La Medicina del Lavoro

Med Lav 2012; *103*, 5: 372-381

Environmental factors affecting the urinary excretion of inorganic arsenic in the general population

P. Lovreglio, Maria Nicolà D'Errico, P. De Pasquale, Maria Enrica Gilberti*, I. Drago, Laura Panuzzo, Antonella Lepera, Rosaria Serra, F. Ferrara, Antonella Basso, P. Apostoli*, L. Soleo

Dipartimento Interdisciplinare di Medicina, Sezione di Medicina del Lavoro "E.C. Vigliani", Università di Bari, Bari, Italy

* Dipartimento di Medicina Sperimentale ed Applicata, Sezione di Medicina del Lavoro ed Igiene Industriale, Università di Brescia, Brescia, Italy

Key words

Inorganic arsenic; fish; seafood; drinking water; biological monitoring

Parole chiave

Arsenico inorganico; pesce; molluschi e crostacei; acqua da bere; monitoraggio biologico

Summary

Objective: *To assess the critical issues concerning the use of urinary inorganic arsenic (iAs), including As^3, As^5, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), as biomarker of internal dose in order to monitor environmental and occupational exposure to inorganic As, considering the influence of diet and drinking water on excretion of iAs.* **Methods:** *The design protocol stipulated collection of weekly urine samples from 6 male subjects for 5 consecutive months. In all the urine samples, iAs was determined by Hydride Generation-Atomic Absorption Spectrophotometry (HG-AAS). In the subjects with iAs higher than 35 μg/L, Biological Exposure Index (BEI) proposed by the American Conference of Governmental Industrial Hygienists (ACGIH), urinary arsenic speciation was performed by HPLC-ICP-MS. Exposure to airborne As was evaluated monthly using personal environmental samplers worn for 8 hours. Throughout the study, the participants filled out a daily food diary, also detailing types of water drunk.* **Result:** *Exposure to airborne As was invariably below the limit of detection, equal to 1 ng/m^3. A total of 77 urine samples were collected. iAs was always detectable and was higher in 7 urine samples, obtained from 5 of the 6 subjects examined, than the BEI. Among foods with a high As content, the intake of seafood and fish within 72 hours before providing the sample seems to be the principal source of the iAs concentrations, while the intake of rice or drinking water showed no influence on this biological marker. Instead, drinking wine within 24 hours before urine sample collection can cause a significant increase in the excretion of iAs.* **Conclusions:** *In populations that eat large amounts of fish and seafood, the use of iAs to monitor occupational and environmental exposure to inorganic As seems to present some problems, and urinary As speciation may be essential at least in cases with iAs measurements above the biological limit values. In any case, a diet sheet reporting all foods eaten within 3 days of urine collection seems to be an indispensable tool to ensure a correct interpretation of the results.*

Pervenuto il 9.3.2012 - Accettato il 14.6.2012

Corrispondenza: Dr. Piero Lovreglio, Dipartimento Interdisciplinare di Medicina, Sezione di Medicina del Lavoro "EC Vigliani", Università di Bari, Piazza G. Cesare 11, 70124 Bari, Italy - Tel.: ++390805478218 - E-mail: piero.lovreglio@uniba.it

The results of this research were presented at the 8th International IOHA Conference 2010 "Health, Work And Social Responsibility. The occupational hygienist and the integration of environment, health and safety", Rome, 28 September - 2 October 2010

Riassunto

«Fattori ambientali determinanti l'escrezione urinaria di arsenico inorganico in soggetti della popolazione generale». **Obiettivo:** *Valutare le criticità presentate dall'arsenico inorganico urinario (iAs), comprendente As^3, As^5, acido monometilarsonico (MMA) ed acido dimetilarsinico (DMA), quale indicatore biologico di dose interna nel monitoraggio dell'esposizione ambientale ed occupazionale ad As inorganico, considerando l'influenza che la dieta e l'acqua da bere possono avere nel condizionare la sua escrezione urinaria.* **Metodi:** *Il protocollo dello studio prevedeva la raccolta dei campioni di urine in 6 soggetti maschi ogni settimana per 5 mesi consecutivi. In tutti i campioni urinari l'iAs è stato determinato in spettrofotometria ad assorbimento atomico con la tecnica di generazione degli idruri (HG-AAS). Nei soggetti che hanno presentato un iAs superiore a 35 μg/L, Indicatore Biologico di Esposizione (BEI) proposto dall'American Conference of Governmental Industrial Hygienists (ACGIH), è stata eseguita la speciazione dell'As urinario con HPLC-ICP-MS. L'esposizione ad As aerodisperso è stata determinata mensilmente con un campionamento ambientale personale della durata di 8 ore. I partecipanti per l'intera durata dello studio hanno compilato un questionario alimentare giornaliero comprendente anche informazioni sul tipo di acqua consumata.* **Risultati:** *L'esposizione ad As aerodisperso è risultata invariabilmente al di sotto del limite di rilevabilità pari a 1 ng/m^3. Sono stati raccolti complessivamente 77 campioni di urine. iAs è risultato sempre rilevabile e superiore al BEI in 7 campioni di urine raccolti da 5 dei 6 soggetti esaminati. Tra gli alimenti ad alto contenuto di As, il consumo di crostacei/molluschi e di pesce sino a 72 ore precedenti la raccolta delle urine sembrano essere i principali determinanti delle concentrazioni di iAs, mentre il consumo di riso o acqua non ha mostrato alcuna influenza su questo indicatore biologico. Il consumo di vino nelle 24 ore precedenti la raccolta delle urine, invece, è risultato in grado di determinare un incremento significativo dell'escrezione di iAs.* **Conclusioni:** *In popolazioni con una dieta caratterizzata da elevato consumo di prodotti ittici, l'utilizzo dell'iAs nel monitoraggio dell'esposizione occupazionale ed ambientale ad As inorganico presenta alcune criticità; in questo caso la speciazione dell'As urinario sembra essere indispensabile, almeno per le determinazioni di iAs risultate superiori ai valori limite biologici. Un diario alimentare relativo ai 3 giorni precedenti la raccolta delle urine appare uno strumento comunque indispensabile per l'interpretazione dei risultati.*

Introduction

Even nowadays despite the severe restrictions imposed on the presence of arsenic (As) in drinking water, food and air in urban areas and workplaces by many international organizations, exposure to inorganic As is responsible for the onset of carcinogenic effects on the lungs, skin and bladder in man, as well as non-carcinogenic effects mainly involving the skin and peripheral circulation system (10, 11, 34). In the general population, inorganic As is absorbed largely through the digestive tract, where the sources of inorganic As include both drinking water and foods such as rice, seafood (mollusks and crustaceans) and fish. Absorption by inhalation seems to be less frequent, affecting mainly residents near large industrial plants that release inorganic As in the surrounding area. Instead, in the occupational field inorganic As is absorbed largely by inhalation. This can occur, even if at lower concentrations than in the past, in non-ferrous metals industries producing copper, in particular, as well as in coal-fired electric power stations, in artistic glass works and the semiconductor production industry (1).

Unlike inorganic As, organic As is generally considered non-toxic, like arsenobetaine, or to have a poorly defined toxicity profile, like the arsenosugars, arsenolipids and dimethylarsinic acid (DMA) (12). The main source of exposure to all these As compounds is the consumption of seafood, fish and seaweed, while there is a significant content of DMA also in other foods such as rice and poultry (10).

The differences between inorganic and organic As compounds are not confined to the toxicity profile but also include their different metabolic processing in man after absorption by inhalation or digestion. As^3, absorbed as such or derived from the

reduction of As^5, largely in the blood and liver, undergoes a liver biotransformation process that, after two successive oxidative methylation reactions, leads to the synthesis of monomethylarsonic acid (MMA) and DMA (32). Moreover, this process also seems to induce the formation of two highly reactive intermediate trivalent products, namely MMA^3 and DMA^3, that may contribute to cause the toxic effects of inorganic As, by turning the methylation sequence into a metabolic activation rather than a detoxification process (20). The two methylated inorganic As metabolites are then excreted in the urine together with an unmodified quota of As^3 and As^5. Measurement of the relative urine concentrations of the various As species after the intake of high quantities of inorganic As through digestion has shown that 10-30% consists of As^3+As^5, 10-20% of MMA and 60-80% of DMA (31). Instead, as regards the organic As forms, arsenosugars and arsenolipids are metabolized in man with the formation also of DMA and then eliminated in the urine, while arsenobetaine and DMA, absorbed as such, do not seem to undergo any further biotransformation process and are also eliminated in the urine (12, 23, 27).

Inorganic urinary As (iAs) is the biological marker most commonly used for the purposes of monitoring occupational and environmental exposure to inorganic As (9). It is determined by Atomic Absorption Spectrophotometry with the hydride generation technique (HG-AAS) and includes both the inorganic As compounds (As^3+As^5) and their methylated compounds (MMA+DMA). The introduction in 1980 of this analytical method to measure urinary inorganic As made it possible to exclude from the analysis arsenobetaine, that accounts for the proportionally highest quota of urinary As absorbed in the diet when consuming fish and seafood (5). However, in view of the different origin of DMA in man, when obtaining high excretion values of iAs, of which DMA can be as much as 2/3 of the entire value, it is difficult to understand whether the iAs level is a true reflection of the degree of exposure to inorganic As.

The aim of the present study was to assess the critical issues concerning the use of iAs as biomarker of internal dose for the purposes of monitoring environmental and occupational exposure to inorganic arsenic, bearing in mind the influence of diet and drinking water in influencing the excretion of iAs.

Methods

Subjects

The study lasted 5 consecutive months (July-November), during which 6 healthy males, all resident in a coastal area in southern Italy (Manfredonia-Apulia), and all office workers at a company in the service sector, were examined. At baseline all the participants were administered a questionnaire detailing personal data, current and previous working activities, lifestyle and diet, as well as medical history to exclude liver and renal disease. Thereafter, throughout the study, the subjects filled out a daily diet sheet from Tuesday to Friday specifying the types and quantities of food, alcoholic beverages and drinking water consumed throughout the day (figure 1). No dietary restrictions were imposed at any time during the study. All subjects gave written informed consent prior to enrolment.

Environmental monitoring

To exclude a possible exposure to airborne As at the workplace, monthly environmental monitoring of As was performed (figure 1), employing active personal samplers worn by the subjects in the respiratory zone for the whole 8-hour work shift. Airborne particulate was collected on cellulose ester membranes to assess the total As concentration in the inhalable dust fraction. The analyses were conducted by inductively coupled plasma mass spectrometry (ICP-MS) with an ELAN 5000 (Perkin–Elmer SCIEX). The limit of detection (LOD) was 1 ng/m^3 and the coefficient of variation (CV) was 6-9%.

Biological monitoring

The design protocol stipulated collection of a urine sample every Friday between 4 and 5 pm, 3

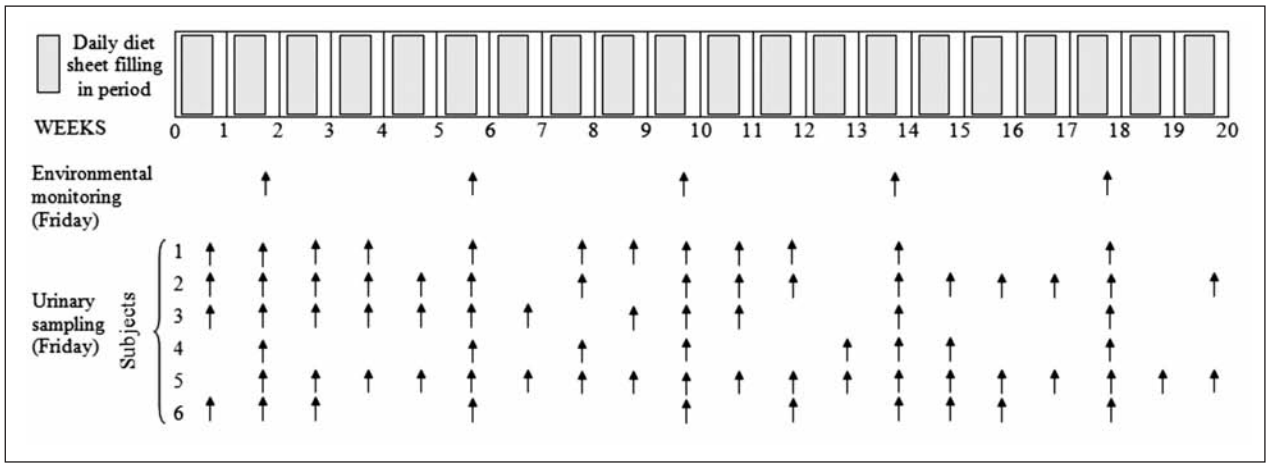

Figure 1 - Temporal sequence of environmental sampling, urinary sampling and dietary data collection in the 6 study subjects throughout the study period

hours after lunch (figure 1). All samples were immediately code labelled and frozen to –20°C, then kept at that temperature until the time of analysis, conducted blind.

IAs was determined by HG-AAS 5100 (Perkin–Elmer). The LOD was 0.1 μg/L and the CV 4.5%. In the urine samples with iAs concentrations exceeding the Biological Exposure Index (BEI) of the American Conference of Governmental Industrial Hygienists (ACGIH), equal to 35 μg/L, urinary speciation was performed, determining As^3, As^5, MMA, DMA and arsenobetaine by liquid chromatography coupled with mass spectrometry (HPLC-ICP-MS), adopting the analytical method previously reported by Apostoli et al. (2-4). For all the As species the LOD was 0.5 μg/L, while the CV was 2.5% for As^3 and Arsenobetaine and 5.0% for As^5, MMA and DMA. Urinary creatinine was determined with the colorimetric method (16); the LOD was 0.01 mg/dL and the CV 5%. The urine samples with urinary creatinine values exceeding the range 0.3-3.0 g/L, recommended by WHO for acceptable biological samples, were excluded from subsequent analyses (35).

The laboratory that carried out the determinations adheres to the external quality control programme organized by the Institute of Occupational Social and Environmental Medicine of the University of Erlangen, Nuremberg.

Statistical Analysis

SPSS software (version 14.0, Chicago, IL, USA) was employed for statistical analysis. A normal distribution was verified by the Kolmogorov-Smirnov test and non normally distributed variables were analyzed by parametric tests after logarithmic transformation. The level of significance was set at $p<0.05$.

Results

There was an ample range of age and BMI among the 6 examined subjects. They all drank almost exclusively bottled mineral water, and only 1 referred a smoking habit at the time of the study (table 1). Among foodstuffs with a high As content, fish and seafood (including all the types of mollusks and crustaceans) were those most commonly consumed at all the times investigated, namely within 24, 48 and 72 hours before urine collection, followed by rice and poultry, whereas mushrooms were rarely eaten by these subjects and were not therefore included in subsequent analyses (table 2). The types of seafood they most commonly consumed were cuttlefish (Sepiidae) and octopus (Octopus vulgaris) among cephalopod seafoods, mussels (Mytilus galloprovincialis) among bivalve mollusks, and prawns (Caridea) among crustaceans.

Table 1 - General characteristics of the 6 study subjects

	N (%)	Mean±SD	Median	Range
Age (years)	6	46.2±11.3	49.0	27-57
BMI (Kg/m^2)	6	27.9±7.9	27.7	19.4-41.5
Smoking habit				
- Smoker	1 (17%)			
- Non-smoker	3 (50%)			
- Ex-smoker	2 (33%)			
Alcohol				
- <10 g/day	2 (33%)			
- 10-30 g/day	3 (50%)			
- >30 g/day	1 (17%)			
Type of drinking water				
- Mineral	5 (83%)			
- Mineral + tapwater	1 (17%)			
- Tapwater	0 (0%)			

Table 2 - Percentage of urine samples of the 6 study subjects who consumed food with a high As content within 24, 48 and 72 hours before collection

Food	Urine samples (N. 77)		
	24 hours	48 hours	72 hours
Seafood (mollusks and/or crustaceans)	20.8	36.4	41.6
Fish	32.5	45.4	58.4
Rice	11.7	20.8	27.3
Poultry	13.0	19.5	24.7
Mushrooms	1.3	6.5	14.3

The environmental monitoring results showed that the airborne As concentrations were invariably below the LOD of 1 ng/m^3.

A total of 77 urine samples were collected, all showing the concentrations of iAs higher than the LOD (table 3). Moreover, in 7 urine samples obtained from 5 of the 6 subjects examined, the iAs concentrations were above the BEI of the ACGIH, and urinary As speciation was performed. The results showed that the As^3 and As^5 concentrations were always below the LOD whereas the MMA, DMA and arsenobetaine concentrations were always above (table 4). Arsenobetaine was always the most common species, and showed particularly high concentrations in 5 of the 7 samples, followed by DMA and MMA. The sum of inorganic As and the urinary methylated metabolites measured singly by speciation exceeded the limits by 35 μg/L in 6 of the 7 urine samples.

The influence of a dietary intake of food with a high As content and wine on the excretion of iAs was studied by subdividing all the urine samples according to the number of meals of seafood, fish, rice or poultry eaten and to the number of glasses of wine drunk within 24, 48 and 72 hours before urine collection (figure 2). Both for the consumption of fish and seafood, regardless of the time interval after eating these foods, the iAs concentra-

Table 3 - Excretion of iAs (μg/L) in each of the 6 subjects analyzed individually

Subject	N Urine samples collected	Mean±SD	Geometric mean	Coefficient of variation (%)	Range	Percentage of samples with iAs > 15 μg/L*
1	12	22.5±17.7	17.1	78.9	2.4-65.2	41.7%
2	16	11.1±9.6	7.0	86.3	0.1-37.2	25.0%
3	12	12.3±9.0	7.8	73.6	0.1-36.3	25.0%
4	8	23.2±17.1	18.4	73.8	8.4-50.8	50.0%
5	19	14.2±11.6	9.5	81.5	1.1-46.0	42.1%
6	10	9.3±10.1	4.9	108.6	0.1-34.8	20.0%
Total	77	14.8±13.1	9.3	88.0	0.1-65.2	33.8%

*Italian reference values: 2-15 μg/L

Table 4 - Concentrations of the different As species (μg/L) in the urine samples of the 6 subjects with iAs values exceeding the BEI of the ACGIH

Subject	Hours since last seafood meal	iAs	As^3	As^5	MMA	DMA	Arsenobetaine	Sum of As^3+As^5+ MMA+DMA	Total As
1	21	65.2	<0.5	<0.5	5.2	54.9	178.0	60.6	635.0
1	>72	49.5	<0.5	<0.5	4.2	40.3	85.3	45.0	214.0
2	29	37.2	<0.5	<0.5	1.8	33.0	33.2	35.3	145.0
3	18	36.3	<0.5	<0.5	2.4	33.3	408.0	36.2	567.0
4	20	47.2	<0.5	<0.5	15.0	32.4	415.0	47.9	582.0
4	14	50.8	<0.5	<0.5	6.3	35.0	119.0	41.8	167.0
5	3	46.0	<0.5	<0.5	5.4	28.3	322.4	34.2	448.0

tions showed a significant increase according to how many of such meals had been eaten, with the sole exception of fish within 72 hours before urine collection. The intake of wine within 24 hours was also associated with a significant increase in the excretion of iAs. Neither rice nor poultry consumption showed any influence on the excretion of iAs, nor was any relation observed with the consumption of beer or with a high or low (>2L vs <2L) consumption of bottled mineral water within 24 hours before urine collection.

Lastly, to study the association between iAs values exceeding the BEI and the intake of seafood, fish, rice, poultry or wine within 24 hours before urine collection, the urine samples were subdivided according to whether the subject had eaten these foods or not in the time interval. A significant difference was found only for seafood (p=0.003) and wine (p=0.004) (figure 3).

Discussion

The aim of the present research was to investigate to what extent the excretion of iAs, used as a biological marker of exposure to inorganic As, is critically affected by an influence of the main environmental sources of As, especially the dietary intake of foods with a high As content within 3 days of urine collection.

The study subjects had a low exposure to inorganic As and high exposure to organic As, because they ate a lot of fish and seafood, whereas they were not occupationally exposed to inorganic As by inhalation or due to airborne pollution. Nevertheless, the iAs concentrations were invariably above the LOD, and 33.8% of the determinations were over 15 μg/L, the top limit of the reference range for the Italian population (19). The overall geometric mean of iAs was 9.3 μg/L, similar to the to-

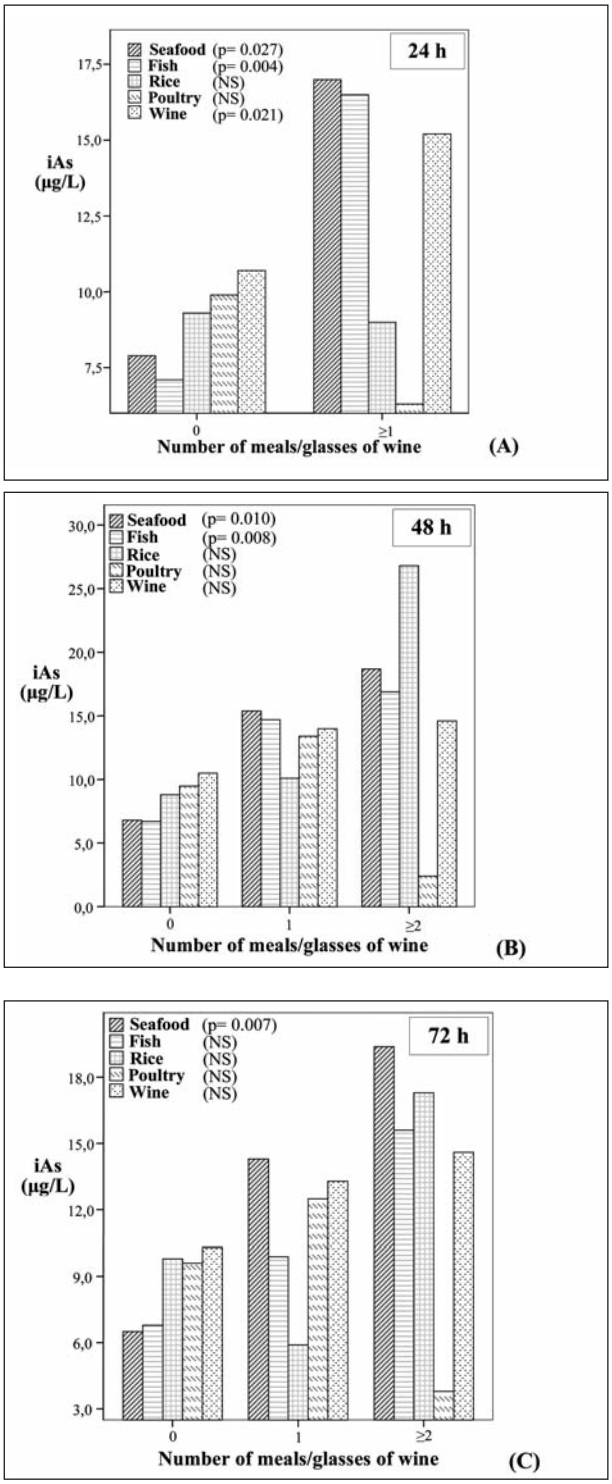

Figure 2 - Trend of iAs concentrations (geometric mean) in the 77 samples of the 6 subjects grouped according to the number of meals of seafood, fish, rice and poultry eaten or to the number of glasses of wine drunk in the 24 (A), 48 (B) and 72 (C) hours before urine collection

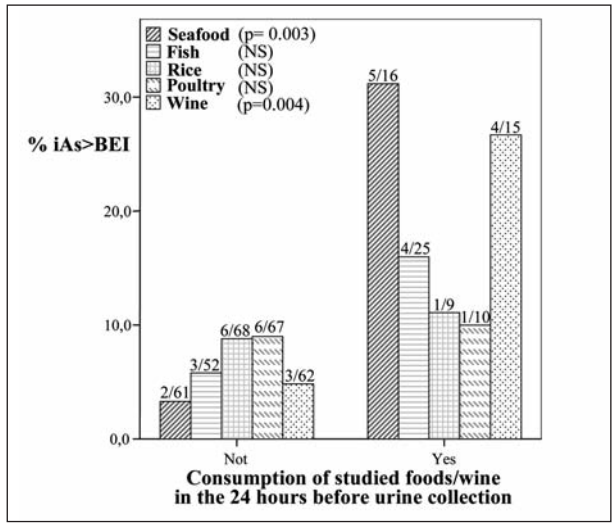

Figure 3 - Percentage of iAs determinations over the BEI in all the urine samples grouped according to the consumption of foods with a high As content or wine in the 24 hours before urine collection

tal As median minus the arsenobetaine value observed in US subjects of the general population enrolled in the NHANES study, who had eaten seafood within 24 hours before urine collection (11.0 µg/L) (25). According to previous studies, in our subjects the excretion of iAs was also found to be associated with the number of fish or seafood meals eaten within 72 hours before urine collection, whereas no relation was found with the intake of rice, poultry or water (figure 2) (8). Therefore, the high intake of seafood and fish was confirmed to be the cause of the concentrations of iAs exceeding the reference values in our subjects, according also to the high content of As in fish from the Adriatic Sea (6, 29, 30).

DMA is the urinary metabolite that contributes most heavily to the excretion of iAs, as seen in our results, too. Overall, these results also seem to confirm that in subjects who eat a lot of fish and seafood, it is not possible to distinguish the organic or inorganic source of the As, and in particular of DMA, by determining iAs. Moreover, only the finding of evidence of a delayed urinary excretion peak of DMA in the 12-24 hours after eating these foods would allow us to claim that the endogenous metabolism quota prevails over the quota absorbed

as such in the diet (17, 22). These results reveal an important limit in the use of iAs to assess the carcinogenic risk of exposure to inorganic As. In fact, while DMA values of inorganic origin undoubtedly reflect a carcinogenic exposure for man, it is still difficult to interpret the toxicological contribution of the DMA form via the metabolism of organic As compounds. This is especially true of the arsenosugars that, unlike arsenobetaine that is believed to be non-toxic, are still regarded with some doubt because a possible carcinogenic effect has not been excluded (12).

To check the influence of eating fish and seafood on iAs concentrations, subjects undergoing biological monitoring are often asked to fill out a diet sheet detailing what they ate for at least 3 days before the urine collection, or else they are asked to refrain from eating these foods for the 3 days before. However, the speciation of urinary As seems to be a more valid method of gaining information on the origin of the As absorbed by the organism (14, 15, 29). In our study speciation was done only on the samples with iAs concentrations above the BEI of the ACGIH, that demonstrated levels of As^3+As^5 that were always below the LOD, while arsenobetaine was invariably the species showing the highest level, and there were always higher percentage values of DMA than MMA. In agreement with the suggestion in previous studies, therefore, the sum of As^3+As^5 or of As^3+As^5+MMA could prove to be a more valid marker of internal dose showing recent environmental or occupational exposure to inorganic As than iAs (4, 24). In fact, the sum of As^3+As^5 cannot be detected unless the subject has been exposed to inorganic As, while MMA, being practically only a product of the metabolism of inorganic As, could provide further indications on the exposure to inorganic As.

Among foods with a high As content, rice may be the most important source of inorganic As and DMA, and could pose an even higher carcinogenic risk in populations that eat it in large quantities, than that posed by the type of drinking water (21, 33). In our study no relationship was observed between the intake of rice and excretion of iAs, unlike what has been reported in volunteers following a controlled diet and in ethnic groups that use rice as staple food (7, 26). This absence of a relationship between rice intake and iAs could be attributed to a masking effect of the large intake of seafood and fish, which could mask the proportionally lower intake of inorganic As and DMA in the rarer meals of rice. This demonstrates the poor validity of iAs in assessing the further contribution to the As intake induced by eating rice.

Even today, in many areas of the world water contaminated by As is still the main source of exposure to inorganic As, that can have both carcinogenic and non-carcinogenic effects on human health. During the present study the 6 subjects drank almost exclusively bottled mineral water, but with different trademark labels even during the same day. Even if Italian legislation stipulates the upper limit of 10 μg/L of As, as recommended by WHO, for both mineral and tap water, mineral water could anyway be an occasional source of exposure to inorganic As, as demonstrated in an investigation of mineral water samples with 40 different bottling trademark labels, all available on the market in the area where our study was conducted and some of which showed As concentrations that were even higher than 10 μg/L (18, 28). Nevertheless, in our subjects the mineral water intake seems to have had a negligible role in influencing the excretion of iAs.

Cigarette smoking was for many years an important non-occupational source of exposure to inorganic As, attributed to the wide use of lead arsenate as a pesticide in the cultivation of tobacco. More recently, since pesticides with an arsenic content have been abandoned, the As content in cigarettes has drastically reduced and smoking no longer seems to be a factor influencing the excretion of iAs, as confirmed also by the results of our study (6, 13, 29). Instead, conflicting results have been obtained in the literature on the influence of alcohol on the intake of arsenic. Although determinations of the As content in wine and beer have generally yielded low concentrations, it has been estimated that the intake of these beverages in the U.S. general population contributes 12% of the dietary inorganic As quota (36). Moreover, a relationship with alcohol has been shown with the excretion of iAs in subjects resident in areas with a natural soil contamination by As (10, 13). Our

study confirmed the role of wine as a source of As, which must be borne in mind when interpreting the results of biological monitoring.

In conclusion, our results are in line with other scientific studies that have warned that in populations that eat large quantities of fish and seafood, the use of iAs as a biological marker to monitor environmental and occupational exposure to inorganic As can be critically affected by the high intake of these foods before urine collection. In these cases speciation of As seems to be essential at least in samples with values of iAs exceeding the biological limit values. In any case, a food diary reporting all food and drink intake for at least 3 days before urine collection seems to be an indispensable tool to ensure a reliable interpretation of the results of iAs measurements.

No potential conflict of interest relevant to this article was reported

References

1. Agency for Toxic Substances and Disease Registry: *Toxicological Profile for Arsenic (Update)*. Atlanta, GA: ATSDR, 2007
2. American Conference of Governmental Industrial Hygienists: *TLVs and BEIs for chemical substances and physical agents*. Cincinnati, OH: ACGIH, 2011
3. Apostoli P, Alessio L, Romeo L, et al: Metabolism of arsenic after acute occupational arsine intoxication. J Toxicol Environ Health 1997; *52*: 331-342
4. Apostoli P, Bartoli D, Alessio L, Buchet JP: Biological monitoring of occupational exposure to inorganic arsenic. Occup Environ Med 1999; *56*: 825-832
5. Buchet JP, Lauwerys R, Roels H: Comparison of several methods for the determination of arsenic compounds in water and in urine. Their application for the study of arsenic metabolism and for the monitoring of workers exposed to arsenic. Int Arch Occup Environ Health 1980; *46*: 11-29
6. Buchet JP, Lison D, Ruggeri M, et al: Assessment of exposure to inorganic arsenic, a human carcinogen, due to consumption of seafood. Arch Toxicol 1996; *70*: 773-778
7. Cascio C, Raab A, Jenkins RO, et al: The impact of a rice based diet on urinary arsenic. J Environ Monit 2011; *13*: 257-265
8. Choi BS, Choi SJ, Kim DW, et al: Effects of repeated seafood consumption on urinary excretion of arsenic species by volunteers. Arch Environ Contam Toxicol 2010; *58*: 222-229
9. Ciarrocca M, Tomei G, Palermo P, et al. Environmental and biological monitoring of arsenic in outdoor workers exposed to urban air pollutants. Int J Hyg Environ Health 2011; doi: 10.1016/j.ijheh.2011.11.014
10. European Food Safety Authority: Scientific Opinion on Arsenic in Food. EFSA Journal 2009. Available at http://www.efsa.europa.eu/en/scdocs/doc/1351.pdf
11. European Parliament: Directive 2004/107/EC of the European Parliament and of the Council of 15 December 2004 relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air. Off J Euro Union 2005; L 23: 3-16
12. Feldmann J, Krupp EM: Critical review or scientific opinion paper: Arsenosugars-a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs? Anal Bioanal Chem 2010; *399*: 1735-1741
13. Fillol C, Dor F, Labat L, et al: Urnary arsenic concentrations and speciation in residents living in an area with naturally contaminated soils. Sci Total Environ 2010; *408*: 1190-1194
14. Foà V, Buratti M, Calzaferri G, et al: Significance of metabolites of arsenic in the urine and optimization of analytic methods for their determination. Med Lav 1982; *73 (*Suppl 3): 365-380
15. Foà V, Colombi A, Maroni M, et al: The speciation of the chemical forms of arsenic in the biological monitoring of exposure to inorganic arsenic. Sci Total Environ 1984; *34*: 241-259
16. Harmoinen AP: Bilirubin and metamizol do not interfere with the randox enzymatic creatinine test. An evaluation of a new enzymatic creatinine determination method. Eur J Clin Chem Clin Biochem 1996; *34*: 975-976
17. Heinrich-Ramm R, Mindt-Prüfert S, Szadkowski D: Arsenic species excretion after controlled seafood consumption. J Chromatogr B Anal Technol Biomed Life Sci 2002; *778*: 263-273
18. Italian Government: Decree of Ministry of Health of 29 December 2003. Attuazione della direttiva n. 2003/40/CE della Commissione nella parte relativa ai criteri dei valutazione delle caratteristiche delle acque minerali naturali di cui al decreto ministeriale 12 novembre 1992, n. 542, e successive modificazioni, nonché alle condizioni di utilizzazione dei trattamenti delle acque minerali naturali e delle acque di sorgente. Off J Ital Rep 2003; 302
19. Italian Society of Reference Value. Available at http://www.valoridiriferimento.it/ (last accessed 7th February 2012)

20. Kitchin KT: Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol Appl Pharmacol 2001; *172*: 249-261
21. Liang F, Li Y, Zhang G, et al: Total and speciated arsenic levels in rice from China. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2010; *27*: 810-816
22. Lovreglio P, D'Errico MN, Gilberti ME, et al: The influence of diet on intra and inter-individual variability of urinary excretion of arsenic species in Italian healthy individuals. Chemosphere 2012; *86*: 898-905
23. Marafante E, Vahter M, Norin H, et al: Biotransformation of dimethylarsinic acid in mouse, hamster and man. J Appl Toxicol 1987; *7*: 111-117
24. Morton J, Leese E: Arsenic speciation in clinical samples: urine analysis using fast micro-liquid chromatography ICP-MS. Anal Bioanal Chem 2011; *399*: 1781-1788
25. Navas-Acien A, Francesconi KA, Silbergeld EK, Guallar E: Seafood intake and urine concentrations of total arsenic, dimethylarsinate and arsenobetaine in the US population. Environ Res 2011; *111*: 110-118
26. Pearson GF, Greenway GM, Brima EI, Haris PI: Rapid arsenic speciation using ion pair LC-ICPMS with a monolithic silica column reveals increased urinary DMA excretion after ingestion of rice. J Anal At Spectrom 2007; *22*: 361-369
27. Schmeisser E, Goessler W, Francesconi KA: Human metabolism of arsenolipids present in cod liver. Anal Bioanal Chem 2006; *385*: 367-376
28. Signorile G, Neve A, Lugoli F, et al: Evaluation of toxic chemical parameters and ecotoxicity levels in bottled mineral waters. J Prev Med Hyg 2007; *48*: 10-16
29. Soleo L, Lovreglio P, Iavicoli S, et al: Significance of urinary arsenic speciation in assessment of seafood ingestion as the main source of organic and inorganic arsenic in a population resident near a coastal area. Chemosphere 2008; *73*: 291-299
30. Storelli MM, Marcotrigiano GO: Organic and inorganic arsenic and lead in fish from the South Adriatic Sea, Italy. Food Addit Contam 2000; *17*: 763-768.
31. Vahter M: Methylation of inorganic arsenic in different mammalian species and population groups. Sci Prog 1999; *82*: 69-88
32. Vahter M: Mechanisms of arsenic biotransformation. Toxicology 2002; *181-182*: 211-217
33. Williams PN, Price AH, Raab A, et al: Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. Environ Sci Technol 2005; *39*: 5531-5540
34. World Health Organization: *Guidelines For Drinking-Water Quality*, second ed. Geneva: WHO, 1993
35. World Health Organization: *Biological Monitoring of Chemical Exposure in the Workplace*, vol. 1. Geneva: WHO, 1996
36. Xue J, Zartarian V, Wang SW, et al: Probabilistic Modeling of Dietary Arsenic Exposure and Dose and Evaluation with 2003-2004 NHANES Data. Environ Health Perspect 2010; *118*: 345-350