Biological monitoring of occupational exposure to inorganic arsenic

P Apostoli, D Bartoli, L Alessio, J P Buchet

Abstract

Objectives—This study was undertaken to assess reliable biological indicators for monitoring the occupational exposure to inorganic arsenic (iAs), taking into account the possible confounding role of arsenicals present in food and of the element present in drinking water.

Methods—51 Glass workers exposed to As trioxide were monitored by measuring dust in the breathing zone, with personal air samplers. Urine samples at the end of work shift were analysed for biological monitoring. A control group of 39 subjects not exposed to As, and eight volunteers who drank water containing about 45 µg/l iAs for a week were also considered. Plasma mass spectrometry (ICP-MS) was used for the analysis of total As in air and urine samples, whereas the urinary As species (trivalent, As³⁺; pentavalent, As⁵⁺; monomethyl arsionic acid, MMA; dimethylarsinic acid, DMA; arsenobetaine, AsB) were measured by liquid chromatography coupled with plasma mass spectrometry (HPLC-MS).

Results—Environmental concentrations of As in air varied widely (mean 84 µg/m³, SD 61, median 40) and also the sum of urinary iAs MMA and DMA, varied among the groups of exposed subjects (mean 106 µg/l, SD 84, median 65). AsB was the most excreted species (34% of total As) followed by DMA (28%), MMA (26%), and As³⁺As⁵⁺ (12%). In the volunteers who drank As in the water the excretion of MMA and DMA increased (from a median of 0.5 to 5 µg/day for MMA and from 4 to 13 µg/day for DMA). The best correlations between As in air and its urinary species were found for total iAs and As³⁺As⁵⁺.

Conclusions—To avoid the effect of As from sources other than occupation on urinary species of the element, in particular on DMA, it is proposed that urinary As³⁺As⁵⁺ may an indicator for monitoring the exposure to iAs. For concentrations of 10 µg/m³ the current environmental limit for iAs, the limit for urinary As³⁺As⁵⁺ was calculated to be around 5 µg/l, even if the wide variation of values needs critical evaluation and application of data. The choice of this indicator might be relevant also from a toxicological point of view. Trivalent arsenic is in fact the most active form of the element, such as cancer.

Keywords: arsenic; speciation; biological monitoring

When assessing occupational exposure to inorganic arsenic (iAs) by biological monitoring, it is necessary to consider the biotransformation pathway of the element itself.

In the human body, iAs changes in oxidation state in both directions: pentavalent species can be found in the urine after a dose of the trivalent As and it has been shown that subjects who ingested meals rich in pentavalent As excreted mainly the trivalent species in urine. The reduction capacity of pentavalent As can be very efficient but results in increased toxicity: indeed, trivalent As has high affinity for thiol groups of proteins and molecules such as lipoic acid.

Biotransformation of arsenic, however, involves mainly methylation, leading to the formation and excretion of monomethylated and dimethylated compounds (monomethylarsionic acid, MMA; dimethylarsinic acid, DMA). Experimental and human observations suggest that two enzymatic activities with a different reaction rate are involved in this methylation.

Arsenobetaine (AsB) which represents, with tetramethylarsonium, the most important species of As in food does not undergo biotransformation and is almost completely eliminated through the urine: one single meal of seafood can induce, for example, a urinary excretion of total As of up to 1000 µg/l.

Other possible, quantitatively less important sources of As in biological media are smoking habits and air pollution of the general environment—for example, living in surroundings of some industrial settings such as non-ferrous foundries and glass factories.

Drinking water may be a relevant source of iAs in some geographical areas, and this natural kind of water pollution has been related to skin alterations, cardiovascular diseases, and cancer.

The absorption of As from food, water, and air explains the presence, in variable amounts, of the element in the urine of the general population. The urinary concentrations of As vary considerably, between 5 and 50 µg/l, and therefore groups from different countries could be distinguished on this basis. The urinary As, for example is much higher in Japan and the United States than in European countries, where it varies.

In occupationally exposed subjects, the urinary excretion of As metabolites varies in accordance to the dose absorbed from the working environment but may also be influenced by...
other sources among which food and water can play an important part.

After several years of biological monitoring of occupational exposure to As the measurement of some urinary species of the element (iAs itself, MMA, and DMA) has been suggested. The basic argument for justifying this choice is that these urinary As species are not influenced by the presence of organoarsenicals of dietary origin.40–44

The correlation between As in ambient air and urinary As in exposed workers has been studied in several surveys with as biological indicator, usually the sum of these species of As. This correlation varied considerably, due to different working exposures investigated, different air sampling methods, and different analytical methods adopted both for environmental and biological samples37 38 45–50 and to the possible confounding role of some methylated forms of dietary origin as recently emphasised.51

The aim of the present study is to investigate the relation between exposure to iAs and its species excreted in urine, to assess accurate biological indicators of exposure, considering the possible confounding contribution of arsenicals present in food and drinking water.

Materials and methods

OCCUPATIONALLY EXPOSED SUBJECTS

We examined 51 male workers employed in art glass manufacture and with different degrees of exposure to dust containing as the main toxic compound As trioxide. As documented in a previous paper,52 other elements were also present in airborne dusts but in the working conditions examined and for the concentrations measured they did not seem to influence the urinary excretion of As, when compared with other published data.

In the present investigation therefore we dealt with the study of the relation between the exposure to iAs and the excretion of total inorganic element and of its species.

The workers studied were 28 oven chargers, 10 batch mixers, and 13 moulders or finishers. The number of workers already investigated was increased by taking into consideration other people employed in the same tasks, who usually drank water containing As, in particular, seafood.

Each subject, was asked to drink 1–1.5 l/day of water and to avoid the consumption of meals rich in As, in particular, seafood.

Urine was collected during the entire day, its volume was measured, and a representative sample frozen at −20°C until analysis.

The current procedures for informed consent were strictly applied.

CONTROL SUBJECTS

To ascertain the excretion of arsenic species in the general population, 39 men were assessed. They were not occupationally exposed to As, lived in areas not polluted by As from industrial plants, consumed drinking water with As concentrations <1 µg/l, and ate a diet poor in seafood.

The urine samples (second void) were collected in the morning and frozen at −20°C until analysis.

ENVIRONMENTAL AND BIOLOGICAL MONITORING

Environmental monitoring was conducted by collecting the airborne particulate matter in the breathing zone, on cellulose ester membranes by personal air samplers for a period of 6 hours on a normal working day, generally on Thursday. During environmental monitoring the workers did not regularly use respiratory masks.

For biological monitoring urine samples at the end of work shift were collected and frozen at −20°C until analysis. All urine samples had a specific gravity ranging between 1.018 and 1.032 and therefore the urinary As concentrations were expressed as µg/l.

ANALYSIS

An inductively coupled plasma mass spectrometer (ICP-MS) ELAN 5000 (Perkin Elmer SCIEX), was used for the analysis of As in the environmental and urine samples, applying methods published elsewhere.9

The urinary As species were measured with liquid chromatography coupled with plasma mass spectrometry (MS-HPLC), with a weak anion exchange column and a mobile phase prepared with methanol ammonium dihydrogen phosphate, ammonium acetate, and glacial acetic acid. The measurement of single As species was carried out by standard additions.39

The sensitivity of the method was 0.5 µg/l for the iAs, with a relative SD for environmental and biological determinations that ranged between 5% and 15%, being higher for AsB, DMA, and MMA. For the single As species the sensitivities were: As3 and As5 0.5 µg/l; MMA 2 µg/l; DMA 1.5 µg/l; and AsB 1 µg/l. The recovery varied from 92% to 105%.

Table 1. Concentrations of arsenic in air (µg/m³)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch mixers</td>
<td>10</td>
<td>59</td>
<td>56.4</td>
<td>26</td>
<td>10–154</td>
<td></td>
</tr>
<tr>
<td>Oven chargers</td>
<td>28</td>
<td>127.0</td>
<td>89.4</td>
<td>123</td>
<td>10–312</td>
<td></td>
</tr>
<tr>
<td>Moulders, finishers</td>
<td>13</td>
<td>4.1</td>
<td>3.7</td>
<td>39</td>
<td>1.5–15</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>82.9</td>
<td>87.4</td>
<td>42</td>
<td>1.5–312</td>
<td></td>
</tr>
</tbody>
</table>

The workers studied were 28 oven chargers, 10 batch mixers, and 13 moulders or finishers. Each subject, was asked to drink 1–1.5 l/day of water and to avoid the consumption of meals rich in As, in particular, seafood.
Table 3 Urinary excretion of As species in eight volunteers drinking water with arsenic (µg/day)

<table>
<thead>
<tr>
<th>Days of water intake</th>
<th>Ar³⁺ + Ar⁵⁺</th>
<th>MMA</th>
<th>DMA</th>
<th>AsBet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Day after the end of water intake</td>
<td>1.3 0.8 0.6</td>
<td>nd 6.2 3.0</td>
<td>1.8 3.3</td>
<td>nd 2.6 10.2 2.9 8.6</td>
</tr>
<tr>
<td>3 Days after the end of water intake</td>
<td>1.3 0.8 0.6</td>
<td>nd 6.2 3.0</td>
<td>1.8 3.3</td>
<td>nd 2.6 10.2 2.9 8.6</td>
</tr>
</tbody>
</table>

nd = Not detectable.
EKA value by DFG (for environmental concentration of As trioxide of 10 µg/m³) is the same, while the tentative biological maximum permissible concentration for As, proposed by Lauwerys and Hoet is 30 µg/g creatinine. As our values are expressed in µg/l and considering that creatinine for men is normally higher than 1 g/l, the exposure is probably overestimated.

The species excreted most was AsB, accounting for 34% of total As in exposed workers and 66% in the control group. Next was DMA, with 28% in workers and 36% in controls, and MMA with 21% and 26%, respectively. Urinary iAs were around 11% of the total As excreted in workers but it was measured only in 13 controls, in whom values were 0.5–1 µg/l. This was greater than the value for all As³, and its presence and concentration in urine were probably due to variations in As metabolism (methylation capacity) of the small quantity of iAs ingested by the general population.

To verify the possible role as confounding factors of arsenic drunk in water the measurement of As species (µg/day) was performed in volunteers who drank water with an As content of 42–46 µg/l, and the results are reported in table 3. The species excreted most was DMA, whereas MMA showed a more rapid increase in urine (10 times the basal excretion).
the dietary regimen, required by survey protocol, which limited food rich in As, AsB gradually decreased. Trivalent As, in concentrations of 1–2 µg/day was measured in three people. The excretion of different species decreases between 19% (iAs) and 42% (MMA) after the end of drinking the water high in As.

We also found that the biological markers related to As drunk in water varied greatly probably as a result of different methylation capacities of different people.

The regressions between As in air and urinary species of As measured in exposed individuals show that iAs+MMA+DMA and As3+As5 correlated well with As in air. The R values ranged between 0.87 and 0.73 and covariance between 0.59 and 0.79. The bootstrap variable selection (table 6) shows that iAs+MMA+DMA and As3+As5 were best correlated with concentrations of As in air.

**Discussion**

Mammals excrete the iAs in urine as methylated metabolites and partially as trivalent or pentavalent species. As the excretion rate is closely related to the metabolic capacity of the organism clear cut differences are found between animal species.11

Buchet et al11 showed in humans a mean excretion in 4 days of 45% of a single dose of 500 µg trivalent As administered through drinking water, whereas Pomroy et al10 found urinary excretion of around 38% of As drunk during 48 hours by volunteers who ingested As5.

After ingestion of a dose of iAs, part of the dose is methylated into MMA and DMA but a considerable proportion is excreted without transformation. During the two consecutive 24 hour periods after the ingestion of 500 µg As, 8% and 2.3%, 5.3% and 2.3%, 9.3% and 8.5% of the dose were eliminated in urine as iAs, MMA, and DMA, respectively.11

Similar proportions of iAs (15% of total As excreted in urine) have been found in urine of people consuming drinking water contaminated with As.11

A more efficient methylation for low doses of As may be hypothesised on the basis of observations related to possible inhibition of DMA formation after high As doses in humans57 and in rats.12

Organoarsenical compounds are excreted in urine at a higher rate than the inorganic forms. This is particularly the case with As from seafood. As an example, Brown et al58 gave a small dose of radiolabelled AsB to six volunteers and found an excretion of 67% of the dose within 24 hours and of >98% within 24 days.

A more recent investigation51 failed to show a biologically significant absorption of iAs either present as such in the food or formed from organoarsenicals during cooking or digestion.

The measurement of As in urine may therefore be regarded as the most reliable indicator of recent exposure to iAs from drinking water, and around 60%–75% of ingested As is excreted by urine.11

The results of our study indicate that ingestion of drinking water in which the concentration of iAs was around 45 µg/l even for a short period can rapidly induce excretion of high amounts of element, with a progressive increase of methylated species of As (MMA and DMA). The percentage of total As excreted was around 50% of ingested As and the increase from basal values was higher for iAs and MMA (from 0.06 to 1.6 and from 0.4 to 5.2 µg As/l, respectively), even though DMA remains the most excreted species (50% of total As). Overall, there was no evidence of a ceiling in methylation capacity even though a wide difference between people was shown, due to the fact, for example, that As3 was not measurable in some people before and after the end of drinking the water high in As. The absorption of As through drinking water does not seem to modify the pattern of excretion of urinary metabolites of As.

The MMA:DMA ratio ranging from 0.3 to 0.4 proved to be similar to published values, whereas lower ratios (0.1–0.2) were found by Hopenhayn et al. In that survey the concentrations of As in drinking water were much higher (<680 µg/l) and the corresponding mean of urinary As concentrations varied from 60 to 580 µg/l, with percentages of iAs from 14% to
18%. The authors stated that the main differences found for MMA:DMA were due to exposure, smoking, residence, and ethnicity, and together these variables explained about 30% of the variability in MMA:DMA.

The importance to health of ingestion of iAs from water is estimated by some authors to be a risk of cancer, which, for water containing 50 µg iAs/l, is comparable with environmental tobacco smoke and radon in homes. A reduction of the currently accepted limit of 50 µg/l for As in drinking water, which is able to cause measurable amounts of iAs in urine, was therefore recommended.

For occupational exposure, as previously recalled several studies attempted to assess the relation between occupational exposure to As and the urinary excretion of its metabolites in different industrial settings: the urinary concentrations of arsenic calculated for exposure to 50 µg/m² of the element varied from 78 to 220 µg/l. The discrepancy among surveys may be explained by some variable factors, such as the different methods of environmental monitoring, or the collection of urine samples, or even the different analytical methods for urinary As and its species.

In accordance with some authors, we also found an influence on the amount of As ingestion due to the use of respirators, the role of hand contamination, and oral ingestion in As sometimes relevant in workplaces such as glass manufacturing and metal smelting.

Considering the quality of analytical methods, problems may arise from lack of standardisation of analytical procedures (mainly for the As speciation in urine), from the absence of certified material, and from the difficulty in planning quality control programmes.

Different estimates of urinary As may, however, be due to the characteristics of urinary biomarkers. Individual metabolic capacity affects metabolism, by oxidation, reduction, and methylation of an element and so affects the presence of different metabolites. Furthermore, sources other than occupational may have a relevant influence. As previously seen, the absorption of arseno-organic compounds through seafood can modify the urinary excretion of As, due to AsB and probably DMA. The DMA does not appear as the methylation product of iAs in seafood, but rather as a product of organoarsenical catabolism. Buchet et al concluded that in occupational exposure the sum of iAs, MMA, and DMA can be misleading for biological monitoring, as when urine samples are collected even 2 days after a meal containing As in seafood the unmetabolised form of the element can be measured.

The measurement of the urinary excretion of iAs, MMA, and especially DMA for biological monitoring of exposure to iAs could therefore be revised to avoid the possibly confounding effect of consumption of As in seafood from other sources.

Our data are similar to those from other investigations in which As speciation was carried out (table 7). Farmer in particular, measured As³, As⁵, MMA, and DMA in urine from workers engaged in the electronics industry, wood preservation, glass works, and synthesis of arsenicals. The mean of the urinary As metabolites were <10, 49.9, 79.4, and 245 µg/g creatinine, respectively. For the most exposed groups, the means of single As species were 1%–6% for As³, 11%–14% for As⁵, 14%–18% for MMA, and 63%–70% for DMA.

This evidence is similar to that of our present investigation. In our case the oxidation to As⁵ was evident only for higher concentrations of iAs in air, as As³ was not detectable by our ICP and HPLC-MS method for 10 workers in the group at lower exposure. This suggests that the oxidation of As³ into As⁵ might be related to absorbed As dose.

Punish authors suggested a biological monitoring method involving only the measurement of iAs: they found that a urinary excretion of 5 µg iAs/l could be expected after an 8 h exposure to 10 µg As/m³.

From our present investigation when the exposure concentration is 10 µg/m³, the following concentrations of urinary As species are to be expected:

- As³: 4.3 µg/l; As⁵ + As³: 5.3 µg/l; MMA: 7.5 µg/l; DMA: 26.9 µg/l; sum of iAs: MMA, DMA: 43.7 µg/l.

The variability of the data already mentioned, mainly for low values of iAs in air suggests, however, that evaluation and application of these data should be cautious.

As well as considerations about correlation between air As and urinary As species, the significance of measurement of the iAs species in urine must also be emphasised. From a toxicological point of view, trivalent As is the most critical species, due to both its reactivity with thiol groups and its easy diffusion through biological membranes. When exposure increases, an increase of iAs in tissues and urine would be expected, as a consequence of the progressive saturation of methylation capability. It has been postulated that a decreased As⁵ methylation might be related to the appearance of effects of As—such as cancer—given that methylation is considered to be a detoxifying mechanism.

In our experience, however, only severe acute intoxications by iAs (suicide attempt or acute arsine intoxication, for example) can lead to complete saturation of metabolic capability, and this occurrence can hardly happen in usual, even bad, occupational exposure situations.

Also, Hopenhayn et al did not find a saturation of methylation in subjects who drank water with a high concentration of As.
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However, because methylation reactions are never fully efficient (a small iAs is always excreted in urine as well as MMA and DMA), a proportion must exist for As between the body binding sites, including methyltransferases and many proteins. Critical sites—for example, for cancer development—may be protected by many other less critical sites. These are progressively occupied in cases of chronic absorption, "opening" the access to the most critical sites. According to this hypothesis methylation reactions allow As3+ binding sites to be cleared by formation of MMA and DMA with lower affinity for binding sites.

Nevertheless, it seems that during formation of MMA and DMA (As3+), trivially methylated As can be formed and impair the cellular response to oxidation.66 Another point worth stressing is that differences in methylating capacity are probably due to methyltransferase gene polymorphism as suggested by Vahter et al.65 Inorganic As in urine could, therefore, be more closely related to the most critical effect (cancer) than other biological indicators of the element. A limiting factor for the choice of iAs as an indicator for routine biological monitoring of occupational exposure to As is that the analytical method for iAs is more difficult because of the lower concentration of iAs to be measured and the complexity of procedures to separate it.

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