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Article

μLC–ICP-MS Determinations of Unexposed UK Urinary Arsenic Speciation Reference Values

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This study provides background levels for five arsenic species in urine, based on urinary data obtained from 95 nonoccupationally exposed volunteers based in the UK. Using a novel, sensitive, robust and reliable speciation methodology, five species of arsenic (arsenobetaine [AB], arsenite [As^{3+}], arsenate [As^{5+}], monomethylarsonic acid [MMA^{5+}] and dimethylarsinic acid [DMA^{5+}]) were determined in urine samples collected from 95 adults. The analytical instrumentation used to analyze the urine samples was a hyphenated micro liquid chromatography (μLC) system coupled to an inductively coupled plasma mass spectrometry (ICP-MS). Separation was achieved using an anion exchange micro-sized column. The results presented give the 95th percentile of concentrations, both uncorrected for creatinine (μg/L) and creatinine corrected (μmol/mol) in urine for the 95 volunteers. Statistical analysis was performed on the dataset using a Bayesian model to determine and quantify effects of gender, smoking and diet. The statistical results show that the consumption of fish, shellfish and red wine has a significant elevating effect on AB, DMA and MMA urinary concentrations; however, no significant effect was observed for smoking. The regression model results indicate that creatinine correction was effective for arsenic species As^{3+}, MMA, DMA and AB. The background levels established here can be used as reference values to help aid interpretation of arsenic speciation results and better assess exposure.

Introduction

The International Agency for Research on Cancer (IARC) has classified inorganic arsenic as a Group 1 agent, defined as carcinogenic to humans (1). Thus, it is imperative that exposure be adequately controlled. Biological monitoring assesses whether a person has been exposed to a substance or its metabolites, as it encompasses all routes of potential exposure. Inorganic arsenic exists as arsenite (trivalent, As^{3+}) and arsenate (pentavalent, As^{5+}). When humans are exposed to inorganic arsenic, the arsenic is reduced and methylated in the body to produce a range of organic arsenic species, including dimethylarsinic acid (DMA^{5+}) and monomethylarsonic acid (MMA^{5+}) (2). Pentavalent methylated species are considered relatively nontoxic, since it is thought that they cannot bind with molecules in the body (3). In the UK, the most common route of exposure to arsenic is from dietary sources. Dietary species of arsenic known as tetra alkylarsonium compounds include arsenobetaine (AB), arsenocholine and trimethylarsine oxide, and are thought to be nontoxic (4). The dietary sources of these species of arsenic include seafood, shellfish, poultry and rice (4–8), and following ingestion, they are eliminated in the urine unchanged (4).

Urine is the main excretory pathway for elimination of arsenic from the body, in the general proportion 10–30% of inorganic arsenic, 10–20% of MMA^{5+} and 60–80% of DMA^{5+} are eliminated primarily via this route after inorganic exposure (2). The ease and noninvasive sample collection makes it the biological sample of choice in most studies, although determination of arsenic levels in blood (9, 10) and serum (11) has been reported.

Currently, there are no unexposed background reference values or exposed UK guidance values for any individual arsenic species in urine. The US biological exposure index (BEI) (12) and the German biological tolerance value (BAT) (13) are 35 and 50 μg/L, respectively, which are based on the summation of inorganic arsenic and its metabolites, but not including AB. This is a controversial, less well-understood area, because there seems to be increasing numbers of foodstuffs containing other arsenic compounds, e.g., DMA (6, 7), and therefore, exposure to arsenic compounds either from environmental or from occupational sources could be overestimated. The BEI and BAT approach of the sum of the species less AB is an attempt to correct for dietary exposure, but does not account for dietary DMA, MMA and arsenosugars. To better assess environmental and occupational exposure, arsenic speciation must be undertaken as a routine assay.

There are several population studies from around the world that have reported urinary arsenic concentrations in unexposed individuals. The two most comprehensive are the National Health and Nutrition Examination Survey (NHANES) (14) in the USA, where 2557 participants' urine samples were measured for seven species of arsenic, and the Human Biomonitoring of Environmental Chemicals in Canada (HBECC) (15), where ~6400 participants' urine samples were measured for six species of arsenic. In addition, similar national surveys have been conducted in France ($n = 2102$ participants) (16) and Korea ($n = 5087$ participants) (17). However, both these studies used a total inorganic arsenic and the metabolites minus AB technique to measure arsenic (16, 17). There is also urinary arsenic speciation data for unexposed individuals in smaller scale clinical studies from around the world such as Germany (18), UK (19, 20), Italy (21) and Spain (22).

It is generally accepted that urinary arsenic speciation provides the most coherent and detailed picture of exposure to arsenic species and compounds. However, there is still a lack of established reference values to use when trying to assess exposure to individual species. By improving and modifying a novel micro liquid chromatography (μLC)–inductively coupled plasma mass spectrometry (ICP-MS) speciation method, previously developed at the Health and Safety Laboratory (20) to have greater sensitivity and lower limits of quantification (LOQs), this paper aims to provide reference background values of five arsenic species in the urine of unexposed individuals from a cohort of UK samples. Information on diet and lifestyle was collected by questionnaire, as exposure to arsenic can occur through diet and environmental sources. Such data will provide a valuable contribution to the knowledge in this area.

Experimental

Instrumentation

Separation was achieved using a hyphenated μLC system with an ICP-MS (XSERIES 2, Thermo Fisher Scientific, Hemel Hempstead, UK). The separation of arsenic species As^{3+}, As^{5+}, MMA^{5+}, DMA^{5+} and AB was achieved using a 5-cm anion exchange guard column (Dionex AG7 4 mm × 50 mm i.d., Thermo Fisher Scientific). The micro-flow delivery of sample and mobile phase was accomplished using an ESI OneFAST system (Elemental Scientific, Warrington, UK), which consists of a six port switching value and a 1-mL sample loop. The delivery of mobile phase and sample onto the column was controlled by the ICP-MS peristaltic pump at a constant rate of 0.2 mL/min. The ICP-MS was operated in a collision cell mode using 7% hydrogen in helium (~3.5 mL/min). The ICP-MS conditions were optimized using a 10-μg/L tuning solution containing arsenic, cobalt, indium and uranium [made in 1% (v/v) nitric acid from 1000 mg/L stock standards (ICP-MS standards, BDH, Poole, UK)].

Reagents

Arsenic speciation compounds sodium arsenite ($NaAsO_2$), sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) and sodium cacodylate (DMA; $Na(CH_3)_2AsO_2{\cdot}3H_2O$) were all purchased from Fisher Scientific (Loughborough, UK). Disodium methyl arsenate (99% MMA; $CH_3AsO(OH)_2$) was purchased from ChemService (West Chester, PA, USA). AB ($C_5H_{11}AsO_2$) was purchased from Sigma-Aldrich (Dorset, UK). Single standard stock solutions of 1000 mg/L were prepared in 1% (v/v) nitric acid (Romil, Cambridge, UK) of As^{3+}, As^{5+}, MMA, DMA and AB in plastic volumetrics. All stock solutions were stored at 4°C. The separation of five arsenic species used a mobile phase of ammonium carbonate purchased from VWR (Leicestershire, UK). Two mobile phases were prepared daily by dissolving ammonium carbonate in ultrapure deionized water (18.2 MΩhm cm) from a Millipore system (Merck Millipore, Billerica, MA, USA) to make 2 mM (mobile Phase A) and 70 mM (mobile Phase B) solutions.

A 1-mg/L mixed species solution of all the five species was prepared in 1% (v/v) nitric acid from the individual 1000-mg/L single standard stock solutions on a weekly basis. Using the 1-mg/L mixed species solution standards of 0.5, 1, 2, 5, 10 and 20 μg/L were prepared daily in 2 mM of ammonium carbonate (mobile Phase A).

Study group and sample collection

The volunteers in this study ($n = 95$) consisted of 53 males and 42 females who were not occupationally exposed to arsenic. All volunteers were over the age of 18 years, with the mean age of people in this study 41.1 years. Individuals volunteered in response to an email outlining the study sent to everyone at the Health and Safety Laboratory (HSL), UK, and the Biomedical Research Centre, Sheffield Hallam University, Sheffield, UK. Participating volunteers were provided with informed consent, as granted by the NRES Committee East Midlands–Leicester, REC number 12/EM/0314. Volunteer information was collected via a questionnaire, which was returned by post with the urine sample. The information collected included: gender, age, smoking habits and diet including vitamin and supplement use in the previous 7 days. All urine samples were collected in 25 mL urine collection bottles (Sterilin Ltd., Newport, UK) and sent to the HSL by first class post. All urine samples had creatinine content analyzed upon arrival and were then kept frozen at −80°C until analysis.

Sample preparation and analysis

Urine samples were thawed and mixed on a roller mixer before analysis. Samples were diluted 1 in 15 with 2 mM ammonium carbonate solution. All samples were analyzed in duplicate.

The certified reference materials (CRMs) used were Clinchek urine control for arsenic species Level 1 and 2 (Lot 923) (Recipe, Munich, Germany). In addition, a blank urine and spiked urine sample (blank urine with all the five species of arsenic added at a concentration of 10 μg/L) was analyzed at the beginning and the end of each series of analyses and after every 10 samples. External quality assurance for four of the arsenic species (not AB) was undertaken in November 2012 by participation in the G-EQAS (Friedrich-Alexander-University, Erlangen-Nuremberg, Germany) trace element quality schemes.

Urinary creatinine concentrations were determined on all urine samples by the Jaffe alkaline picrate method (23) on a Cobas Mira Plus (Horiba Diagnostics, Northampton, UK). Creatinine (a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body) allows a urine sample to be normalized for dilution or concentration. In determining the creatinine levels using mathematical modeling, it was possible to investigate the effectiveness of this correction for each arsenic species.

Statistical analysis

The relationship was modeled between urinary concentration and several factors (including gender, smoking and dietary habits) using a multiple regression model. The Bayesian regression model used in this study had the form

$$\begin{aligned}\ln(\mathrm{As}_i) = {} & \mu + \beta_1 \ln(\mathrm{creatinine}_i) + \beta_2 I(\mathrm{smoking}_i) + \beta_3 I(\mathrm{gender}_i) \\ & + \beta_4 I(\mathrm{supplements}_i) + \beta_5 I(\mathrm{fish}_i) + \beta_6 I(\mathrm{shellfish}_i) \\ & + \beta_7 I(\mathrm{seaweed}_i) + \beta_8 I(\mathrm{rice}_i) + \beta_9 I(\mathrm{mushrooms}_i) \\ & + \beta_{10} I(\mathrm{red\,wine}_i) \beta_{11} I(\mathrm{realale}_i) + \beta_{12} I(\mathrm{poultry}_i) \\ & + \beta_{13} I(\mathrm{ricemilk}_i) \beta_{14} I(\mathrm{bran}_i) + \varepsilon_i\end{aligned}$$

where As_i is the urinary concentration of arsenic on the ith individual; μ, the mean concentration (on the log scale) of a nonsmoking female who has not taken dietary substances represented in the model; $\mathrm{creatinine}_i$, the creatinine concentration with associated effect β_1; $I(\mathrm{smoker}_i) = 0$, if sample was from a nonsmoker and 1 if smoker (β_2 represents the smoking adjustment to the mean concentration); $I(\mathrm{gender}_i) = 0$, if the urine sample was from a female and 1, if male (β_3 represents the male adjustment). Indicator variables and adjustments for the intake of supplements, fish, shellfish, seaweed, rice, mushrooms, red wine, real ale, poultry, rice milk and bran are similarly represented. ε_i are normally distributed residual errors with mean zero and standard deviation σ.

One of the difficulties in analyzing urinary data is that measurements are often below the LOQ (referred to as nondetects).

However, it is known that these measurements lie between zero and the LOQ, i.e., they are left censored. The data were modeled using Markov Chain Monte Carlo (MCMC) methods in WinBUGs (24) within a Bayesian framework, treating the nondetects as left-censored.

Results

Analytical method

The method described in this study is a sensitive, robust and reproducible method for the routine determination of five species of arsenic in a urine sample. The chromatograms in Figure 1 show that this optimized speciation method achieves full separation in <6 min. The calibration range was 0–20 μg/L for all the five species of arsenic. The limit of detection (LOD) was calculated as three times the standard deviation of the blank; this was between 0.003 and 0.051 μg/L for all the five species of arsenic. The LOQ for undiluted urine was calculated as 15 times the mean background equivalent concentration (BEC) for all the five species of arsenic. The BEC, LOD and LOQ for each arsenic species are summarized in Table I. Of the 95 volunteer urine samples measured, no level was below the LOQ for DMA and only 1% of AB, 2% of MMA and 12% of As^{3+} were less than the LOQ. However, 63% of the As^{5+} values were less than the LOQ of 0.04 μg/L

Analytical validation

The results for the in-house spiked urine quality control sample shown in Table II gave an average recovery of 93% for As^{5+}, MMA, DMA and AB and 90% for As^{3+} in the daily spiked urine samples ($n = 29$). The external quality assurance and CRMs analyzed are presented in Table II. Clinchek Levels 1 and 2 are certified for arsenic species; analyses gave good results for arsenic species As^{5+}, MMA, DMA and AB, which were all within target range, whereas As^{3+} did not show good agreement and gave a result below the target range. However, the method is continually validated through participation in the German External Quality Assessment Scheme (GEQAS); the results for the 50th GEQAS in November 2012 being shown in Table II. The results were within the target criteria for all the five species of arsenic. This may suggest possible instability of As^{3+} in the Clinchek CRMs.

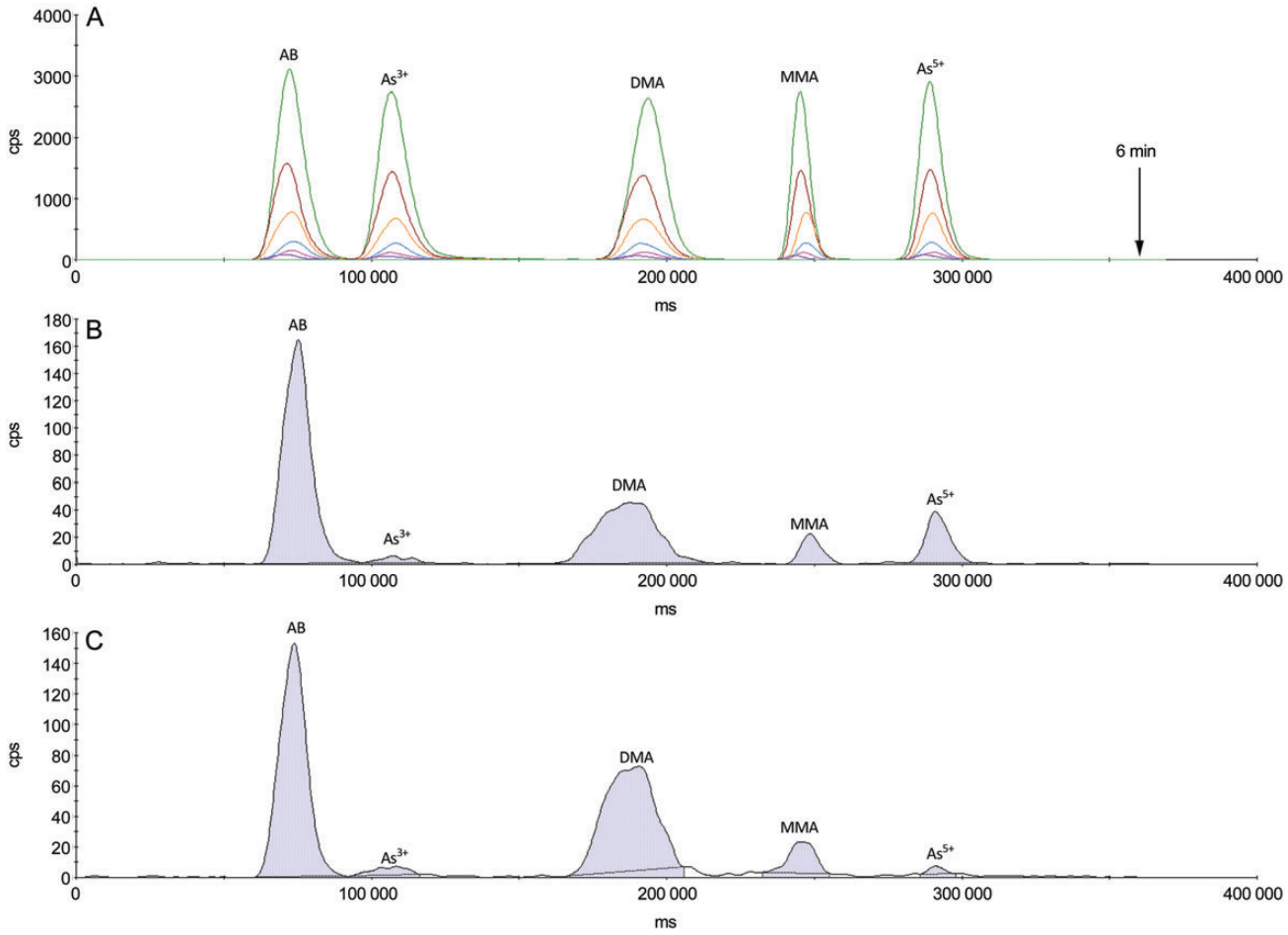

Figure 1. Chromatograms showing full separation of five arsenic species in <6 min using an ESI OneFAST system coupled to a 5-cm Dionex AG7 anion exchange column, with mobile phases 2 and 70 mM of ammonium carbonate solution. (A) Overlay of the individual standard calibration chromatograms of 0–20 μg/L calibration. (B) CRM Clinchek 1 for arsenic species in urine. (C) A urine sample of an unexposed individual adult.

Study results

Of the 95 volunteers who participated, 82 were nonsmokers (33 females and 49 males) and 13 were smokers (9 females and 4 males). The urine results are summarized in Table III; the median and the 95th percentile concentrations are expressed both uncorrected for creatinine (μg/L) and creatinine corrected (μmol/mol) in urine to adjust for urinary dilution. Creatinine concentration ranged between 0.89 and 26.45 mmol/L. Nine individuals had a low (below 3 mmol/L) or high (above 30 mmol/L) creatinine; however, creatinine concentration was not an exclusion criterion (the reason being that excluding these individuals would exclude a much higher proportion of females than males). As 63% of As^{5+} measurements were below the LOQ, the median As^{5+} concentration could not be quantified. The 95th percentiles for As^{3+} and As^{5+} were 0.54 (0.99 μmol/mol creatinine) and 0.23 μg/L (0.35 μmol/mol creatinine), respectively. MMA, DMA and AB gave 95th percentiles of 2.37 (3.08 μmol/mol creatinine), 12.68 (16.08 μmol/mol creatinine) and 126.7 μg/L (174.7 μmol/mol creatinine), respectively. The median and 95th percentile concentrations for male and female urinary arsenic species are also presented in Table III. Females were observed to have lower median and 95th percentiles of uncorrected for creatinine (μg/L) concentrations for arsenic species As^{3+}, As^{5+} and MMA. However, for the creatinine-corrected data, females show a lower median concentration than males, but a higher 95th percentile for As^{3+}.

Statistical results

General statistical analysis using Prism Graphpad version 4 for Windows was performed, comparing each arsenic species in relation to gender and smoking habits. Using a two-tailed Mann–Whitney nonparametric statistical test, there was no significant difference between smokers and nonsmokers for inorganic arsenic. Furthermore, no significant difference was found between females and males for As^{3+}, As^{5+}, MMA or AB; however, a statistically significant higher value for DMA was found in females in the creatinine-corrected values only. The most likely reason for this could be that women tend to have lower creatinine levels than men (23), resulting in a higher corrected DMA value for women than for men.

The Bayesian regression model was fitted separately for As^{3+}, MMA, DMA and AB; however, due to the large proportion (63%) of As^{5+} measurements below the LOQ, the analysis was not carried out for As^{5+}. When the model was fitted to the data, a creatinine coefficient of one was highly plausible for As^{3+}, MMA, DMA and AB (i.e., the credible interval for the parameter β_1 for each of these species included unity). This indicated that a creatinine correction may be effective for these species. The model was subsequently refitted to the data with β_1 set to unity, which corresponds to fitting a regression model to creatinine-corrected concentrations. The model considered all the variables including gender, smoking and dietary factors. Variables that were considered to be nonsignificant at the 5% level were subsequently removed from the model.

Table I

The background equivalent concentration (calibration curve intercept), LOD (signal-to-background noise) and limit of quantification for all the five species of arsenic in μg/L, in addition to the number and percentage of samples less than the LOQ

Arsenic species	BEC (μg/L)	LOD 3× SD of the blank (μg/L)	LOQ (μg/L)	No. of samples <LOQ	% <LOQ
As^{3+}	0.001	0.004	0.02	11	12
As^{5+}	0.003	0.051	0.04	60	63
MMA	0.002	0.030	0.04	2	2
DMA	0.003	0.003	0.04	0	0
AB	0.002	0.003	0.03	1	1

Table II

Results obtained for five species of arsenic in urinary CRM and an external quality assurance scheme

Arsenic species	In-house spiked urine (10 μg/L) % recovery ($n = 29$)	Clinchek Level 1–923 (Cert As species μg/L)		Clinchek Level 2–923 (Cert As species μg/L)		G-EQAS—Round 50 (μg/L)			
						50–2A		50–2B	
		Target	Result	Target	Result	Target	Result	Target	Results
As^{3+}	9.0 ± 0.8 (90%)	1.44–3.84	0.5 ± 0.1	7.05–11.8	2.9 ± 0.6	1.7–4.1	2.2	4.9–9.1	8.1
As^{5+}	9.3 ± 0.9 (93%)	2.10–4.90	4.2 ± 0.3	18.9–31.5	30.5 ± 1.8	3.8–8.6	6.0	13.5–24.3	21.8
MMA	9.3 ± 1.0 (93%)	1.50–3.50	2.8 ± 0.5	5.03–8.38	7.2 ± 0.5	1.0–2.8	1.7	6.8–11.6	10.9
DMA	9.3 ± 1.1 (93%)	5.88–13.7	9.5 ± 0.5	32.6–54.3	43.6 ± 1.8	8.3–14.9	11.6	64.8–96	95.9
AB	9.3 ± 1.8 (93%)	12.6–21.0	15.4 ± 1.1	23.0–34.6	27.7 ± 1.2	–	–	–	–

Table III

Statistical summary of median and 95th percentile concentrations for all the five species of arsenic in both μg/L and μmol/mol creatinine, for both total urine samples and female and male urine samples

	As^{3+}		As^{5+}		MMA		DMA		AB	
	μg/L	μmol/mol creatinine	μg/L	μmol/mol creatinine	μg/L	μmol/mol creatinine	μg/L	μmol/mol creatinine	μg/L	μmol/mol creatinine
Total: $n = 95$										
Median	0.11	0.19	<LOQ	<LOQ	0.56	0.90	2.44	4.30	3.87	10.0
95th Percentile	0.54	0.99	0.23	0.35	2.37	3.08	12.68	16.08	126.7	174.7
Females: $n = 42$										
Median	0.07	0.15	<LOQ	<LOQ	0.48	0.95	2.43	5.35	6.77	19.4
95th percentile	0.44	1.05	0.15	0.38	2.10	3.42	12.68	26.7	333.9	672.8
Male: $n = 53$										
Median	0.15	0.22	<LOQ	<LOQ	0.57	0.90	2.44	4.10	3.42	6.10
95th percentile	0.6	0.97	0.31	0.97	2.67	2.69	12.59	11.1	101.5	129.4

The results of the Bayesian model showed that there was no significant dietary difference between females and males. However, it did show a statistically significant difference between gender for arsenic species AB and DMA only. The mean urinary AB and DMA values for females were ~77 and 28% higher than males. In agreement with the nonparametric statistical test, the Bayesian model found no significant difference between smokers and nonsmokers; however, there were only 13 smokers (14% of the study group) in the dataset. As expected, the consumption of fish resulted in increased urinary AB and DMA concentrations. Volunteers who had eaten fish had urinary DMA concentrations twice as high as that in those who had not eaten fish and AB concentrations 10 times higher than that in those who had not eaten fish. The Bayesian model also showed that the mean concentration for urinary MMA was twice as high for those who had consumed fish than that in those who had not. However, the consumption of shellfish did not have an effect on urinary MMA concentrations and showed only a 27% increase in urinary DMA concentrations and a 2-fold increase in urinary AB concentrations. Red wine also had a statistically significant effect on urinary AB; the consumption of red wine was found to increase AB concentrations by 79% even after adjusting for consumption of fish and shellfish (of the 31 individuals who had consumed red wine, nine had consumed neither fish nor shellfish). Other dietary factors such as rice, mushrooms, real ale, poultry, rice milk and bran were not found to be statistically significant in the model for any of the four arsenic species.

To quantify and compare the variation of lognormally distributed measurements between the different arsenic species, the geometric coefficient of variation (GCV) was determined. The GCV quantifies intrasubject variability and is calculated as:

$$\mathrm{GCV} = \sqrt{e^{s^2} - 1}$$

where s is the standard deviation of the data after a lognormal transformation. The greater the GCV, the greater the variability in the measurements. AB showed the greatest variability with a GCV of 2.96, and DMA showed the lowest variability with a GCV of 0.77. As^{3+} and MMA had a GCV of 1.38 and 0.80, respectively.

The correlation between species was determined by calculating Pearson's correlation coefficient (a measure of the linear association between two variables) for the natural logarithm of the pairs of species. Nondetects were dealt with by imputation, whereby their values were replaced with the corresponding central estimates from the MCMC analysis, rather than by a common fixed value. This ensured that the nondetects did not introduce an artificial pattern to the data at low concentrations that could potentially dominate the dataset (25). A strong positive correlation was found between MMA and DMA; a moderate correlation was found between As^{3+} and MMA, As^{3+} and DMA, MMA and AB as well as DMA and AB. A very weak correlation was found between As^{3+} and AB. The correlation coefficient was not calculated for As^{5+} due to the very high proportion (63%) of measurements below the LOQ.

Discussion

Exposure to arsenic can occur through diet, environmental or occupational sources. Reported here is the determined urinary arsenic speciation concentrations collected from 95 volunteers who are not occupationally exposed to arsenic. The median uncorrected for creatinine (μg/L) concentrations for all the five species of arsenic gave good agreement with other studies where 'control' unexposed samples were reported and for background level studies (14, 15, 19, 21). Both the NHANES (14) and HBECC (15) studies quantified DMA and AB only, due to the large proportion of reported nondetects for As^{3+}, As^{5+} and MMA. The LODs reported in the NHANES study were 1.2 μg/L for As^{5+}, 1 μg/L for As^{3+}, 0.9 μg/L for MMA, 1.7 μg/L for DMA and 0.4 μg/L for AB (26), and most of the 95th percentiles reported in the HBECC study are marked as 'use with caution' or 'unreliable to publish' (15). It could be argued that both of these arsenic speciation methods are not suitable for the analysis of control samples. The LOD and LOQ values reported for this novel arsenic speciation method demonstrated in this study are lower and, therefore, better suited to the analysis of background samples. The LOQs reported in both Heitland and Koster (18) and Morton and Leese (20) could not achieve LOQ concentrations of <0.1 μg/L for any of the five species of arsenic, whereas in this study the LOQ ranges from 0.02 to 0.04 μg/L. Despite differing LOD and LOQ, the 95th percentile uncorrected for creatinine concentrations (μg/L) for all the arsenic species apart from AB gave very good agreement with those reported by both the NHANES (14) study for US unexposed residents and Heitland and Koster (18) for German unexposed residents ($n = 98$). The median and the 95th percentile for creatinine-uncorrected concentration (μg/L) for total inorganic arsenic and methylated metabolites (not including AB in this study) are 3.1 and 15.1 μg/L, respectively, which are both lower than the BEI (12) and BAT (13) values of 35 and 50 μg/L, respectively. In this study, the 95th percentile concentration for AB gave a much higher value of 126.7 μg/L (174.7 μmol/mol creatinine), compared with 35.0 (13) and 22.7 μg/L (18) reported in other studies. However, the HBECC (15) study gave a slightly higher 95th percentiles of 4.5 μg/L for As^{3+} and 30 μg/L for DMA, although their reported value of 110 μg/L for AB is similar to that obtained in our study. The predominant dietary species of arsenic (AB) is found primarily in seafood, shellfish, algae and seaweed products; however, it can also be found in poultry (5) and cereal products such as rice and bran (6). AB is thought to be the end point of arsenic metabolism in marine animals. It is not, however, believed to be accumulative in humans, and following ingestion, AB is eliminated unchanged in urine, within a couple of days. However, a study by Newcombe *et al.* (7) reported that AB was present in the urine of volunteers after 12 days of an AB-free diet (no seafood or shellfish, chicken or mushrooms), however rice was consumed. Newcombe *et al.* (7) suggested that dietary arsenic (AB) is present in rice. In our study, 11 urine samples had AB present, when no fish, seafood or seaweed products had been eaten in the preceding 7 days, but red wine, poultry and rice had been consumed. The median and 95th percentile concentrations for DMA in this study are 2.44 (4.30 μmol/mol creatinine) and 12.68 μg/L (16.08 μmol/mol creatinine), respectively. The presence of methylated metabolites in urine samples, which show no exposure to inorganic arsenic, suggests that dietary sources are not only responsible for the presence of AB in urine, but also DMA and to a lesser extent MMA. Pearson *et al.*, (8) also reported that methylated metabolites can be from dietary sources, showing that excretion of DMA in urine increased after eating American long-grain rice.

Elevated DMA in urine after the consumption of different types of fish has also been reported (4).

The regression model allowed us to investigate whether creatinine correction to the uncorrected data is appropriate. The results indicated that creatinine correction was effective for each of the arsenic species, where regression modeling was carried out (As^{3+}, MMA, DMA and AB).

Conclusion

The arsenic speciation method described in this paper has been used to determine urinary arsenic speciation results in a cohort of nonoccupationally exposed volunteers in the UK. This speciation method demonstrates high sensitivity, enabling accurate determinations of five species of arsenic in control urinary samples. Despite this, 63% of samples had no As^{5+} present.

Statistical analysis indicated that creatinine correction was effective for each of the arsenic species, where regression modeling was carried out (As^{3+}, MMA, DMA and AB). Fish consumption was associated with higher levels of MMA, DMA and AB, shellfish with higher levels of DMA and AB and red wine with higher levels of AB.

It can be expected that dietary sources of arsenic are not only responsible for the presence of AB, but also for DMA and possibly MMA. The existence of methylated metabolites in a urine sample does not necessarily equate to evidence of inorganic arsenic exposure and its subsequent methylation. Therefore, a method where an amalgamated value (total less AB) is determined is not an accurate measurement of exposure. To make an inferred exposure assessment, a speciation result is required and this could be further improved by keeping a diet log. There is a need for a detailed characterization of arsenic content in foodstuffs and beverages to make a more informed assessment. It would also be useful to establish further occupational exposure levels based on speciation results.

The results from this study mean that 95th percentile background reference ranges have been established for As^{3+}, As^{5+}, MMA, DMA and AB. This will allow better interpretation of any subsequent exposure.

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