Urinary Arsenic as a Biomarker: Speciation Analysis for the Assessment of Dietary Exposure

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Speciation Analysis and Implications for Dietary Exposure

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Abstract

Chronic adverse health effects due to groundwater contamination of inorganic arsenic (iAs) has long been known in many parts of the world. Carcinogenic effect of iAs due to low-level dietary exposure has become a serious matter of concern even in noncontaminated regions of the world. For the effective health risk management of dietary iAs intake, a quantitative dose-response relationship has to be established based on reliable analytical epidemiologic studies. Urinary levels of iAs and its metabolites, monomethylarsonic acid (MMA) and

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dimethylarsinic acid (DMA), are a suitable biomarker of exposure, and urine analysis serves as a reliable tool of exposure assessment of individuals in such epidemiologic studies. This review summarizes a method of urine As speciation analysis, and advantages and limitations of the use of urine As species as a biomarker of exposure to dietary iAs in environmental epidemiologic studies are discussed. Use of urine As speciation as a biomarker of susceptibility is also referred.

Keywords

Arsenic · Monomethlarsonic acid · Dimethylarsinic acid · Urine · Exposure assessment · Biomarker of exposure · Biomarker of susceptibility · Cancer · Epidemiology · Risk assessment · Risk management

Abbreviations

AAS	Atomic absorption spectrometry
AB	Arsenobetaine
AC	Arsenocholine
AFS	Atomic fluorescence spectrometry
As	Arsenic
CRM	Certified reference material
DMA	Dimethlarsinic acid
DMA^{III}	Dimethylarsinious acid
DR	Diet recall
FFQ	Food frequency questionnaire
iAs	Inorganic arsenic
ICC	Intra class correlation
ICP-AES	Inductively coupled plasma- atomic emission spectrometry
ICP-MS	Inductively coupled plasma- mass spectrometry
LC	Liquid chromatography
MMA	Monomethylarsonic acid
MMA^{III}	Monomethylarsenious acid
NMSC	Non- melanoma skin cancer
SCC	Squamous cell carcinoma
TMA	Trimethylarsine
TMAO	Trimethylarsine oxide

Introduction

Arsenic (As) is a toxic metalloid and known as a poison from ancient time. Acute high-level exposure to As results in neurological and gastrointestinal symptoms which can be fatal (ATSDR 2007). Chronic exposure to excessive As has been known to be a cause of skin lesions and cancers of the lung, bladder, kidney, liver, and prostate in addition to cancer of skin (IARC 2012). Endemic occurrence of these

symptoms has been well documented among the general populations in Taiwan, China, South and Southeast Asian countries, and South American countries due to groundwater contamination of As. The number of affected people is estimated to be nearly hundred million or more in the world. The As in the groundwater in these regions of the world was mostly of natural origin, though anthropogenic origins, such as mining activity, were present in some cases in the past. Thus, health problems with As in the world are more as natural disaster than as an anthropogenic, industrial pollution at present.

The abovementioned toxicities of As are exclusively from inorganic As (iAs), i.e., arsenate (As(V)) and arsenite (As(III)). It is well known that a variety of organic forms of As are present in the environment particularly in marine biota. More than 100 organic As compounds have ever been identified in the nature. In contrast to the toxic iAs, most of the organic As compounds are much less toxic. Humans are exposed to organic As compounds mainly via the consumption of marine products, such as seafood and seaweeds. Some foods of terrestrial origin contain detectable levels of iAs and some methylated As compounds of simpler structure, e.g., dimethylarsinic acid.

For the assessment of health effects of As in humans at environmental levels, speciation analysis of As is recognized essential at present because of the great variation in toxicity of different chemical forms. Total As analysis can only be applicable to a limited situation where the sole involvement of iAs as a contaminant but not of others is clearly known.

This review summarizes analytical method of As speciation, liquid chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS), and its application to the field of environmental health. In the field of environmental health, WHO's definition of biomarker (IPCS 1993) has been widely used: biomarker is defined as any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical, or biological. Three classes of biomarkers were identified as (i) biomarker of exposure, (ii) biomarker of effect, and (iii) biomarker of susceptibility (IPCS 1993). In this review, described is urinary As speciation analyzed by LC-ICP-MS as a biomarker of exposure to iAs, which will be more valuable in future epidemiologic researches of As exposure and cancer and/or neurodevelopment. Urinary As speciation as a biomarker of susceptibility to iAs toxicities will also be referred.

Arsenic Species in the Environment and Biota

There are many organic As compounds in the environment, mainly in marine organisms, which include methylated As (monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethyl As compounds, etc.; Fig. 1) and more complicated organic As compounds, e.g., arsenosugars and arsenolipids (Fig. 2). These organic As compounds are generally much less toxic, except for a couple of possible exceptions referred to later, when compared with the toxic iAs. The comparison of lethal dose 50 (LD_{50}) values of inorganic and some organic As

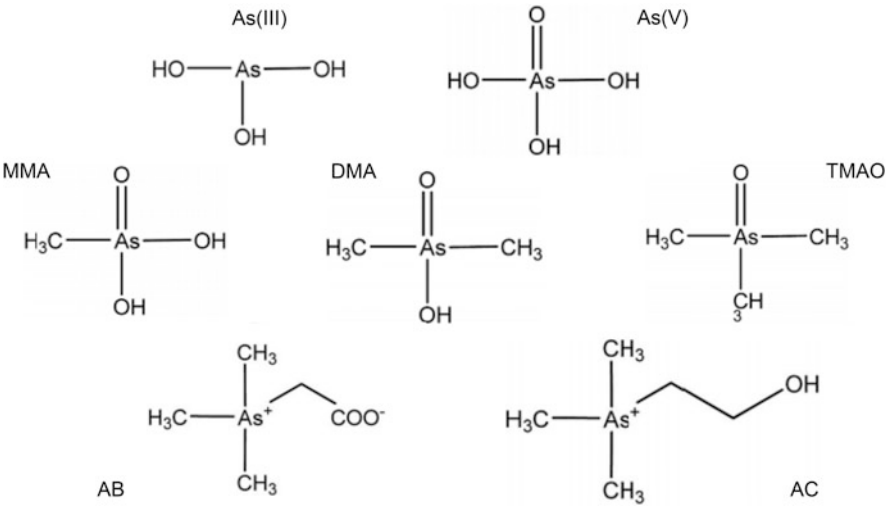

Fig. 1 Structure of arsenic species (1)

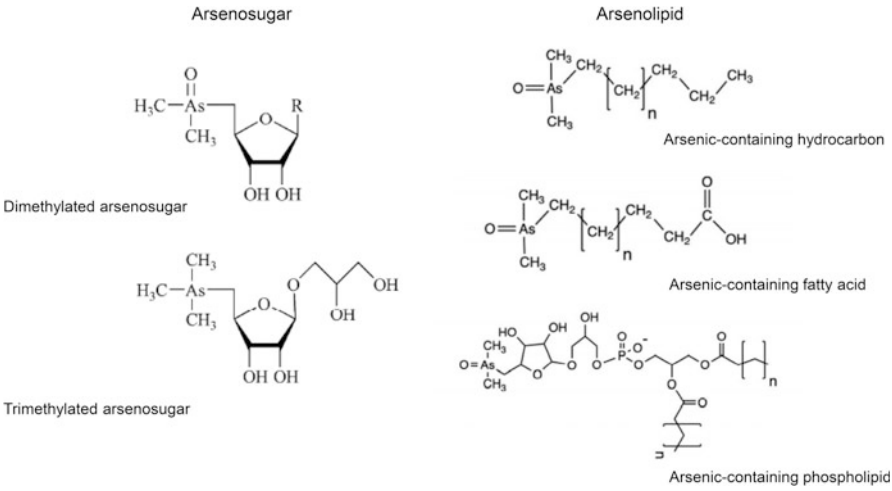

Fig. 2 Structure of arsenic species (2). R in dimethylated arsenosugar is either one of the following: H, OH, glycerol, phosphate, sulfonate, or sulfate

compounds in rodents (Kaise et al. 1985, 1989, 1992) is shown in Fig. 3. It is particularly interesting to note that LD_{50} of arsenobetaine (AB), a trimethylated As compounds commonly and abundantly found in marine animals, is as much as >10 g/kg (Kaise et al. 1985), suggesting that it is virtually nontoxic.

Arsenosugars are found in plankton and seaweed, and arsenolipids are in fish and seaweed. Some of the arsenolipid compounds found in fish and seaweed are known to have potent cell toxicity (Meyer et al. 2014), but most others do not. Occurrence of

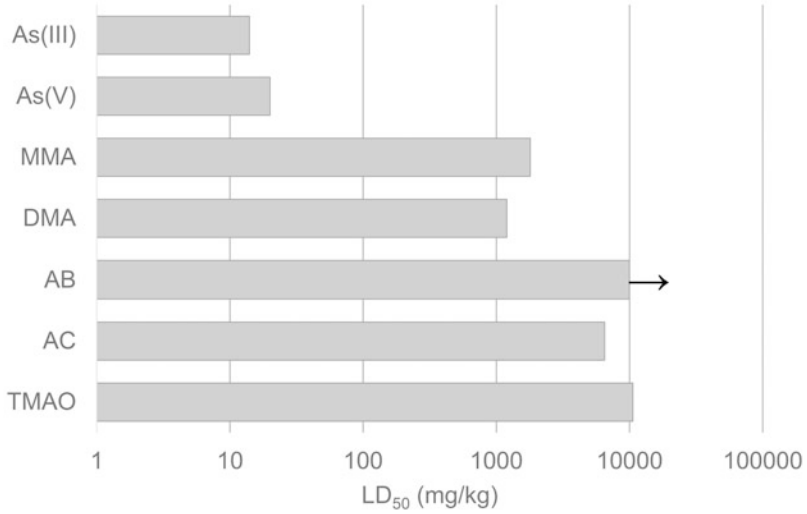

Fig. 3 Acute toxicity of arsenic species. Lethal dose 50 (LD_{50}, mg/kg) in rodents. As(III), arsenite; As(V), arsenate; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AB, arsenobetaine; AC, arsenocholine; TMAO, trimethylarsine oxide. (Source: Kaise et al. 1985, 1989, 1992)

Table 1 Major As species in food category

Food category		Major As species
Terrestrial plant	Rice	iAs, DMA
	Mushroom	iAs, DMA, AB
	Others	iAs
Aquatic plant	Seaweeds	iAs, MMA, DMA, Assugars, As-lipids
Terrestrial animal	Meat	iAs, MMA, DMA
Marine animal	Fish	MMA, DMA, AB, AC, TMAO, As-lipids
	Shellfish	MMA, DMA, AB, TMAO, As-sugars, As-lipids
	Crustaceans	MMA, DMA, AB, AC, TMAO, As-lipids
Freshwater animal	Fish	DMA, AB

MMA monomethylarsonic acid, *DMA* dimethylarsinic acid, *iAs* inorganic As, *AB* arsenobetaine, *AC* arsenocholine, *TMAO* trimethylarsine oxide

organic As compounds other than MMA and DMA is not common in terrestrial environment and biota, where iAs is usually found at lower concentration.

Humans are exposed to various As compounds via the diet in addition to iAs from drinking water. Table 1 shows major As species in some food categories. People are exposed to these As species depending on the food choices.

Other exposure routes including inhalation and dermal exposure do not significantly contribute to aggregated As exposure of general population. Oral ingestion of soil and house dust, which were known to contain only iAs, was not a significant contributor to As exposure (Oguri et al. 2013).

HPLC-ICP-MS for Speciation Analysis of As

Speciation of As was analyzed in early days by selective extraction of iAs in hydrochloric acid into solvent followed by atomic absorption (AAS) detection (Yasui et al. 1978). Later, hydride generation (HG) technique was widely employed for speciation of inorganic and methylated As species (Welna et al. 2020). This technique utilizes different boiling points of liquid nitrogen-trapped hydrides of different As compounds or gas chromatographic separation of hydrides of the As compounds. Atomic absorption spectrometry (AAS) and atomic fluorescence spectrometry (AFS) were typically used as a detector of As in HG technique. However, this HG technique was applicable only to iAs, MMA, and DMA speciation because these are the only compounds that form volatile hydride by sodium borohydrate reduction. Other organic As compounds of biological or environmental significance, e.g., AB, do not form hydride: speciation analysis based on HG technique had only a limited value in As speciation of biological and clinical samples unless chromatographic separation and As species digestion are preceded (mentioned later in this section).

Subsequently, liquid chromatographic separation with inductively coupled plasma-atomic emission spectrometry (ICP-AES) detection was developed for As speciation in the early 1980s (Morita et al. 1981). They employed cation and anion exchange columns for the separation of 5 As species including AB and applied anion exchange to hijiki extract. Use of LC is much more advantageous over HG technique because As compounds that do not form hydride could be speciated without tedious sample pretreatment. The ICP-AES detection was quickly replaced by ICP-MS detection because the latter was much more sensitive for the detection of As than was ICP-AES. Detection limit of As by LC-ICP-MS was around 0.1 μg/L, which was lower by 100 times than that by ICP-AES. The improvement of detection limit made LC-based technique applicable to urinary As speciation analysis of non-exposed individuals.

However, use of ICP-MS as a detector of LC had a drawback: it limited the composition of mobile phase of LC because ICP-MS did not favor the introduction of high-concentration salts or organic solvent. This is because introduction of salts or organic solvent makes ionizing source (argon plasma) unstable and buildup of salt or carbon (soot) at the interface between ICP and mass spectrometer, which significantly deteriorates stability and sensitivity of measurement. For this reason, ion exchange or reversed phase column with mobile phase containing dilute salts has been favored for As speciation with ICP-MS detection.

Anion or cation exchange separation has widely been used for separation of iAs, MMA, and DMA for ICP-MS detection (Ardini et al. 2020; Reid et al. 2020). Diluted salts are used for mobile phase (e.g., 10 mM ammonium phosphate). Cation exchange separation was used for the speciation of trimethylated and tetramethylated As compounds. Ion-pair, reversed phase separation has also been the method of choice for the separation and determination of iAs, methylated compounds, and arsenosugars. Employment of three different separation modes including two ion-pair, reversed phase separations coupled with ICP-MS detection enabled

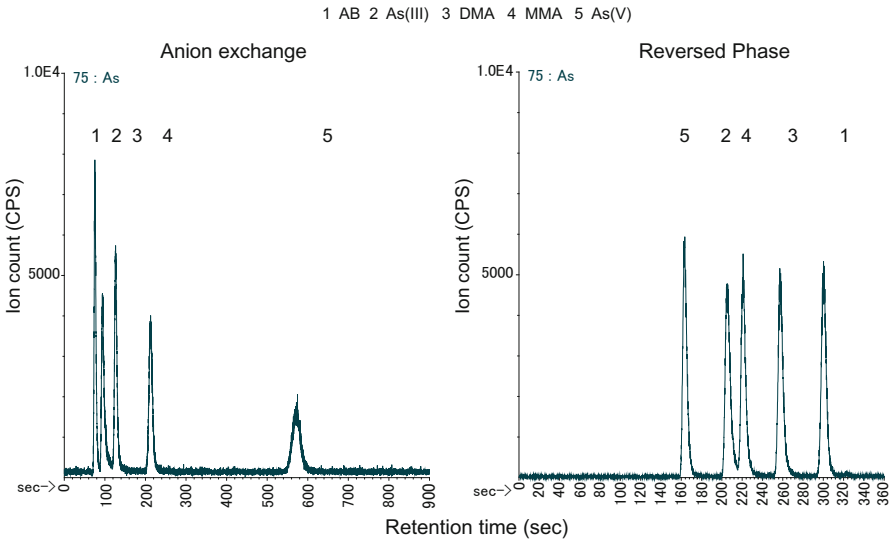

Fig. 4 Chromatograms of arsenic species standard by anion exchange and reversed phase separation modes. Anion exchange: Hamilton PRP X-100 (10 μm, id 4.1 × 150 mm), 10 mM $(NH_4)_2HPO_4$ (pH 8.25). Reversed phase: Shiseido CAPCELL PAK C_{18} (3 μm, id 4.6 × 150 mm), 10 mM Sodium 1-butanesulfonate/4 mM malonic acid/4 mM tetramethylammonium hydroxide/0.05% methanol (pH 3.0) (Narukawa and Chiba 2010). Standard mixture solution (20 μL injection) containing 20 μg As/L each of (1) AB, (2) As(III), (3) DMA, (4) MMA, and (5) As(V). For abbreviations, see legend to Fig. 3. (Chromatograms provided by Dr. T. Narukawa, National Institute of Advanced Industrial Science and Technology, Japan)

full separation of 15 As compounds, and it was applied to As speciation of human urine (Shibata and Morita 1989). Figure 4 shows comparison of anion exchange and reversed phase separations of five major As compounds. Column used for anion exchange was PRP X-100, the most widely used one, and that for reversed phase was C_{18} ODS with mobile phase composition originally developed by Shibata and Morita (1989). Since the elution order is As(III), AB, MMA, DMA, As(V) in anion exchange (Fig. 4 left), peak width of As(V) becomes wider, leading to lower signal to noise. In addition, As(III) is not fully separated from other cationic compounds (e.g., trimethylarsine oxide and tetramethylarsine), and co-elution can occur, requiring combined use of cation exchange for the full separation of wider range of compounds. For the latter, effort has been made to make separation better by careful selection of composition and pH of mobile phase. On the other hand, ion-pair, reversed phase separation provides full separation of toxicologically important species from cationic As compounds. Thus, generally speaking, reversed phase separation is better than anion exchange in terms of accurate determination of As(III) and As(V), important analytes in terms of health effect assessment.

HG-based speciation technique was developed by including online oxidation module in the system to render non-hydride-forming As compounds to hydride-forming compound. Liquid chromatographically separated As species were

individually oxidized to form hydride-generating compound (e.g., iAs) online, which were subsequently detected by HG-based analytical method. Thus, complicated system, e.g., LC-online oxidation-HG-ICP-MS (or other detectors), was developed (e.g., Nakazato and Tao 2006). Chemical reagent, microwave, or ultraviolet radiation, sometimes a combination of these, was used for online oxidizer in such system. The most remarkable merit of this complicated system was higher sensitivity, e.g., 1–2 ng/L of detection limit, which resulted from higher sample introduction efficacy of gaseous hydride to the detector as well as low background due to freedom from coexisting components.

Urinary As Species as a Biomarker of Exposure

Since toxicity and carcinogenicity are expected virtually for iAs exposure only for the moment, human exposure assessment is necessary for iAs but not for other organic As species. It is well known that absorbed iAs is metabolized to MMA and DMA in the liver in humans by the combination of oxidation, reduction, and methylation (Watanabe and Himeno 2013; Stýblo et al. 2021). Although methylation was previously regarded as detoxifying process of toxic iAs in organisms, it turned out it's not necessarily the case because intermediate metabolites produced during methylation process, monomethylarsenious acid (MMA^{III}) and dimethylarsinious acid (DMA^{III}), were found to be more toxic than iAs. The MMA^{III} and DMA^{III} in cells are eventually oxidized to less toxic MMA and DMA (MMA and DMA without superscript denote MMA^{V} and DMA^{V}, respectively, throughout this review) in the organisms and are excreted.

Urine As concentration has been used for biomarker of iAs exposure in humans because absorbed iAs is excreted in urine as iAs, MMA, and DMA with a relatively short biological half-time (1–2 days, Buchet et al. 1981). Urinary total As concentration was used in earlier studies, and it is still acceptable as a biomarker of exposure when source of exposure is known and species exposed is limited to iAs. For instance, for a population living in groundwater-contaminated area and who do not consume marine products, the exposed As species is almost exclusively inorganic except for a minor portion of DMA from crops (Table 1). In this case, As in urine is expected to be composed of iAs and its metabolites MMA and DMA: thus total As concentration is equal to the sum of As species resulting from iAs exposure, and total As analysis is justified in this case. However, for a population who consume marine products, such as seafood and seaweed, they are exposed to a variety of nontoxic As species; therefore, urinary total As analysis is never an option for As exposure assessment because organic As species are also excreted in urine unchanged or after metabolism. Speciation analysis of As in urine, namely, iAs, MMA, and DMA, is essential in such populations for the assessment of toxic iAs exposure. Here, iAs means the sum of As(III) and As(V) because it is difficult to completely control analytical artifact that induces valency conversion, e.g., oxidation of As(III) to As(V) or reduction of As(V) to As(III), during storage and/or analysis of urine sample (Crecelius and Yager 1997). In this review only the sum of As(III) and As(V) is dealt with and expressed as "iAs."

As a reliable biomarker of exposure to iAs, urinary concentrations of iAs, MMA, and DMA should be measured and summed for the quantitative estimation of iAs exposure level. Although MMA and DMA are contained in some foods of terrestrial origin, the levels are generally so low that the direct contribution from dietary MMA and DMA to urinary MMA and DMA has been ignored, and use of iAs + MMA + DMA in urine has been justified as a biomarker of iAs exposure. However, for a population consuming marine products, urinary iAs + MMA + DMA cannot be used as a biomarker of iAs exposure because appreciable contribution is expected from DMA produced from metabolism of some organic As compounds. Arsenosugars are the typical example: DMA was the most abundant metabolite excreted from arsenosugar-dosed humans (Ma and Le 1998; Francesconi et al. 2002). This meant that urine iAs + MMA + DMA could overestimate iAs exposure in seaweed-consuming populations due to DMA metabolized from arsenosugars contained abundantly in seaweeds. DMA is also produced from the metabolism of arsenolipids (Schmeisser et al. 2006), which are known to be contained in seaweeds and seafood.

Hata et al. (2016) suggested the use of iAs + MMA as a biomarker of iAs exposure in such population. They found a highly significant positive correlation ($r = 0.962$, $p < 0.0001$) between iAs + MMA and iAs + MMA + DMA in urine samples from Bangladeshi people ($n = 330$) who do not consume marine products. The authors successfully applied urinary iAs + MMA for the assessment of occupational inhalation exposure to iAs in Japanese workers, who habitually consume substantial amount of seafood and seaweeds. Thus, urinary iAs + MMA + DMA or iAs + MMA is regarded as a biomarker of iAs exposure depending on whether the target population consume marine products or not.

Due to the widespread notion that speciation information is essential, recent human biomonitoring programs include not only urinary total As but also As species. Table 2 shows urinary As speciation results of the US, French, and Canadian

Table 2 Average levels of arsenic species reported in human biomonitoring programs (μg/L otherwise indicated)

Country	N	Year of sampling	As(III)	As(V)	MMA	DMA	AB	iAs + MMA +DMA	Others	Ref
USA	2568	2003–2004	<1.0	<1.2	<0.9	3.90	1.00		AC <0.6 TMAO <1.0	Caldwell et al. (2009)
USA	9537	2003–2014			0.73	3.84	1.98			Wang et al. (2018)
France[a]	1515	2006–2007						3.53		Saoudi et al. (2012)
Canada	2615	2016–2017	0.36	<0.14	0.40	3.1		4.1	AB +AC 1.3	Health Canada (2019)

For abbreviations, see footnote to Table 1

[a]Unit: μg/g creatinine

Table 3 Analytical results of arsenic species in certified reference materials of urine matrix. (unit: ng/mL)[a]

	NIST SRM 2669 Frozen Human Urine Level I		NIST SRM 2669 Frozen Human Urine Level II	
	Certified	Measured ($n = 8$)	Certified	Measured ($n = 8$)
As(V)	2.41 ± 0.30	3.81 ± 0.13[b]	6.16 ± 0.95	11.43 ± 0.24[b]
As(III)	1.47 ± 0.10		5.03 ± 0.31	
MMA[c]	1.87 ± 0.39	1.83 ± 0.08	7.18 ± 0.56	6.97 ± 0.35
DMA[c]	3.47 ± 0.41	3.52 ± 0.19	25.3 ± 0.70	25.3 ± 0.30
AsB[c]	12.4 ± 1.9	12.5 ± 0.55	1.43 ± 0.08	1.46 ± 0.02

[a]Yoshinaga J and Narukawa T (2020). (Reproduced with permission)
[b]Sum of As(III) and As(V) on the chromatogram was shown because transformation of valence could be possible
[c]Abbreviation: *MMA* methylarsonic acid, *DMA* dimethylarsinic acid, *AsB* Arsenobetaine

populations (Caldwell et al. 2009; Saoudi et al. 2012; Wang et al. 2018; Health Canada 2019). Anion exchange LC-ICP-MS was used for the determination in these programs. As can be seen from this table, As(III), As(V), and MMA were hardly detectable in the US, French, and Canadian populations: they were detectable only <10% of the subjects of each population. DMA and AB were detectable. This was due to lower abundance of iAs and MMA in urine, suggesting that the populations were, on average, not exposed to excessive iAs. Moderate sensitivity of LC-ICP-MS system might also be the reason of low detectability. Detection of AB in these programs indicated that the US, French, and Canadian populations consume some seafood: this implies that a fraction of the DMA detected in urine might be from the metabolism of arsenolipids in seafood and thus overestimation of iAs exposure levels could occur when iAs + MMA + DMA was used as a biomarker of exposure.

Analytical quality assurance of LC-ICP-MS analysis is a relevant issue when using the urinary concentrations of iAs, MMA, and DMA as a biomarker of iAs exposure. Certified reference materials (CRMs) from the National Institute of Standards and Technology (NIST), USA, are widely used for this purpose. Table 3 shows an example of analytical quality assurance of urinary LC-ICP-MS analysis for iAs exposure assessment based on NIST CRMs (Yoshinaga and Narukawa 2020). Urine-based CRM for As speciation is also available from the National Institute for Environmental Studies, Japan (NIES CRM No. 18 Human Urine). Presentation of the analytical results of urine-based CRM is required for any studies that involve urinary As speciation analysis.

Urinary iAs Speciation Analysis in Environmental Epidemiology

Intake level of iAs is crucial information for the assessment of health risks of individual and population. In earlier epidemiologic studies on the health effects of groundwater As contamination, total As levels in drinking water were used as an exposure proxy of a population. Concentration of total As in public well water was used as exposure proxy

of all the people who use the well in some epidemiologic studies. Apparently, this exposure assessment did not provide accurate exposure of individuals and population because water consumption can be variable among individuals of the target population, and this did not take into consideration possible intake from diet. However, this approach has been justified because (1) this could provide cost-effective semiquantitative estimate of exposure levels of large number of individuals under the situation of urgent research needs and (2) intake of As from drinking water was assumed to be much greater than that from diet in groundwater-contaminated region. This semiquantitative approach was applicable only to the exposure assessment of ecological epidemiologic studies in groundwater-contaminated area.

Public awareness of carcinogenetic effect of iAs has stimulated concern over the cancer risk of general populations in the regions where groundwater is not contaminated. More quantitative approach of exposure assessment on individual basis has become necessary to establish a reliable dose-response relationship in an analytical epidemiologic study. Establishment of a dose-response relationship is indispensable for accurately assessing health risk. For the quantitative assessment of iAs exposure, not only iAs from drinking water but also the dietary iAs intake must be assessed in the regions where groundwater contamination is not present. There are two quantitative approaches for the assessment of dietary intake of a chemical. Food frequency questionnaire (FFQ) or diet recall (DR) coupled with an appropriate food composition table is one of the approaches. This approach has often been taken in a large-scale cohort study because it is based on questionnaire and less time-, cost-, and labor-consuming for obtaining semiquantitative exposure/intake information from a large number of subjects. There were several studies that estimated iAs intake of individuals by FFQ or diet recall approach (Signers-Pastor et al. 2008; Sawada et al. 2013; Wong et al. 2013).

A duplicate diet study is another approach, which measures the concentration of the target chemical in a duplicate diet sample collected from subject to calculate daily intake on individual basis. It provides fully quantitative intake information of the day of diet sampling; however, apparently it is much more time-, cost-, and labor-consuming than FFQ/DR. The most problematic point is that duplicate diet approach requires extensive sample pretreatment including sample collection, homogenization, extraction of iAs, as well as subsequent measurement. Thus, duplicate diet approach is not practical for assessing exposure in a large-scale epidemiologic study. Daily iAs intake estimation of a population based on duplicate diet approach has been reported in several publications (Ruangwises et al. 2011; Hayashi et al. 2019).

Use of urinary levels as a biomarker of iAs exposure has advantages over the estimation based on diet iAs. Urine analysis is much less time- and labor-consuming than duplicate diet because it does not require extensive sample pretreatment but only dilution. It is possible to measure a large number of samples. Moreover, urine concentration of iAs + MMA + DMA reflects internal dose of iAs, MMA, and DMA and includes contributions from both diet and drinking water. It is not affected by, e.g., erroneous recall in FFQ/DR approach or missing food items in duplicate diet approach. Increasing number of epidemiologic studies has been published in which levels of urinary iAs species were used as an exposure metrics. Gilbert-Diamond

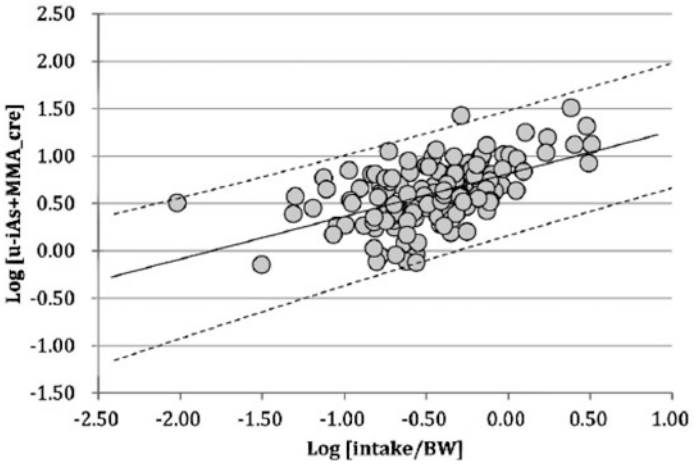

Fig. 5 Scatter plot of inorganic arsenic intake and sum of urinary levels of inorganic As and monomethylarsonic acid. (Reproduced from Yoshinaga and Narukawa (2020) with permission)

et al. (2013) conducted a case-control study on incidence of squamous cell carcinoma (SCC) and urinary iAs, MMA, and DMA levels in New Hampshire, USA. Three hundred and twenty-three case and 319 control subjects were involved in the statistical analysis. The mean concentration of urinary iAs + MMA + DMA of case and control was 5.27 and 4.76 μg/L, respectively, indicating that both cases and controls were not excessively exposed (see Table 2). The authors found a positive association between SCC and urinary iAs + MMA + DMA concentration, as well as that between SCC and MMA, and SCC and DMA. These results indicated that iAs exposure could be involved in carcinogenesis of skin at the levels general public are exposed (Fig. 5).

Kim et al. (2017) also conducted a case-control study on the association between As exposure and non-melanoma skin cancer in Korea. Geometric mean urinary iAs + MMA + DMA concentration of the patients was 34.52 μg/L ($n = 124$) and higher than the USA mean of Gilbert-Diamond et al.; this must be due to higher DMA (32.78 μg/L) in these Korean study subjects. Urinary iAs concentrations were significantly higher in the cases (0.140 μg/L) than in controls (0.055 μg/L), but the difference was not significant for organic As (MMA + DMA). A significant difference was found when basal cell carcinoma and SCC cases were separately tested. Higher DMA concentrations of the cases and controls in this study suggested the contribution of seafood and/or seaweed consumption, as Koreans are known to consume marine products. If this was the case, use of iAs + MMA + DMA was not appropriate as a biomarker of iAs exposure in this study, and this might be in part the reason of the absence of significant difference in urinary iAs + MMA + DMA between cases and controls.

Signers-Pastor et al. (2019) cross-sectionally examined the relationship between iAs exposure and neuropsychological development among children in Spain. Mean

urinary concentration of iAs + MMA + DMA was 4.85 μg/L ($n = 361$) indicating that the children had low-level exposure. The authors found a negative association between urinary iAs + MMA + DMA and some of the motor functions of children. Since urinary AB concentration was rather high for the children (mean, 67% of iAs + MMA + DMA + AB), some of the DMA must have been metabolic product of arsenolipids so that use of iAs + MMA + DMA as a biomarker of exposure to iAs might be erroneous.

Quantitative Relationship Between Urinary As Speciation and Dietary Intake

Since there is no animal model for some of the adverse effects of iAs, e.g., cancer and neurobehavioral development, a threshold value, a value below which unacceptable adverse effect is not expected, has to be obtained from dose-response relationship established in epidemiologic studies. When urinary As concentrations are used as an exposure metrics in the epidemiologic studies, then the threshold value would be obtained as a urinary concentration. Risk managers have to express the threshold as daily intake, such as tolerable daily intake or reference dose, for the protection of public health. Therefore, it would become necessary to convert urinary iAs concentration to daily intake of iAs. Quantitative relationship between iAs intake and urinary iAs and metabolites (MMA and DMA; iAs, MMA, and DMA are collectively designated "iAs species" hereafter) concentrations has not been extensively investigated to date.

Uchino et al. (2006) estimated total As intake from drinking water and diet and urinary total As concentrations in 147 subjects living in West Bengal, India, where groundwater As contamination is known. Sum of total As intakes from drinking water and diet was estimated to be 37.1–1098 μg/person/day. Although total As was measured, it was estimated to be mostly iAs. They found a positive correlation between urinary As and estimated daily As intake ($r^2 = 0.134$, $p < 0.001$). They presented a regression equation: [daily As intake] = 0.341 × [urinary As conc] + 244.106. It is not known if transformation of urinary As concentration or daily As intake was in fact needed or not in calculating Pearson correlation coefficient and regression equation. This regression equation may be used for converting urinary As (most probably iAs + MMA + DMA) to dietary iAs intake in exposed population.

Yoshinaga and Narukawa (2020) measured iAs intake and urinary As species concentrations of 150 Japanese subjects. The general Japanese is known to be exposed to iAs via the consumption of rice and hijiki seaweed (Oguri et al. 2014), while drinking water does not significantly contribute to iAs intake. The intake of iAs was obtained by duplicated diet approach, and urinary speciation analysis was performed to obtain the concentrations of iAs species and other organic As compounds. Since the Japanese habitually consume seaweed and seafood, urinary iAs + MMA concentrations but not iAs + MMA + DMA were related to daily intake of iAs in this study. Geometric mean daily intake of iAs was 20.5 μg/person/day or

0.349 μg/kg/day, and median urinary iAs, MMA, and DMA were 2.66, 2.63, and 28.2 μg/L (specific gravity-corrected), respectively, for the 150 subjects. They found a significant positive correlation between the urinary iAs + MMA and daily iAs intake ($r = 0.544$, $p < 0.001$, Fig. 4) and obtained a regression equation: $\log_{10}$[daily intake] $= 0.451 \times \log_{10}$[iAs + MMA] + 0.814 where iAs + MMA is a creatinine-corrected concentration (μg As/g creatinine). The equation was also obtained for a specific gravity-corrected concentration of iAs + MMA as follows: $\log_{10}$[daily intake] $= 0.400 \times \log_{10}$[iAs + MMA] + 0.899.

Quantitative relationship between intake and excretion of iAs has to be investigated for a variety of situations, e.g., contaminated or non-contaminated, marine food eater or nonmarine food eater, etc., because the knowledge is population-specific and would be relevant for managing health risks in the future.

Problem in Using Urinary As Speciation as a Biomarker of Exposure

One important issue in using urinary iAs species concentration as a biomarker of exposure to iAs is that urinary concentration reflects only the levels of recent exposure to iAs because biological half-time of iAs species is not long. In contrast to this fact, what is needed is an assessment of long-term exposure to iAs of individuals when exposure is related to incidence of cancer and other chronic pathological conditions.

To what extent levels of urinary iAs species reflects long-term exposure was investigated by Kile et al. (2009). They measured iAs, MMA, and DMA concentrations in urine samples repeatedly collected from 196 subjects for 3 months in Bangladesh. The median urinary iAs, MMA, and DMA concentrations were 3.8, 2.6, and 22.8 μg/L, respectively, which were higher than corresponding average concentrations obtained in human biomonitoring as shown in Table 2. This is because the subjects in Kile et al. were exposed to groundwater As contamination. The levels of urinary iAs species were relatively constant within-subject, and intraclass correlation (ICC) coefficients were 0.35–0.49. This result indicated that iAs species concentration in a single spot urine sample collected from an individual represents long-term iAs exposure levels of the individual.

In contrast, ICC coefficient of urinary iAs + MMA concentrations collected from Japanese subjects over 4–5 months was 0.15, indicating that within-subject variation was much greater than between-subject variation (Oguri et al. 2012). The discrepancy between the results of Bangladeshi and Japanese studies was due to the difference in iAs exposure pattern between the two: In Bangladesh, subjects were exposed to drinking water iAs, while in Japan, people were exposed to dietary iAs particularly from hijiki seaweed that is usually eaten as a side dish. Hijiki seaweed is well known to contain high levels of iAs (up to 100 mg/kg dry; Yasui et al. 1978; Almela et al. 2006) and consumed almost exclusively in Japan. Frequency of hijiki seaweed consumption in Japan was reported to be 2–3 times a month. Therefore, iAs intake is expected to elevate only on the day hijiki seaweed is eaten. Exposure to drinking water iAs must be more constant than iAs intake from hijiki seaweed within-individual.

Thus, usability of urinary concentrations of iAs species as a biomarker of exposure can be different depending on the exposure pattern of the subject population. It must be confirmed if the exposure pattern can be regarded as constant when urinary iAs species are used as a biomarker of iAs exposure. Oguri et al. (2012) proposed four repetitions of urine sampling measurement for the estimation of long-term iAs intake of the Japanese based on ICC values obtained in their study.

Urinary As Speciation as a Biomarker of Susceptibility

In this review, urinary As speciation has been discussed in terms of biomarker of exposure to dietary iAs. It must be noted that urinary As speciation has also been used as a biomarker of susceptibility, a term according to WHO's definition (IPCS 1993), and more and more attention has been paid to this aspect in analytical epidemiologic studies.

Not only the concentration of iAs species but also the percentage MMA (%MMA) or DMA (%DMA) of urinary iAs + MMA + DMA was found to be associated with a variety of pathological conditions including cancers in the previous studies (Tseng 2007). Pierce et al. (2013) found a significant association between percent iAs or %MMA and skin lesion, while negative association between %DMA and skin lesion in As-exposed Bangladeshi subjects ($n = 2060$). Gamboa-Loira et al. (2017) carried out a meta-analysis, involving 13 non-ecological epidemiologic studies, on the association between urinary %MMA or %DMA and risk of cancers. They found consistent positive associations between %MMA and incidence of various cancers (e.g., bladder and negative association between %DMA and some cancer). Thus, association of higher %MMA with the elevated incidence of pathological conditions has been consistently found in many epidemiologic studies, while the association of lower %DMA with elevated disease risk was less consistent.

The %MMA and %DMA are regarded as a measure of methylation capacity of absorbed iAs: high %DMA and low %MMA represent enhanced methylation capacity, while low %DMA and high %MMA represent low methylation capacity. The abovementioned epidemiologic studies indicated that an individual with low methylation capacity is likely to develop skin lesion and cancers as a result of iAs exposure. It was speculated that low methylation capacity (=high urinary MMA) was associated with more occurrence of MMA^{III} in the cells, which is subsequently oxidized and excreted in urine as MMA (Tseng 2007). Lower methylation capacity thus results in more adverse effects. Interindividual variation in the methylation capacity in a population was attributed to genetic and environmental factors (Tseng 2009; Agusa et al. 2011). The %MMA and %DMA are derived from urinary As speciation analysis and taken as a biomarker of susceptibility to iAs toxicity/carcinogenicity.

Thus, along with iAs exposure levels, methylation capacity of individuals is now regarded as indispensable information in the epidemiologic assessment of toxicity/carcinogenicity of iAs. Speciation analysis of urinary As serves both as a biomarker of exposure and biomarker of susceptibility.

Concluding Remarks

More emphasis will be placed on conducting analytical epidemiologic studies on low-level dietary iAs exposure and cancers and other pathological conditions. Exposure information of individuals in the subject population is indispensable in such studies, particularly to establish a quantitative dose-response relationship between exposure and occurrence of adverse effects. Urinary As speciation analysis will be the unique method of choice for such studies because urinary concentration of iAs + MMA + DMA or iAs + MMA is the most appropriate biomarker of exposure to iAs. Furthermore, speciation analysis provides %MMA and %DMA, a biomarker of susceptibility, that should be included in the assessment of adverse effects. In doing so, dietary habit of the subjects must be cautiously considered for the selection of a suitable biomarker of exposure, e.g., iAs + MMA should be selected in place of iAs + MMA + DMA if the subjects consume marine food. For this consideration, measurement of AB, in addition to iAs species, is recommended as a marker of seafood consumption. It should also be considered if multiple urine sampling is required or a single spot urine sampling is sufficient for assessing long-term exposure levels. These mean that dietary habit of the subjects or a population must be carefully assessed when exposure is to be assessed.

Applications to Other Diseases or Conditions

In this chapter, reviewed is an application of urinary As speciation analysis to an analytical epidemiologic study for the establishment of dose-response relationship between inorganic As exposure and cancer, neurodevelopment, and other chronic pathological conditions. This technique has also been applied to clinical settings where internal iAs kinetics monitoring for diagnosis and treatment of acute As poisoning cases and acute promyelocytic leukemia (APL) patients dosed with arsenic trioxide for therapeutic purpose are required.

Acute arsenic poisoning cases due to accidental, suicidal, and criminal situations have been known worldwide. Arsenic speciation analysis of urine and serum has been applied to the cases for diagnosis and monitoring (e.g., Yoshimura et al. 2011). Adverse health outcomes among residents of a region in Japan due to diphenylarsinic acid (DPAA) poisoning were known. DPAA was a decomposition product of warfare agent produced during World War II in Japan, and the DPAA-containing waste was buried underground of the region for unknown reasons and the waste-contaminated groundwater of the region (Ishii et al. 2004). LC-ICP-MS analysis of biological fluids of the patients was developed and applied for the clinical investigation of DPAA-affected patients (Shibata et al. 2004).

In 1996, intravenous administration of arsenic trioxide (As_2O_3, 0.16 mg/kg dose) was found effective for the treatment of APL. Arsenic therapy is now considered as an effective therapeutic choice for APL patients resistant to all-trans retinoic acid therapy, the standard therapy of APL. Serum and/or urine As speciation analysis is a routine analytical method for monitoring of administered patients (Wang et al. 2004; Fukai et al. 2006).

Mini-Dictionary of Terms

Arsenobetaine. The most well-known organic arsenic compound. It was first purified and identified in 1977 in the muscle of Western Rock Lobster. Fishes and crustaceans contain AB at high concentration. It is nontoxic and humans excrete AB unchanged into urine quickly.

Arsenosugars. Major water-soluble organic arsenicals found in seaweeds. It was first identified in 1981. Arsenosugar contains 5-deoxypentose moiety and an arsinoyl or arsinothioyl group to C5 atom (see Fig. 2). Considered as nontoxic.

Arsenolipids. The presence of lipid-soluble forms of arsenic has long been known in marine animals and seaweeds, but identification and characterization of the compounds have recently been done. Arsenic-containing fatty acids, hydrocarbons, and phospholipids are the major arsenolipids studied so far (see Fig. 2). Most of the compounds are not toxic but some of the hydrocarbon types are found cytotoxic.

Hijiki. *Sargassum fusiforme*. It is a unique seaweed in that it contains inorganic arsenic at elevated concentration. Other seaweeds contain arsenosugars and methylated arsenicals. The Japanese traditionally consume hijiki. In 2004, UK government announced not to eat hijiki because it contains inorganic arsenic.

Methylation. Methylation of inorganic arsenic has been considered a detoxifying process of organisms because methylated arsenic is much less toxic than inorganic arsenic. Methylation was first postulated in the 1940s by reduction of arsenic atom followed by oxidative methylation (Challenger pathway). Recent evidence suggests that methylation of trivalent As atom can occur with the conjugation with glutathione. Production of highly toxic MMAIII and DMAIII during either pathway indicated that methylation is not simply a detoxifying process but it can activate arsenic toxicity at the same time.

Key Facts of Urinary Arsenic as a Biomarker: Speciation Analysis for the Assessment of Dietary Exposure

Key Facts of Urine As Species as a Biomarker of Exposure

- Humans are orally exposed to a variety of As species via drinking water and diet.
- In human urine, inorganic As (iAs), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) are detectable after iAs exposure.
- Other organic As compounds (e.g., arsenobetaine) are detectable in urine of marine food eaters.
- There is a highly significant positive association between sum of urinary iAs, MMA, and/or DMA levels and dietary iAs exposure levels.
- Significant associations were found between the levels of urinary iAs species and incidences of cancer and other pathological conditions in the previous epidemiologic studies.

- The %MMA and %DMA in urine as a methylation capacity of an individual are increasingly attracting interest in terms of its association with occurrence of pathological conditions.

Key Facts of Urinary As Speciation Analytical Method

- Liquid chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS) is a suitable method of choice for urine As speciation analysis.
- Anion exchange mode is used commonly, but reversed phase mode provides better separation of iAs species in human urine.
- Sensitivity for iAs species of LC-ICP-MS (detection limit around 0.1 μg As/L) is moderate for accurately determining iAs and MMA in urine of nonexposed subjects.
- Use of hydride generation module in LC-ICP-MS system can enhance the sensitivity by more than one order of magnitude.

Summary Points

- Inorganic arsenic (iAs) is a human carcinogen as well as the cause of other pathological conditions.
- Adverse health outcomes have been found in many parts of the world where groundwater iAs contamination is present.
- A biomarker of exposure to iAs is necessary for establishing a dose-response relationship in humans by epidemiologic studies of population who is exposed to low-level dietary iAs.
- Sum of the concentrations of urinary iAs and its metabolites, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), namely, iAs + MMA + DMA, is a suitable biomarker of exposure to iAs.
- Liquid chromatography combined with inductively couple plasma-mass spectrometric detector (LC-ICP-MS) is a suitable analytical method for arsenic speciation.
- The number of analytical epidemiologic studies involving urinary LC-ICP-MS analysis for biomarker of exposure is increasing and will be more in the future.
- Use of urinary As speciation as a biomarker of exposure has a couple of cautions, i.e., interference of arsenosugar- and/or arsenolipid-derived DMA in the urine of marine products eaters and representativeness of spot urine iAs species as a long-term exposure.
- Urinary As speciation can provide methylation capacity of an individual, which is now recognized as a relevant factor for occurrence of iAs-derived pathological conditions.

References

Agency for Toxic Substances and Disease Registry [ATSDR]. Toxicological profile for Arsenic. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2007.

Agusa T, Fujihara J, Takeshita H, Iwata H. Individual variations in inorganic arsenic metabolism associated with AS3MT genetic polymorphisms. Int J Mol Sci. 2011;12:2351–82.

Almela C, Clemente MJ, Vélez D, Montoro R. Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. Fd Chem Toxicol. 2006;44:1901–8.

Ardini F, Dan G, Grotti M. Arsenic speciation analysis of environmental samples. J Anal At Spectrom. 2020;35:215–37.

Buchet JP, Lauwerys R, Roels H. Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. Int Arch Occup Environ Heakth. 1981;48:111–8.

Caldwell KL, Jones RL, Verdon CP, et al. Levels of urinary total and speciated arsenic in the US population: national health and nutrition examination survey 2003–2004. J Exp Sci Environ Epidemiol. 2009;19:59–68.

Crecelius E, Yager J. Intercomparison of analytical methods for arsenic speciation in human urine. Environ Health Perspect. 1997;105:650–3.

Francesconi KA, Tanggaard R, McKenzie CJ, Goessler W. Arsenic metabolism in human urine after ingestion of an arsenosugar. Clin Chem. 2002;48:92–101.

Fukai Y, Hirata M, Ueno M, et al. Clinical pharmacokinetic study of arsenic trioxide in an acute promyelocytic leukemia (APL) patient: speciation of arsenic metabolites in serum and urine. Biol Pharm Bull. 2006;29:1022–7.

Gamboa-Loira B, Cebrián ME, Franco-Marina F, et al. Arsenic metabolism and cancer risk: a meta-analysis. Environ Res. 2017;156:551–8.

Gilbert-Diamond D, Li Z, Perry AE, et al. A population-based case-control study of urinary arsenic species and squamous cell carcinoma in New Hampshire, USA. Environ Health Perspect. 2013;121:1154–60.

Hata A, Kurosawa H, Endo Y, Yamanaka K, Fujitani N, Endo G. A biological indicator of inorganic arsenic exposure using the sum of urinary inorganic arsenic and monomethylarsonic acid concentrations. J Occup Health. 2016;58:196–200.

Hayashi A, Sato F, Imai T, Yoshinaga J. Daily intake of total and inorganic arsenic, lead, and aluminum of the Japanese: duplicated diet study. J Food Comp Anal. 2019;77:77–83.

Health Canada Fifth Report on Human Biomonitoring of Environmental Chemicals in Canada. 2019. https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/fifth-report-human-biomonitoring.html

International Agency for Research on Cancer [IARC]. Arsenic and Arsenic Compounds. In: IARC monographs vol. 100C Arsenic, metals, fibres and dusts. Geneva: World Health Organization; 2012. p. P41–93.

International Programme on Chemical Safety [IPCS]. Biomarkers and risk assessment: concept and principles. Geneva: World Health Organization; 1993.

Ishii K, Tamaoka A, Otsuka F, et al. Diphenylarsinic acid poisoning from chemical weapons in Kamisu, Japan. Anal Neuorol. 2004;56:741–5.

Kaise T, Watanabe S, Itoh K. The acute toxicity of arsenobetaine. Chemosphere. 1985;14:1327–32.

Kaise T, Yamauchi H, Horiguchi Y, et al. A comparative study on acute toxicity of methylarsonic acid, dimethylarsinic acid and trimethylarsine oxide in mice. Appl Organomet Chem. 1989;3:273–7.

Kaise T, Hriguchi Y, Fukui S, et al. Acute toxicity and metabolism of arsenocholine in mice. Appl Organomet Chem. 1992;6:369–73.

Kile ML, Hoffman E, Hsueh Y-M, et al. Variability in biomarkers of arsenic exposure and metabolism in adults over time. Environ Health Perspect. 2009;117:455–60.

Kim T-H, Seo J-W, Hong Y-S, et al. Case-control study of chronic low-level exposure of inorganic arsenic species and non-melanoma skin cancer. J Dermatol. 2017;44:1374–9.
Ma M, Le XC. Effect of arsenosugar ingestion on urinary arsenic speciation. Clin Chem. 1998;44: 539–50.
Meyer S, Matissek M, Müller SM, et al. In vitro toxicological characterization of three arsenic-containing hydrocarbons. Metallomics. 2014;6:1023–33.
Morita M, Uehiro T, Fuwa K. Determination of arsenic compounds in biological samples by liquid chromatography with inductively coupled argon plasma-atomic emission spectrometric detection. Anal Chem. 1981;53:1806–8.
Nakazato T, Tao H. A high-efficiency photooxidation reactor for speciation of organic arsenicals by liquid chromatography-hydride generation-ICPMS. Anal Chem. 2006;78:1665–72.
Narukawa T, Chiba K. Heat-assisted aqueous extraction of rice flour for arsenic speciation analysis. J Agric Food Chem. 2010;58:8183–8.
Oguri T, Suzuki Y, Hisada A, Yoshinaga J. Intra- and inter-individual variability in urinary concentrations of inorganic arsenic metabolites in Japanese subjects. Biomed Res Trace Elem. 2012;23:33–9. (In Japanese with English Abstract)
Oguri T, Ishibashi Y, Yoshinaga J. Total and inorganic arsenic content of residential soil and house dust. J Environ Chem. 2013;23:43–7. (In Japanese with English Abstract)
Oguri T, Yoshinaga J, Tao H, Nakazato T. Inorganic arsenic in the Japanese diet: daily intake and source. Arch Environ Contam Toxicol. 2014;66:100–12.
Pierce BL, Tong L, Argos M, et al. Arsenic metabolism efficiency has a causal role in arsenic toxicity: Mendelian randomization and gene-environment interaction. Int J Epidemiol. 2013;42: 1862–72.
Reid MS, Hoy KS, Schofield JRM, et al. Arsenic speciation analysis: a review with an emphasis on chromatographic separations. Trend Anal Chem. 2020;123:115770.
Ruangwises S, Ruangwises N, Saipan P. Dietary intake of total and inorganic arsenic by adults in arsenic-contaminated Dan Chang District, Thailand, using duplicate diet approach. Bull Environ Contam Toxicol. 2011;86:208–11.
Saoudi A, Zeghnoun A, Bidondo M-L, et al. Urinary arsenic levels in the French adult population: the French national nutrition and health study, 2006–2007. Sci Total Environ. 2012;433:206–15.
Sawada N, Iwasaki M, Inoue M, Takachi R, Sasazuki S, Yamaji T, Shimazu T, Tsugane S. Dietary arsenic intake and subsequent risk of cancer: the Japan Public Health Center-based (JPHC) Prospective Study. Cancer Causes Control 2013;24:1403–1415.
Schmeisser E, Goessler W, Francesconi KA. Human metabolism of arsenolipids present in cod liver. Anal Bioanal Chem. 2006;385:367–76.
Shibata Y, Morita M. Speciation of arsenic by reversed-phase high performance liquid chromatography-inductively coupled plasma mass spectrometry. Anal Sci. 1989;5:107–9.
Shibata Y, Tsuzuku K, Komori S, et al. Analysis of diphenylarsinic acid in human and environmental samples by HPLC-ICP-MS. Appl Organomet Chem. 2004;18
Signers-Pastor AJ, Mitra K, Sarkhel S, et al. Arsenic speciation in food and estimation of the dietary intake of inorganic arsenic in a rural village of West Bengal, India. Agric Food Chem. 2008;56: 9469–74.
Signers-Pastor AJ, Vioque J, Navarrete-Muñoz EM, et al. Inorganic arsenic exposure and neuropsychological development of children of 4–5 years of age living in Spain. Environ Res. 2019;174:135–42.
Stýblo M, Venkatratnam A, Fry RC, Thomas DJ. Origins, fate, and actions of methylated trivalent metabolites of inorganic arsenic: progress and prospects. Arch Toxicol. 2021;95:1547–72.
Tseng C-H. Arsenic methylation, urinary arsenic metabolites and human diseases: current perspective. J Environ Sci Health Part C. 2007;25:1–22.
Tseng C-H. A review on environmental factors regulating arsenic methylation in humans. Toxicol Appl Pharmacol. 2009;235:338–50.

Uchino T, Roychowdhury T, Ando M, Tokunaga H. Intake of arsenic from water, food composites and excretion through urine, hair from a studied population in West Bengal, India. Fd Chem Toxicol. 2006;44:455–61.

Wang Z, Zhou J, Lu X, et al. Arsenic speciation in urine from acute promyelocytic leukemia patients undergoing arsenic trioxide treatment. Chem Res Toxicol. 2004;17:95–103.

Wang X, Mukherjee B, Park SK. Associations of cumulative exposure to heavy metal mixtures with obesity and its comorbidities among U.S. adults in NHANES 2003–2014. Environ Int. 2018;121:683–94.

Watanabe T, Himeno S. Metabolism of arsenic and its toxicological relevance. Arch Toxicol. 2013;87:969–79.

Welna M, Szymczycha-Madeja A, Pohl P. Non-chromatographic speciation of as by HG technique — analysis of samples with different matrices. Molecules. 2020;25:4944. https://doi.org/10.3390/molecules25214944.

Wong WWK, Chung SWC, Chan BTP, et al. Dietary exposure to inorganic arsenic of the Hong Kong population: results of the first Hong Kong total diet study. Fd Chem Toxicol. 2013;51:379–85.

Yasui A, Tsutsumi C, Toda S. Selective determination of inorganic arsenic (III), (V) and organic arsenic in biological materials by solvent extraction-atomic absorption spectrophotometry. Agric Biol Chem. 1978;42:2139–45.

Yoshimura Y, Endo Y, Shimoda Y, et al. Acute arsine poisoning confirmed by speciation analysis of arsenic compounds in plasma and urine by HPLC-ICP-MS. J Occup Health. 2011;53:45–9.

Yoshinaga J, Narukawa T. Association of dietary intake and urinary excretion of inorganic arsenic in the Japanese subjects. Regul Toxicol Pharmacol. 2020;116:104745.