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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

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Summary. Several arsenic species (inorganic tri- or pentavalent arsenic, mono- and dimethylated arsonic acids) can be determined in water samples by electrothermal atomic absorption spectrometry after appropriate acidification procedures (concentrated HCl or a mixture $HCl/HClO_4/HBr$) and extraction by toluene in the presence or absence of KI; the determination of aromatic derivatives and of arsenic thiol complexes needs a wet or dry ashing step. The procedures for water analysis are not directly applicable to urine samples; in the best conditions, total inorganic plus 85% on the average of the methylated arsenicals present in urine are measured after acidification with concentrated HCl and extraction by toluene in the presence of KI. Total arsenic content (including arsenic from marine origin) is measured only after a drastic mineralization step like MgO treatment at 600° C. The results obtained by the electrothermal atomic absorption technique and those obtained by neutron activation analysis are in excellent agreement.

When the presence of arsenic of marine origin is suspected in urine, the analysis of inorganic arsenic and its metabolites is preferably performed by an arsine generation technique. The sum of inorganic arsenic and of its mono- and dimethylated derivatives determined by such a technique is identical with the results obtained by electrothermal atomic absorption spectrometry after complete mineralization of the samples as long as no arsenic from marine origin is present.

After oral ingestion of As_2O_3 by man, the urinary excretion of inorganic arsenic and its metabolites is important and rapid (approximately 60% are eliminated by the oral route with a half life of 30 h).

While the excretion occurs in the form of inorganic species during the first hours following the ingestion, a methylating process is rapidly triggered and leads to a preponderant excretion of dimethylarsinic acid 1 day after

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ingestion. In the case of ingestion of seafood containing arsenic, the urinary excretion occurs at a higher rate (half life 18 h) apparently without transformation.

The absence of interference of arsenic from marine origin and the capacity of measuring separately inorganic arsenic and its main urinary metabolites makes the arsine generation technique the best suited for the monitoring of workers exposed to inorganic arsenic. However, since the technique may sometimes be too elaborate and time-consuming for routine work, the biological monitoring of workers can be performed by determining total arsenic concentration in urine after mineralization with MgO. Samples with high arsenic content are then re-analyzed to distinguish between occupational exposure and ingestion of the organic arsenic present in marine organisms. This is carried out either by the arsine generation method or, if this technique is not available, by a direct extraction procedure in the presence of KI of a sample acidified with HCl. With the latter procedure, 85% of the methylated arsenic is measured on average without interference of arsenic from marine origin.

Key words: Trivalent arsenic – Pentavalent arsenic – Monomethylarsonic acid – Cacodylic acid – Metabolism – Man – Biological monitoring

There is an abundant and often controversial literature on the determination of arsenic in biological material mainly because this element may exist in different chemical forms in vivo. The forms studied most are inorganic trivalent and pentavalent arsenic with their well known different toxicities. Their mono- and dimethyl derivatives represent the main metabolites in vivo. Another organic derivative of low toxicity, mainly from marine organisms, has recently been identified as O-phosphatidyltrimethyl arsoniumlactic acid (Cooney et al. 1978) the degradation of which yields trimethylarsoniumbetaïn (Edmonds et al. 1977).

The interest in estimating separately the inorganic and organic arsenicals present in human urine has been stressed recently by several authors (Lauwerys et al. 1979; Penrose 1974; Talmi and Bostick 1975). Indeed, it is very useful to distinguish between occupational exposure to arsenic (mainly exposure to arsenic oxide and less frequently to some organic pesticides) and exposure to arsenicals present in marine organisms.

We report in this paper an evaluation of some of the methods proposed for determining the total arsenic content of urine and its chemical forms.

We have first compared various sample pretreatments for measuring several arsenic compounds (inorganic tri- or pentavalent, aliphatic and aromatic derivatives) in aqueous solution in order to define the experimental conditions allowing their specific determination. The possible application of these procedures for the measurement of the arsenicals present in human urine was then tested. A modification of the method of Braman and Foreback (1973) was also evaluated for measuring specifically inorganic, mono- and dimethylated arsenic in urine. Finally, we have considered the applicability of some techniques for the study of arsenic metabolism in man and the biological monitoring of workers exposed to arsenic.

Materials and Methods

Reagents

Except for disodium methylarsinate which was obtained from Carlo Erba (Milano, Italy), all other reagents were purchased from Merck (Darmstadt, Federal Republic of Germany). Arsenic standard stock solutions (0.5 g/l as As) were made either in H_2SO_4 10% (As_2O_3, 3 $As_2O_5 \cdot 5H_2O$) or in NaOH 0.1 N (disodium methylarsonate, dimethylarsinic acid (cacodylic acid), and 4-amino-benzene arsonic acid (arsanilic acid)). Working standards (25 to 200 µg/l) were prepared just before use by appropriate dilution with demineralized water.

Recommended Procedure for the Analysis of Total Arsenic by Electrothermal Atomic Absorption Spectrometry (ET-AAS)

Plastic centrifuge tubes with caps (Sorvall, Norwalk, Connecticut, USA) were utilized for the two extraction steps preceding the atomic absorption measurement. Unless otherwise stated, arsenic determination before or after mineralization was carried out as follows:

Into a 50 ml plastic tube, pipet 5 ml of the standard arsenic solution or the sample to be analyzed; add 0.2 ml 40% KI in water and 15 ml of a concentrated acid mixture ($HCl/HClO_4$/HBr, 10/3/3, vol/vol) (this mixture is replaced by 24 ml of concentrated HCl and KI is omitted when only inorganic As (III) must be analyzed); add 10 ml of toluene; cap the tube and shake vigorously for 3 min; allow the separation of the two phases (a slight centrifugation may be useful when urine samples are extracted without prior mineralization;

transfer 5 ml of the upper phase (toluene) into another 15 ml plastic tube containing 2.5 ml of 0.25% $Co(NO_3)_2$ in 1% HNO_3; cap the tube and shake again for at least 1 min;

after separation, remove the organic phase by aspiration and measure the arsenic concentration of the aqueous phase by electrothermal atomic absorption spectrometry (ET-AAS); an external standard calibration curve is used for the calculations. Measurements were carried out with a Perkin-Elmer atomic absorption spectrometer, model 420, equipped with an electrode-less discharge lamp operating at 8 W, a deuterium background corrector, an auto-sampling system AS-1, a graphite furnace HGA-76B, and a recorder. The spectral line at 193.7 nm and a slit width of 2 nm were selected. The heating program of the graphite furnace was the following:

ramp 15°C/s (rate 3) until 100°C and hold 10 s at this temperature;
ramp 23°C/s (rate 30) until 285°C and hold 10 s at this temperature;
ramp 670°C/s (rate 1) until 1200°C and hold 30 s at this temperature;
atomize at 2500°C for 6 s during which the nitrogen flow is stopped.

Table 1. Toluene extraction of different arsenic compounds in water after various pretreatments (determination by ET-AAS)

As compound	Recovery %; acidification procedure; pretreatment			
	HCl	$HCl/HClO_4$/HBr	HCl + KI	HCl + KI after wet digestion
As_2O_3	99.1	99.6	99.0	99.3
As_2O_5	0	99.6	97.1	99.5
MMA	0	0	99.6	100.9
DMA	0	0	98.8	99.0
ARSAN	0	0	0	100.3

MMA = monomethylarsonic acid; DMA = dimethylarsinic acid; ARSAN = arsanilic acid

Modification of the Braman and Foreback (1973) Procedure for the Analysis of Different As-compounds

In this technique, arsines are generated at low pH with sodium borohydride and are measured by atomic absorption spectrometry (AG-AAS). Technical details are as follows: up to 1 ml of sample is treated with 10 ml 1% HCl in the reaction vessel of a Mercury/Hydride System-1 from Perkin-Elmer; nitrogen is allowed to flow during 30 s at a rate of 300 ml/min and 1 ml of 5% $NaBH_4$ in 0.06% NaOH is introduced within 20 s by means of a peristaltic pump; the volatile arsines are carried by nitrogen flow through an empty 25 ml impinger placed in melting ice and through another empty trap place in an isopropanol-solid carbon dioxide bath; they are frozen out in a liquid nitrogen cooled U-shaped tube (30 cm in length and 4 mm of internal diameter) half-packed with Chromosorb W AW-DMCS, 60–80 mesh (Intersmat Instruments, Pavillons sous Bois, France); the reduction is allowed to proceed for 2 min and then the liquid nitrogen is removed; the arsines are volatilized and carried into a quartz tube heated at 800° C by electrical resistances.

Results and Discussion

I. Analysis of Aqueous Solutions

Arsenic can be found in many chemical forms: inorganic and organic compounds in which its valence state is tri- or pentavalent. Arsenic tri- or pentoxide, arsenous and arsenic acids and their salts, arsenites and arsenates belong to the former class which also include arsenic complexes, in which arsenic is linked to sulfur atoms such as in BAL (2,3-dimercaptopropanol) complex. The organic compounds may be classified as aliphatic or aromatic derivatives according to the nature of the carbon atom linked to arsenic; both classes may be further divided according to the valency of the element.

We have first tested the efficacy of different acidification procedures of the samples in the presence or absence of KI towards several arsenic compounds dissolved in aqueous solution at a concentration of 100 µg As/l and analyzed by ET-AAS as described in Material and Methods. The compounds tested were diarsenic trioxide and pentoxide, disodium methylarsonate (MMA), cacodylic (DMA) and arsanilic (ARSAN) acids. As shown in Table 1, it has been possible to find conditions allowing the specific measurement of several forms of arsenic in water solution: inorganic trivalent arsenic alone is determined after treatment with concentrated HCl as already described by Fischer and Harre (1954), while in the presence of KI inorganic tri- and pentavalent and the sum of methylated As (MMA + DMA) are measured together. If the acidification is made with a mixture of $HCl/HClO_4/HBr$ total inorganic As (III + V) is measured and therefore, by difference, inorganic As (V) and the sum of methylated As can be estimated.

Arsanilic acid can be measured only after a wet digestion step as follows: 5 ml of arsenic solution are treated with 15 ml of a concentrated acid mixture (HNO_3/$HClO_4/H_2SO_4$, 10/2/1 by volume); gentle heating is performed on a hot plate at 130° C for 1 h, heating is then increased to distill $HClO_4$ and stopped when dense white SO_3 vapors evolve; after cooling, water is added up to a known volume and an aliquot of the final solution is extracted as usual in the presence of HCl + KI.

The results were compared with those obtained after dry ashing of the samples. To 20 ml of arsenic solution (As_2O_3 or DMA) are added 2 g of MgO in a porcelain

Table 2. Recovery of different arsenic species after dry ashing (determination by ET-AAS)

As added (μg/l) as			Result (μg/l)	Recovery (%)
Inorganic	ARSAN	DMA		
75	—	—	71.7	95.6
—	75	—	75.8	101.1
—	—	75	72.6	96.8
25	25	25	76.2	101.6
50	—	25	72.6	96.8
25	—	50	77.4	103.2
50	25	—	75	100.0
25	50	—	78.2	104.3
—	50	25	79.9	106.5
—	25	50	76.6	102.1

ARSAN = arsanilic acid; DMA = dimethylarsinic acid

Table 3. Recovery of 2000 μg/l of inorganic trivalent arsenic in the presence of 10^{-2} mol/l thiol compounds (determination by ET-AAS)

Thiol 10^{-2} M	Recovery (%)		
	Direct extraction	Wet acid digestion	Dry ashing procedure
DTT	96	90	96
GHS	95	95	101
Cyst	94	94	97
DMSA	22	99	101
BAL	59	97	95

crucible, the content of which is then dried at low heating (80° C–100° C); when dry, the residue is covered with 2 ml of saturated aqueous $Mg(NO_3)_2$ and the crucible placed in a muffle furnace. Its temperature is brought to 600° C in 2 h and kept at this level for an additional 1.5 h. After cooling, 10 ml of water is added and well mixed with the residue before the successive additions of two 5 ml portions of HCl 6 N and 5 ml of concentrated HCl. The crucible is then placed on a hot plate for 1 h at 120° C–130° C to allow a complete dissolution of the solid material and a reduction of the volume which can easily be brought to 20 ml or even to 10 ml, if necessary. The determination is continued by acidification of an aliquot with the acid mixture, treatment with KI and the two extractions as described under Materials and Methods. In the concentration range 0 to 100 μg As/l the slopes of calibration curves are the same for As_2O_3 measured either directly or after wet or dry ashing and for cacodylic acid after treatment with MgO (t-test on the slopes, $P > 0.05$).

Another test was carried out to further confirm the complete recovery of inorganic (As_2O_3), aromatic (ARSAN), and aliphatic (DMA) arsenic after application

Table 4. Stability of aqueous solutions containing 100 µg/l As (III) or As (V) at three different pHs (determination by ET-AAS)

As	pH	Extraction method[a]	Days of storage before analysis			
			0	1	3	8
	4.8	1	97	96	100	101
		2	100	99	88	76
As (III)	7.0	1	100	96	98	102
		2	104	97	72	8
	9.6	1	98	104	103	99
		2	103	95	77	28
	4.8	1	99	101	98	102
		2	0	0	0	0
As (V)	7.0	1	107	102	99	100
		2	0	0	0	0
	9.6	1	98	104	101	97
		2	0	0	0	0

[a] 1: extraction from $HCl/HClO_4/HBr$ in the absence of KI; 2: extraction from 10 N HCl in the absence of KI

of the dry ashing procedure described above. Different proportions of the arsenic solutions were mixed to obtain a final concentration of 75 µg/l. The results shown in Table 2 demonstrate that the three arsenic compounds are completely recovered after dry ashing and measurement by ET-AAS as described under Materials and Methods.

A special case of arsenic compounds where the element is bound to an organic molecule through a sulfur atom is worth consideration. Such complexes can be present in biological materials, e.g., after BAL treatment. We have tested the recovery of 2000 µg/l of As as As_2O_3, in the presence of 10^{-2} mol/l dithiothreitol (DTT), reduced glutathione (GSH), cysteine (Cyst), 2,3-dimercaptosuccinic acid (DMSA), and 2,3-dimercaptopropanol (BAL). The ET-AAS measurements were performed with and without the wet acid digestion or dry ashing procedure as described above. The recovery tests are summarized in Table 3. DMSA appears to be a better complexing agent than BLA but both wet and dry ashing set the arsenic free from both complexes. The same tests with inorganic pentavalent arsenic (As_2O_5) gave similar results except that BAL did not appear to complex As (V) whereas DMSA is again an effective chelating agent for this arsenic compound (recovery after direct extraction: 30%).

As_2O_3 is slightly soluble in water giving rise to arsenous acid; in solution, the alkaline salts of this acid (arsenites) can slowly absorb oxygen from the air to give arsenates. It was thus important to check the stability of both inorganic compounds (As_2O_3 and As_2O_5) in aqueous solutions of different pH. Standard solutions containing 100 µg As/l as As_2O_3 or As_2O_5 were freshly prepared at pH 4.8, 7.0 and 9.6 and were kept for several days at room temperature in polyethylene bottles. These solutions were analyzed for As directly after preparation and after 1, 3, and

8 days using two different extraction methods: that of Fischer and Harre (1954) which extracts only As (III) from a 10 N HCl solution and that described in Materials and Methods which extracts all inorganic As due to the presence of KI. The results are presented in Table 4. It is clear that whatever the pH, the extraction with the acid mixture ($HCl/HClO_4/HBr$) in the absence of KI allows a complete recovery of both arsenic species. However, using the method of Fischer and Harre (1954) for the extraction of As (III), it appeared that some of the As (III) was lost during storage probably by transformation into As (V). This oxidation is rather rapid: after 1 day, there was already some loss of As (III) which increases with time particularly at neutral and alkaline pH.

In summary, the tests performed with various arsenic compounds in aqueous solution indicate that the application of the ET-AAS method described in Materials and Methods allows:

1. The determination of inorganic As (III) only when the freshly prepared sample is acidified with concentrated HCl before toluene extraction;

2. the determination of total inorganic arsenic (As (III) + As (V)) when the sample is treated in the absence of KI with the concentrated acid mixture, ($HCl/HClO_4/HBr$) before toluene extraction (in the presence of KI, a possible interference can arise from the partial extraction of arsenic-thiol complexes and of methylated derivatives);

3. the determination of all the arsenic species tested (i.e., inorganic As + As-thiol complexes + aromatic and aliphatic As) after wet ashing of the sample, acidification with HCl and extraction with toluene in the presence of KI, or after dry ashing and in this case, followed by any of the two acidification procedures.

II. Analysis in Urine

As long as exposure is mainly to inorganic arsenic and there is no absorption of arsenic from marine organisms, arsenic in urine of man and animals is principally in the methylated form; a small quantity is also excreted as inorganic tri- or pentavalent arsenic (Braman and Foreback 1973; Crecelius 1977). We have first tested whether inorganic arsenic (As (III) + As (V)) could be selectively measured by direct extraction of the urine after acidification with the acid mixture in the absence of KI.

This procedure was applied to determine the concentration of inorganic arsenic in urine of people exposed to inorganic arsenic in industry, before and after addition of $Na\ AsO_2$ to the samples to increase their concentration by 40 and 80 µg As (III)/l, respectively. From the individual values of the spiked urine samples reported in Table 5, it was concluded that inorganic arsenic is quantitatively extracted from urine: the mean recovery equals 97% and 100% when 40 and 80 µg As (III)/l were added, respectively.

Since we have observed that As (III) can be readily oxidized in aqueous solution, we also tested the stability of As (III) and As (V) in urine brought to different pH: 4.8, 7.0 and 9.5. As for the tests on aqueous solutions, urine samples containing 100 µg As (III)/l were analyzed directly after preparation and 1, 3, and 8 days later. We have compared the results obtained with the use of two extraction methods: the first allows the extraction of total inorganic arsenic by toluene in the absence of KI

Sample no.	Arsenic concentration (µg As/l)		
	No addition	+ 40 µg As(III)/l	+ 80 µg As(III)/l
1	63	103 (100)[a]	144 (101)
2	35	73 (96)	113 (97)
3	51	90 (98)	126 (94)
4	7	46 (97)	90 (104)
5	13	51 (95)	92 (99)
6	30	69 (96)	114 (104)
7	20	57 (94)	101 (102)
8	28	68 (100)	112 (105)
9	46	84 (93)	122 (95)
10	11	52 (102)	89 (98)
11	12	50 (94)	97 (106)
12	46	85 (98)	120 (92)

Acidification with $HCl/HClO_4/HBr$ in the absence of KI
[a] percent recovery

Table 5. Direct extraction of inorganic arsenic added as As(III) to different urine samples (determination by ET-AAS)

pH	Extraction method[a]	Days of storage before analysis			
		0	1	3	8
4.8	1	101	94	102	104
	2	105	92	85	86
7.0	1	98	98	105	105
	2	104	97	85	89
9.5	1	96	101	101	103
	2	65	56	52	34

[a] 1: extraction from $HCl/HClO_4/HBr$ in the absence of KI; 2: extraction from 10 N HCl in the absence of KI

Table 6. Stability of As(III) in urine containing 100 µg As(III)/l (determination by ET-AAS)

from a solution acidified with the mixture $HCl/HClO_4/HBr$, the other extracts specifically As (III) from a 10 N HCl mixture. As shown in Table 6, As (III) can be partly oxidized in urine; this oxidation is more important when the pH is alkaline and proceeds slowly with time. This observation was again confirmed by the measurement of As (III) in eight urine samples supplemented with As_2O_3 to increase the concentration by 100 µg As(III)/l. Acidification with HCl and subsequent toluene extraction yield a mean concentration (±SD) of 103 (±4) µg As(III)/l just after preparation but after 1, 3, and 8 days at room temperature, the concentration declined to 100 (±2.5), 81 (±3) and 78 (±5) µg/l, respectively. The pH increased slightly during the test, from 5.9 (±0.6) at the start to 6.2 (±0.8) after 8 days. To confirm that the observed loss was due to oxidation of As (III) and not to absorption on the vessel wall, measurements were also made using the extraction

with the acid mixture ($HCl/HClO_4/HBr$); in that case, a complete recovery of inorganic arsenic was observed. We have then tested whether inorganic arsenic and its methylated derivatives (monomethylarsonic and dimethylarsinic acids) could be determined separately as was possible with water samples (see Table 1). The results were disappointing because under the conditions where only total inorganic arsenic was extracted from aqueous solutions (extraction with the acid mixture), 5% of the monomethylarsonic acid was extracted from urine and under the conditions where methylated arsenic compounds can be measured in aqueous solution (extraction with HCl + KI) the recovery was only partial.

Consequently, the pre-addition values reported in Table 5 are inaccurate. Further tests showed that the recovery of the monomethylated acid varied from 80 to 100% from sample to sample when the acidification was made with HCl and the extraction by toluene in the presence of KI, conditions which were selected for the simultaneous measurement of inorganic and methylated arsenicals in aqueous solution. A wet digestion of the urine samples did not modify these results.

In order to increase the recovery percentage of the methylated arsenic derivatives, we have incubated the urine samples for 1 h at 65° C after acidification with HCl and have used a higher amount of KI before the extraction with organic solvent, as described by Fitchett et al. (1975). Using toluene as organic extractant, recovery tests made with five urine samples spiked either with monomethylarsonic acid (MMA) or with cacodylic acid (DMA) failed to confirm the observations of these authors: with water as back-extracting medium, 70% and 28% of MMA and DMA, respectively, are determined whereas in the presence of 0.005 M dichromate, these percentages amount to 94 and 85%, respectively. The incomplete recovery of the methylated derivatives does not appear to be due to a difference of organic solvent: indeed, Fitchett et al. used chloroform for the extraction and observed a recovery of 88% of DMA from urine. Moreover, they tested diiodomethylarsine as its monomethylated derivative and not monomethylarsonic acid which is the natural metabolite present in urine and which can already be extracted to a large extent with water in the absence of dichromates, while according to Fitchett et al. (1975) inorganic arsenic only is supposed to be measured under these conditions.

The failure to completely recover the methylated derivatives of arsenic added to urine whatever the acidification procedure prompted us to use a more drastic mineralization by MgO at 600° C. Under these conditions, the recovery of 50 µg As/l added as As_2O_3, arsanilic (ARSAN) or cacodylic (DMA) acid in three different urines is complete (Table 7). When the arsenic concentration of urine from the laboratory staff was determined either directly by the Fitchett et al. (1975) technique or after the wet or the dry digestion procedures, striking individual differences between the results were observed (Table 8). While the wet acid digestion does not seem to increase the arsenic concentration above the level found after direct extraction, the dry ashing technique with MgO and $Mg(NO_3)_2$ can enhance markedly the measured arsenic concentration at least in some samples. This confirms that in certain conditions a significant proportion of arsenic is in a special form which is measured only after dry ashing. Since it is known that seafood consumption can alter the urinary arsenic values (Pinto et al. 1976) in a dramatic fashion, we enquired about the food consumed by the subjects during the days preceding the urine collection and found that the subjects (no. 9 and no. 10) with

Urine	As µg/l			
	No addition	+ 50 µg As/l as		
		As_2O_3	ARSAN	DMA
1	16	63	69	65
2	33	89	86	83
3	41	102	104	91

ARSAN = arsanilic acid; DMA = dimethylarsinic acid

Table 7. Recovery of three forms of arsenic in urine after dry ashing with MgO and Mg $(NO_3)_2$ (determination by ET-AAS)

Urine sample	As µg/l		
	Direct extraction[a]	Wet acid digestion	Dry ashing procedure
1	4	12	17
2	9	10	16
3	8	9	18
4	1	5	8
5	2	6	8
6	4	9	9
7	7	12	34
8	6	7	12
9	20	24	109
10	7	17	74

[a] Fitchett et al. (1975) technique using toluene instead of chloroform

Table 8. Arsenic concentration in normal urine samples with or without pretreatment (determination by ET-AAS)

Mean[a]	Standard error	Coefficient of variation
4 µg/l	1.0	0.46
20 µg/l	0.5	0.04
141 µg/l	4.6	0.06
167 µg/l	1.9	0.02

[a] Mean of three determinations

Table 9. Reproducibility of the determination of total arsenic concentration in urine after dry ashing (determination by ET-AAS)

high arsenic concentration in urine had eaten shrimps 2 days before the sampling. The total arsenic content of the urine of 18 adult subjects not occupationally exposed was measured (dry ashing procedure); the mean concentration and the standard deviation of the mean were respectively 7.8 and 5.3 µg As/g creatinine. The reproducibility of the measurements of total arsenic concentration in urine are satisfactory as shown in Table 9. The results obtained with the atomic absorption technique used in our laboratory for the determination of total As

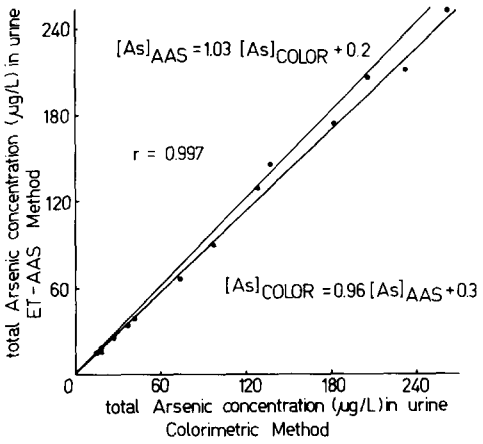

Fig. 1. Comparison of the electrothermal atomic absorption spectrometry (ET-AAS) and the silver diethyldithiocarbamate colorimetric method for the measurement of arsenic concentration in urine samples mineralized with MgO at 600° C

concentration in urine give results similar to those obtained with the colorimetric method of Vasak and Sedivec (1952) at least if the samples are completely mineralized (dry ashing) before analysis (Fig. 1).

A comparison of our technique (ET-AAS after dry ashing) with neutron activation analysis (NAA) was also made on 10 urine samples whose As concentration ranged from 36 to 1180 µg/l (see below Table 14, Mediterranean population with important fish consumption habits). The coefficient of correlation between both sets of results is 0.998 and the regression equations are: $(As)_{NAA} = 1.06\ (As)_{ET\text{-}AAS} + 2.5$ and $(As)_{ET\text{-}AAS} = 0.94\ (As)_{NAA} - 1.5$. At this stage of our investigation, we were thus able to measure (1) the total concentration of arsenic (eventually including As from seafood) in urine after treatment with MgO; (2) the total inorganic (tri- and pentavalent) arsenic concentration plus roughly the total monomethylated derivative concentration and only 85% of the dimethylated compound by the procedure of Fitchett et al. (1975) and (3) only the inorganic trivalent arsenic after extraction with toluene of a 10 N HCl solution in the absence of KI; in the latter case, a fresh neutral or acid urine sample is needed.

These techniques were judged to be insufficient for the study of arsenic metabolism in human. Consequently, we turned to the more selective method of Braman and Foreback (1973) which we used with some modifications as described in Materials and Methods without attempting to distinguish between tri- and pentavalent inorganic As. For purposes of comparison, the total arsenic concentration was measured in urine samples of 34 workers exposed to As_2O_3 and of nine control subjects by ET-AAS after a dry ashing step (X) and also by AG-AAS without any pretreatment (Y); in the case of AG-AAS total arsenic was obtained by summing up the concentrations of the total inorganic, monomethylated and dimethylated derivatives. The subjects were asked not to eat fish or shelfish during the week preceding the urine sampling in order to avoid any interference of the organic arsenic derivative present in marine organisms. Urine concentrations ranged from 2 to 1600 µg/l.

The correlation coefficient between both sets of results was 0.993 and the regression equations were $Y = 0.995,\ X + 15.7$, and $X = 0.991,\ Y - 10.8$. Some

Table 10. Comparison of the total arsenic concentrations in urine samples measured by ET-AAS after dry ashing with MgO and by AG-AAS on native sample

Sample	Arsenic µg/l				
	AG-AAS				ET-AAS after MgO ashing
	Inorganic	MMA	DMA	Total	
Control	3.3	3.0	12.9	19.2	20.1
	0.5	1.6	11.7	13.8	9.5
	1.8	2.8	19.3	23.9	42.5
	0.3	0.9	3.2	4.4	6.5
	2.1	0.2	8.9	11.2	14.7
Exposed workers	165	173	596	934	953
	65	64	186	315	380
	37	22	105	164	168
	49	27	174	250	252
	31	13	30	74	91

examples of the individual values obtained are shown in Table 10. Individual results obtained by AG-AAS show that in the control subjects, the proportions of inorganic As, MMA and DMA are 5.9 ± 5.2, 9.2 ± 8.4 and $83.9 \pm 10.8\%$ (mean $\pm$ SD) of the total concentration, respectively; these figures amount to 23.3 ± 10.3, 16.9 ± 5.2 and $59.9 \pm 10.8\%$, respectively in the group of workers. These results agree particularly well with those reported by Smith et al. (1977).

III. Study of the Urinary Excretion of Arsenic and Its Methylated Metabolites in Human Exposed to As_2O_3

The evaluation of inorganic arsenic exposure by the measurement of its urinary concentration requires a knowledge of the rate of its elimination after exposure. Only two recent studies have been performed with human beings (Crecelius 1977; Mappes 1977). They indicate that an important fraction of inorganic arsenic ingested is excreted via urine and that the biological half-live of the main metabolites is around 30 h. We have checked these findings and simultaneously compared the excretion of the organo-arsenicals present in marine fauna which have also been found to be rapidly eliminated (Pinto et al. 1976; Freeman et al. 1979) without transformation (Crecelius 1977; Edmonds and Francesconi 1977). Five members of the laboratory staff who were asked to avoid eating fish or shellfish before and during the experiment were dosed once with 3 mg As in the form of $Na\ AsO_2$ in water just after voiding the bladder at 9 a.m. For the two first days, urine was collected during two successive 6-h periods followed by a 12-h period and then during three 24-h periods. The volumes of the urine samples and their total arsenic concentrations (ET-AAS after MgO ashing) were measured. The mean excretion rate ($\pm$ SD) during each period of urine collection is given in Fig. 2 A. The total amount excreted in urine after 5

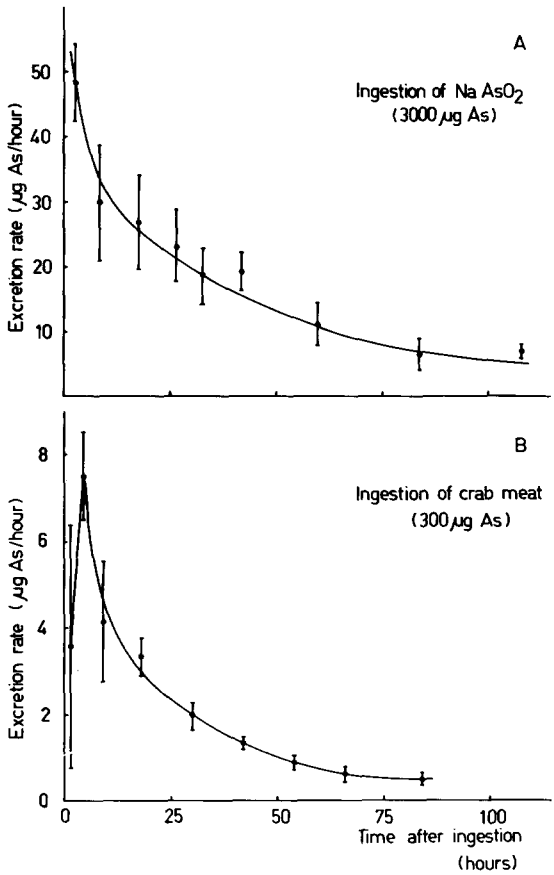

Fig. 2A and B. Urinary arsenic excretion rate as a function of time. **A** in the case of one ingestion of 3000 µg As as $NaAsO_2$ (five subjects); **B** in the case of one ingestion of 135 g crab meat containing 300 µg As (four subjects). (Vertical bars represent the SD of the mean values which are plotted at time corresponding to the mid-period collection)

days amounts to 48% of the quantity ingested (assuming a background daily excretion of 25 µg As). A biological half-live of 30 h can be calculated. The results obtained in four subjects who ate 135 g crab meat containing 300 µg As are shown in Fig. 2B. The urinary As excretion is important (56% of the dose are excreted in 4 days through urine) and somewhat more rapidly than in the case of inorganic arsenic (the biological half-live calculated from the excretion rate during the first 4 days is 18 h).

In order to gain information about the methylation capacity of the human body, the excretion of inorganic As and its MMA and DMA derivatives was followed in urine samples of three volunteers who ingested 500 µg As ($Na AsO_2$ in water). As usual, they were asked to avoid eating seafood. Total arsenic determinations by ET-AAS after mineralization with MgO and the analysis of inorganic As, MMA and DMA by AG-AAS were performed. The total amounts of arsenic measured with both techniques were again in good agreement. The concentrations of total arsenic in the samples and the corresponding percentages of the three forms determined by AG-AAS are shown in Table 11. The balance study during the 4 days following ingestion shows that 39, 49, and 45.5% of

Table 11. Urinary excretion of inorganic arsenic and its methylated derivatives by three volunteers who ingested 500 μg inorganic As(III)

Sampling period	Subject	Total As[a] (μg)	Percentage[b]		
			Inorganic	MMA	DMA
The day before ingestion	1	3.8	6.4	3.2	90.4
	2	8.7	3.0	7.7	89.2
	3	10.8	14.3	0.0	85.7
After ingestion					
0— 4h	1	8.4	100.0	0.0	0.0
	2	14.6	63.3	12.2	24.5
	3	5.5	69.0	13.8	17.2
4— 8h	1	23.2	60.7	15.2	24.1
	2	29.6	31.2	27.7	41.1
	3	25.7	49.0	27.9	23.1
8—12h	1	22.2	33.3	22.8	43.9
	2	35.9	15.8	30.8	53.4
	3	15.8	24.0	39.2	36.8
12—24h	1	36.8	43.5	17.4	39.1
	2	57.6	25.0	19.8	55.2
	3	57.0	28.0	30.0	42.0
24—36h	1	39.7	18.0	19.7	62.3
	2	36.8	5.7	20.0	74.3
	3	59.0	19.3	14.2	66.5
36—48h	1	14.3	10.5	21.0	68.5
	2	26.5	37.0	11.1	51.9
	3	21.7	9.5	28.6	61.9
48—72h	1	26.0	21.4	21.4	57.2
	2	51.8	7.1	10.7	82.2
	3	57.4	17.8	17.8	64.3
72—96h	1	38.7	9.3	12.5	78.2
	2	28.9	5.9	11.8	82.4
	3	28.8	18.8	12.5	68.7

[a] Dry ashing with MgO and determination with ET-AAS
[b] Determination with AG-AAS

the dose were eliminated through the urinary tract by the three subjects, respectively. For these calculations, the background elimination was chosen to be equal to that measured the day before ingestion. It can be observed that after ingestion of inorganic arsenic, a rapid methylating process occurs: during the first hours, arsenic is excreted mainly as inorganic As, but this mode of excretion greatly disappears thereafter leading to the excretion of methylated derivatives, especially DMA. Within the 4 days after ingestion the proportion of the total arsenic excreted in the inorganic form amounts to 32.4, 22.4, and 22.3%, that in the monomethylated form to 18.4, 20.0, and 22.5% and finally that in the dimethylated form to 49.2, 57.6, and 55.2% for the three subjects, respectively. Arsenic excretion measured 2 weeks after ingestion has returned to the normal range.

Table 12. Urinary excretion of inorganic and methylated arsenic as a function of time in two cases of oral ingestion of a high dose of As_2O_3 (attempted suicides)

Subject	Days after ingestion	Total As[a] µg/g creatinine	Percentage[b]		
			Inorganic	MMA	DMA
1	1	6682	85.0	15.0	0.0
	2	5044	93.3	5.6	1.1
	3	5675	62.9	0.9	36.2
	4	4519	49.4	15.3	35.3
	5	4006	32.5	16.5	51.0
	6	2047	6.1	19.4	74.4
	7	2338	1.4	24.5	74.1
	8	1693	13.7	16.9	69.4
2	0	6143	100.0	0.0	0.0
	1	3689	87.9	9.9	2.2
	2	2080	61.4	17.7	20.9
	3	1873	42.9	31.0	26.1
	4	804	25.1	28.6	46.3

[a] Dry ashing with MgO and determination with ET-AAS
[b] Determination with AG-AAS

Table 13. Arsenic content in urine samples after fish consumption

Fish	As (µg/l)			
	AG-AAS			ET-AAS after MgO ashing
	Inorganic	MMA	DMA	
Fresh cod	3.0	0.0	0.0	185
	1.5	2.1	17.4	170
Cured herring	0.6	0.0	13.2	113
Trout	1.0	1.3	14.0	131
Plaice	0.4	0.2	11.3	18,800
	0.2	0.0	11.4	14,070
Sole	0.2	0.0	3.5	150
	0.6	0.1	3.4	136

We had the opportunity to examine the excretion of urinary arsenic in two cases of severe acute exposure to As_2O_3 (attempted suicides), but as the total amount of arsenic ingested was unknown, a balance study was impossible to perform. Nevertheless, the results of some determinations of the different forms of arsenic found in spot urine samples show that after ingestion of a large amount of inorganic arsenic, a methylation process rapidly occurs and leads to a preponderant excretion of the dimethylated derivative (Table 12). It must be

Table 14. Comparison of the urinary arsenic excretion in two populations of workers with different fish consumption habits

Group	Subjects	As (μg/l)					$\frac{I}{II} \times 100$
		AG-AAS				ET-AAS after MgO ashing	
		Inorganic	MMA	DMA	Total		
					I.	II.	
Mediterranean	1	2.8	1.3	7.4	11.5	36	32
	2	1.7	1.3	20.4	23.4	1180	2
	3	3.8	1.0	13.4	18.2	820	2
	4	2.3	1.2	11.7	15.2	417	4
	5	2.3	1.1	11.1	14.5	501	3
	6	1.0	2.2	22.4	25.6	195	13
	7	0.0	0.7	6.2	6.9	378	2
	8	2.4	1.5	9.1	13.0	122	11
	9	1.6	0.6	7.6	9.8	85	12
	10	0.3	1.0	7.9	9.2	92	10
Belgian	1	16.1	5.7	17.8	39.6	34	116
	2	35.5	6.5	10.9	52.9	68	78
	3	7.9	6.8	37.3	52.0	144	36
	4	9.2	6.1	58.0	73.3	69	106
	5	13.3	5.3	16.8	35.4	136	26
	6	39.5	4.7	24.0	68.2	93	73
	7	8.2	12.5	9.7	30.4	379	8
	8	5.3	3.1	7.1	15.5	28	55
	9	6.6	3.1	17.2	26.9	22	122
	10	24.4	14.1	74.6	113.1	170	67

noted that both subjects were under treatment with BAL and diuretics. In order to strengthen the usefulness of the determination of inorganic arsenic and its methylated metabolites as indices of exposure to the inorganic form, urine samples following meals including fish dishes were analyzed by ET-AAS after MgO dry ashing and by AG-AAS without pretreatment. Results are presented in Table 13. Although the total amount of arsenic observed after complete mineralization is high, that observed with the other technique (AG-AAS) corresponds to values of control subjects who did not eat fish. It thus appears to be highly probable that AG-AAS does not measure arsenic from marine origin as long as the sample is not mineralized or treated with NaOH which can set cacodylic acid free from the arsenic complex present in urine of seafood consumers (Crecelius 1977; Edmonds and Francesconi 1977).

The importance of characterizing the different forms of arsenic excreted in urine for the monitoring of workers can be illustrated by the results obtained with two populations potentially exposed to inorganic arsenic but who differ markedly

in their fish consumption habits (Table 14). The first group of workers lives along the Mediterranean Sea, the other in Belgium. The concentrations are expressed in µg/l and correspond to 24 h samples. It is clearly demonstrated that a high total arsenic concentration as measured on MgO dry ashed samples (ET-AAS) is not necessarily associated with a high inorganic plus methylated arsenic content. While the total arsenic concentrations (ET-AAS) are on the average higher in the Mediterranean group, the highest fractions of inorganic plus methylated As are found in the Belgian group and probably reflect a different exposure to arsenic from marine fauna and arsenic of industrial origin.

Conclusions

Recently, we have drawn attention to the fact that several methods proposed to measure total arsenic concentration in urine do not necessarily do so because quite often the mineralization procedure is not drastic enough to completely release arsenic from its covalent bonds (Lauwerys et al. 1979). Usually, the recovery has only been tested by addition of As_2O_3 standards to the samples and therefore, no conclusion can be drawn regarding the recovery of other arsenic compounds. Even when various arsenic species were selectively determined as in Braman and Foreback's method, an independent method (e.g., NAA) has rarely been used to evaluate whether the sum of their concentrations equals the total amount of arsenic present. To our knowledge, only Smith et al. (1977) compared their results obtained by the arsine generation technique with those obtained on mineralizates by the silver diethyldithiocarbamate method.

The fact that a technique like the colorimetric method of Vasak and Sedivec (1952) as applied by Pinto et al. (1976) can detect an increased arsenic concentration in urine after a fish meal is no proof that the total amount of arsenic present is correctly evaluated. Furthermore, it is known that wet treatment of the arsenic compound present in marine fauna may release methylarsonic acids (Uthe et al. 1974; Edmonds and Francesconi 1977) which also react with silver diethyldithiocarbamate. The colored derivative produced has an absorption spectrum different from that obtained with arsine (Peoples et al. 1971) and normally its interference at 560 mm should be minimal. At lower wavelength, however, various proportions of inorganic and organic arsines are measured by the silver diethylditniocarbamate method. No correct estimate of total arsenic concentration can thus be performed if care has not been taken to fully mineralize the sample before arsine production. The dry ashing procedure before ET-AAS measurement as described in this paper allows the determination of total arsenic concentration in urine. Recovery tests with various arsenic compounds and comparison of the results with those obtained by NAA confirm this conclusion.

However, it is well known that the toxic properties of the different arsenic compounds are closely related to the chemical form of As (Penrose 1974). Whatever the material under examination, the measurement of arsenic concentration should not be restricted to the total arsenic content.

Rather few methods have been described that give information about the chemical form of arsenic, particularly in biological materials like urine. We tried selective extraction procedures preceded or not by a mineralization step before

the analysis by ET-AAS. Promising results were obtained with aqueous solutions of the more interesting arsenic species, but unfortunately, they could not be reproduced in a satisfactory manner with urine samples. A modification (AG-AAS) of the arsine selective volatilization technique of Braman and Foreback (1973) was developed allowing the use of an atomic absorption spectrophotometer as detection device instead of the plasma excitation emission spectrometer used by these authors. This method was compared with the classical ET-AAS preceded by dry ashing of the sample with MgO and proved to be quite satisfactory when urine from subjects who had not eaten fish were compared. After seafood consumption, the results differed substantially because the organic form of arsenic from marine origin could not be measured by AG-AAS but was completely mineralized by the treatment with MgO at 600° C and consequently fully determined (this was ascertained by comparison with results obtained by neutron activation analysis).

For all these reasons, the study of the metabolism of inorganic arsenic in the human has to be approached by the use of both the ET-AAS method after dry mineralization and the arsine generation technique. After a single oral intake of As_2O_3, we were able to confirm the existence of a methylation process of inorganic arsenic in man which was recently evidenced by Crecelius (1977). If, during the first hours after ingestion, the main form of urinary arsenic is inorganic As, the relative proportions of the species excreted in urine rapidly change with time leading to a predominant excretion of the dimethylated form (which represents also the most abundant form in the urine of control subjects). The rate of urinary excretion of inorganic arsenic and its metabolites is rapid: a biological half-life of 30 h has been calculated from the balance study with five volunteers who ingested a single dose of 3000 µg As as $NaAsO_2$: exactly the same value has been reported by Crecelius (1977). The importance of the urinary excretion appears to be considerable: 50 to 60% of the ingested dose is eliminated in a few days. This is in agreement with the observation of Mappes (1977): in the case of daily ingestion of 760 µg As as As_2O_3, an equilibrium was reached after 5 days and at that time the daily urinary excretion amounted to 70% of the ingested dose.

Consequently, the determination of urinary arsenic by AG-AAS appears to be the most suitable method for the biological monitoring of workers exposed to inorganic arsenic: it is not influenced by an eventual excretion of arsenic from seafood origin and the relative proportion of the inorganic form versus the dimethylated form can give information about the length of time between exposure and urine sampling.

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