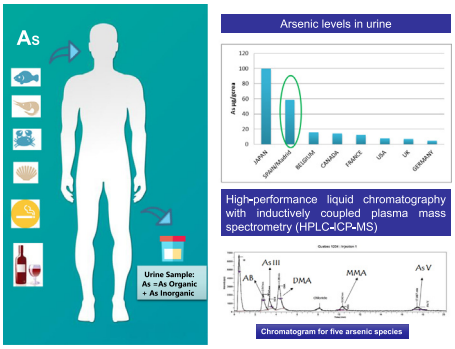
HIGHLIGHTS

- Total urinary arsenic levels in Madrid, a central region of Spain, are higher than in other regions of the Western world.
- Seafood consumption in Spain is among the highest in the world.
- Exposure to arsenic is associated with age, sex and dietary habits.
- Urinary dimethylarsinic acid and urinary arsenobetaine levels are correlated.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Very few studies exist on urinary arsenic exposure in Spain.
Objective: To evaluate total and speciated urinary arsenic (As) levels in a Spanish population sample.
Methods: Demographic, lifestyle and dietary data was collected for 124 volunteers (aged 20–76 years; 88 women and 36 men), who were tested for total arsenic and five arsenic species using high-performance liquid chromatography-inductively coupled plasma mass spectrometry.
Results: Arsenobetaine (AB) and dimethylarsinic acid (DMA) were detected in 96.8% of the study participants (limit of detection (LOD) 1.0 μg/L for AB and 1.9 μg/L for DMA). Monomethylarsonic acid (MMA) and arsenous acid (As(III)) were detected in 5.6% (LOD 1.8 μg/L) and 1.6% (LOD 1.4 μg/L) of the participants, respectively; arsenic acid (As(V)) was not detected (LOD 1.4 μg/L). AB and DMA (geometric mean (GM) 29.1 μg/L and 7.5 μg/L, respectively) were the main contributors to total urinary arsenic levels. Urinary DMA was positively associated with AB.
Conclusion: Total arsenic levels observed in the Spanish population sample were higher than those reported by other European studies. The most recurrent urinary arsenic species was AB, followed by DMA, probably attributable to the high Spanish consumption of seafood. We recommend using inorganic As + MMA as the two main urinary biomarkers for inorganic As exposure. Our results provide reference data for analysing arsenic speciation results and assessing human exposure.

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1. Introduction

Arsenic is a naturally occurring element, widely distributed in the earth's crust, which, in the environment, combines with oxygen, chlorine and sulfur to form inorganic arsenic compounds. In animals and plants, arsenic combines with carbon and hydrogen to form organic arsenic compounds (ATSDR, 2010). For humans, contaminated water or food is the primary route of arsenic exposure.

Via food, average daily intake of total arsenic for the European Union (EU) population is in the range 0.09–0.38 μg/kg body weight (EFSA, 2014). Most foodstuffs — meat, poultry, dairy products and cereals — contain low levels of inorganic and organic arsenic; however, seafood (defined for our purposes as fish, shellfish and molluscs) is the main contributor to dietary arsenic (usually organic compounds). Regional differences in daily intake of total arsenic through food are therefore mainly attributable to variations in seafood consumption; Japan's intake, for example, is higher than in Europe and the USA (WHO, 2000). Inorganic arsenic is a naturally occurring toxicant and carcinogen that contaminates groundwater supply systems in countries around the world. In areas where high levels of arsenic are naturally present, certain foods prepared with water with a high arsenic content or irrigated with contaminated water (e.g., rice) also contribute to total daily intake (WHO, 2010).

Annual per capita seafood consumption in Spain, at 42.9 kg, is second only to that of Japan, at 53.70 kg. Finland, France and China are also important consumers, at over 30 kg per capita (35.6, 34.6 and 32.8, respectively) and also Belgium, Italy and Canada, which lie in the 20–30 kg bracket (25.07 kg, 25.38 kg and 22.30 kg, respectively); other illustrative values are those for the USA (21.70 kg), the UK (18.96 kg), Germany (14.20 kg), Czech Republic (9.5 kg) and Hungary (5.3 kg) (FAOSTAT, 2011).

In humans, urine is the main route for excreting arsenic in any of several chemical forms. Thus, excretion can be via organic arsenic species such as arsenobetaine (AB) and arsenocholine (ACh) and via species such as arsenous acid (As(III)) and arsenic acid (As(V)) associated with exposure to and metabolism of inorganic arsenic (iAs). Excretion can also occur via successive oxidative methylation reactions: dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA), and also trimethylarsine oxide (TMAO), which, however, is not normally detected in human urine (Hata et al., 2007).

Exactly how iAs is methylated has not yet been clearly established (Molin et al., 2015), as toxicity depends on chemical form and state of oxidation. The most toxic species are generally the trivalent or pentavalent associated inorganic species, with a variety of adverse effects in humans. The organic species — in particular, AB, associated with seafood intake (WHO, 2010; Schoof and Yager, 2007) — are relatively less toxic.

While total arsenic concentration in urine can be misinterpreted as an iAs exposure marker, DMA and MMA have often been usefully adopted as exposure markers. However, DMA and MMA can co-occur with AB and others arsenosugars in seafood and other foods and result in direct exposure; it has also been demonstrated that AB and others arsenosugars in seafood may be metabolized to DMA and MMA and so contribute to urinary DMA and MMA levels (Molin et al., 2012; Navas-Acien et al., 2011; Tseng, 2007). Likewise, the analysis of urinary DMA may be misleading as a biomarker for iAs exposure since this might also be produced as a metabolite of organic arsenic species and even by direct exposure to DMA (Aylward et al., 2014; Molin et al., 2015).

The fact that methylation efficiency is influenced by many factors — including the level of arsenic exposure, age, sex and lifestyle and dietary factors (Tseng, 2007) — results in inter-individual differences in human methylation patterns. It has been suggested that inefficient methylation capacity may be reflected by a high urinary MMA content that could increase the risk for toxicity following iAs exposure (Basu et al., 2011).

Speciation of urinary arsenic concentration has become the standard method for biomonitoring arsenic, since it enables relative proportions for biomarkers of exposure to both organic and inorganic arsenic species to be established and, consequently, facilitates evaluation of the health risk of arsenic exposure.

The objective of this research was to evaluate arsenic levels and speciation in urine for the Spanish population in order to facilitate evaluation of the health risk of arsenic exposure. Since Spaniards are high consumers of seafood it is to be expected that significant levels of arsenic will be acquired through food sources.

2. Materials and methods

2.1. Subjects

Included in the study were 88 female and 36 male (n = 124) Spanish subjects, aged 20–76 years and living in the Madrid region (central Spain), who agreed voluntarily to participate in the study and who provided 12-hour overnight urine samples. The samples were immediately stored in a freezer at −20 °C for further analysis. Lifestyle data for the study participants were collected through individual questionnaires with specific questions on age, dietary habits, smoking and alcohol consumption habits and drug intake. The research was reviewed and approved by Research Ethics Committee of the Institute of Toxicology of Defence (ITOXDEF) and written informed consent was obtained from each subject.

2.2. Laboratory methods

Arsenic speciation compounds arsenic (III) oxide, As_2O_3 CAS 1327-53-3, arsenic (V) oxide hydrate, $As_2O_5 \cdot xH_2O$, CAS 12044-50-7, dimethylarsinic acid, $(CH_3)_2As(O)OH$, CAS 75-60-5, and disodium methyl arsenate, $CH_3AsNa_2O_3 \cdot 6H_2O$, CAS 144-21-8 were all purchased from Sigma-Aldrich and arsenobetaine, $C_5H_{11}AsO_2$, CAS 64436-13-1, and trimethylarsine oxide (TMAO), $(CH3)_3AsO$ CAS 4964-14-1, were purchased from Argus Chemicals (Vernio, Italy).

The mobile phase was prepared using ammonium phosphate dibasic solution 2.5 M BioUltra CAS 7783-28-0, ammonium hydroxide solution CAS 1336-21-6, sodium hydroxide solution 1 N CAS 1310-73-2, all purchased from Sigma-Aldrich, and nitric acid 65% Suprapur CAS 7697-37-2 purchased from Merck-Millipore.

Analysis was performed using a high-performance liquid chromatography (HPLC) system consisting of an Agilent 1100 Series with quaternary pump, autosampler, thermostatic column compartment and vacuum degasser, coupled to an ELAN DRC II inductively coupled plasma mass spectrometer (ICP-MS) with two Rheodyne 6-port, 2-position switching valves (Elmer, 2003). The liquid chromatography (LC) column used was a Hamilton PRP-X100 anion exchange column (4.1 mm × 250 mm, 10 μm). Operating systems used were Chemstation for HPLC Software Agilent System and for ICP-MS ELAN Instrument Control Software and Chromera Perkin Elmer Software.

Operating conditions were in accordance with those established by the Centers for Disease Control and Prevention (CDC) (NHANES, 2011–2012; Aylward et al., 2014). Operating parameters for HPLC and ICP-MS are summarized in Tables 1 and 2, respectively. Fig. 1 depicts a chromatogram of urine quality control for five arsenic compounds, with TMAO as the internal standard. Since urine with a high chlorine component could potentially cause ICP-MS polyatomic isobaric spectral overlap interference from the formation of $40Ar35Cl^+$ with the same m/z as 75As, the $ArCl^+$ peak from the arsenic species was resolved by optimizing chromatographic parameters. Retention time for chlorine was approximately 8 min (Fig. 1).

Stock standards of each species were prepared by serial dilution of the starting compounds with 18 MΩ cm of deionized water, for a final concentration of 5 mg/L. These standards were prepared daily by mixing