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Andrea Raab · J. Feldmann

Arsenic speciation in hair extracts

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Abstract Ingested arsenic is known to be not only excreted by urine, but to be stored in sulphydryl-rich tissue like hair, nail or skin. We developed an extraction method for arsenic species from these tissues and studied the stability of different arsenic species during the extraction process. Inorganic and pentavalent methylated arsenic was found to be stable under the extraction conditions, whereas trivalent methylated arsenicals and the thio-analogue of DMA^V (DMAS) showed reduced stability. The absorption ability of hair for these different species was studied as well. Inorganic arsenic is better absorbed by hair than monomethyl- or dimethylarsenicals, whereby the trivalent forms are taken up better than the pentavalent forms. Independent of which methylated arsenical was used for the incubation, the pentavalent form was always the dominant form after extraction. Hair and nail samples from humans suffering from chronic arsenic intoxication contained dominantly inorganic arsenic with small and strongly varying amounts of DMA^V and MA^V present. DMAS was only found in some nail sample extracts containing unusually high amounts of DMA^V and is believed to be formed during the extraction process.

Keywords Arsenic speciation · Sample preparation · Hair analysis · HPLC-ICP-MS · Accumulation potential · Species transformation

Introduction

The health of millions of people worldwide is in danger due to increased intake of inorganic arsenic from contaminated drinking water. However, the metabolism of inorganic arsenic (As^V and As^{III}) in humans has been extensively studied [1]. Ingested inorganic arsenic is, after take-up in the intestine, converted mostly to mono- and dimethylated arsenic species in the liver. This methylation process is regulated by specific enzymes and involves reduction and oxidative methylation with *S*-adenosylmethionine (SAM) as methyl donor. According to the Challenger mechanism [2], trivalent arsenicals are formed during this process, which can readily bind to sulphydryl-groups of proteins or enzymes [3]. About 60–70% of ingested inorganic arsenic is excreted within 48 h [4]. It is mainly excreted via the kidneys as DMA^V (60–80%), MA^V (10–20%) and inorganic arsenic (10–20%) [5]. Although trivalent methylated arsenicals have recently been determined in urine, arsenic is mostly excreted as their pentavalent counterparts [6] due to their increased stability. While most ingested arsenic is excreted as methylated arsenic, some of it is stored in sulphydryl-rich tissue like skin, nail and hair. The ability of hair to accumulate arsenic was realised early in the nineteenth century with the development of the sensitive Marsh-test as a method of determining arsenic in biological material. The determination of arsenic in hair was often used to decide the cause of suspicious death. Often samples were analyzed some years after death occurred and it was soon found that possible external contamination of hair by arsenic needs to be considered [7]. There are various publications dealing with the problems of external contamination [8, 9] and the usefulness of hair analysis in general [10, 11]. But limited knowledge is available about the different arsenic species that accumulate in hair, their distribution, possible correlation with other parameters of arsenic exposure, and the influence of the metabolism of the hair follicle [12].

The aims of this study are threefold:

1. To evaluate an extraction method for the arsenic speciation in hair with the focus on species transformation reactions.
2. To study the binding potential of different arsenic species to hair.

A. Raab · J. Feldmann (✉)
Department of Chemistry, University of Aberdeen,
Meston Walk, Old Aberdeen, AB24 3UE,
Scotland, UK
E-mail: j.feldmann@abdn.ac.uk

3. To demonstrate the occurrence of different arsenic species in hair extracts from people exposed to excess arsenic in drinking water.

Materials and methods

Reagents and standards

All arsenicals are known human carcinogens and need careful handling. All chemicals used were of analytical grade or better from BDH, UK except where stated otherwise. The arsenic standards used for identification of the species and quantification of the chromatograms were prepared from arsenic trioxide (As_2O_3), sodium arsenate ($Na_2HAsO_3{\cdot}7\ H_2O$), disodium monomethyl arsonate (MA^V) (ChemService, USA), arsenobetaine (AB) and sodium cacodylate (DMA^V) (Strem, USA). The trivalent methylarsine oxide (MA^{III}) and dimethylarsinous iodide (DMA^{III}) were synthesised following published procedures [13, 14]. The thio-analogue of DMA^V was prepared by treating DMA^V solution with a mixture of sodium-metabisulphite, sodium-thiosulphate and sulphuric acid [15]. The identification of the thio-analogue of DMA^V is described in Hansen et al [16]. This species is named in the following as DMAS. Ammonium carbonate (90% mixture of carbonate and bicarbonate), pyridine (99 + %) and formic acid (100%) were used for preparing the buffers for the chromatography. Deionised water (18 MΩ Elga UK) was used throughout all experiments. Pyridine buffer was prepared by diluting the appropriate amount of pyridine in 1 L of water and adjusting the pH with formic acid. For the carbonate buffer, 2 g ammonium carbonate were dissolved in 1 L water (pH was 8.0).

Quantitative arsenic determination

The hair was digested by adding 1 mL HNO_3 (conc) and 1 mL H_2O_2 (30%) to 0.1 g hair in polypropylene vials and heating. Standard reference material (human hair GBW09101) was treated in the same way. NIST SRM 2670, hair extracts and solutions used in the different experiments were diluted in 1% nitric acid prior to quantification, 10 µg/L rhodium was used as internal standard.

Extraction procedure

The extraction procedure was optimised by using arsenic contaminated sheep wool and human hair GBW09101 as described in detail elsewhere [17]. Briefly, the hair was mixed with water (0.5 g hair/10 mL water) in loosely capped polypropylene tubes and boiled for 6 h (final volume ~2 mL). The extract was separated from the hair by filtering (0.45 µm Nylon Iso-Disc Filters, Supelco, USA). The extracts were stored at 4 °C overnight and measured the next day.

Arsenic determination by ICP-MS

The inductively coupled plasma mass spectrometer (ICP-MS, Spectromass 2000, Spectro Analytical Instruments, Kleve, Germany) was fitted with a Meinhard nebulizer and a water-cooled cyclonic spray chamber. The instrument was optimised daily for arsenic intensity. It was used either with an autosampler for quantitative determination of arsenic or connected via a 30 cm Teflon tube to the outlet of the HPLC column.

Speciation by HPLC-ICP MS

A commercial HPLC system (LKB, Bromma, Sweden) fitted with a 100 µl sample loop was used; the flow rate was 1.0 mL/min. The column outlet was connected via a T-piece to a second pump supplying a 100 µg/L indium solution (flow rate 0.1 mL/min) used as continuous internal standard. The T-piece was connected via a 30 cm Teflon tube to the nebulizer of the ICP-MS. Beside m/z 75 for arsenic, 77 for chloride interference and 115 for indium were all monitored with a dwell time of 500 ms. Different concentrations of DMA^V were used for calibration, since the response of the ICP-MS to the different As species is not significantly different when peak areas are used for quantification. A cation exchange column [Supelcosil LC-SCX (250×4.1 mm)] and an anion exchange column (Hamilton PRP X 100 [150×4.1 mm]) were used for the separation. The mobile phase for the cation exchange column was a 20 mM pyridine buffer at pH 3.0. 15 mM carbonate buffer at pH 8.0 was used for the anion exchange column. Cation exchange chromatography was used for the separation of extracts from hair incubated with arsenobetaine. All separations were performed at room temperature. Each sample was injected twice for the determination of retention times and peak area.

Hair and nail samples

Standard reference materials NIST SRM 2670 (elevated level) (NIST Gaithersburg, USA) and GBW09101 human hair reference material (Shanghai Institute of Nuclear Research Academia Sinica, Shanghai, China) were used. The GBW09101 was used for quantitative measurements and as ''standard'' material for species determination, whereas the NIST SRM 2670 was used to check the quality of the separation using the values given in [18] as reference values.

Samples for in vitro arsenic incubation experiments

The sample for in vitro binding experiments was taken from a person not exposed to excess arsenic. The hair was not treated chemically by bleaching or other means. It was cut into pieces of about 2 mm length.

Samples of hair/nail contaminated with arsenic in vivo

To test the method developed on "real samples", hair and nail samples from West Bengal (Nadia region) and Central India were measured. Ten hair and nail samples from Central India, Kaudikase, Ambagarh Chouki, Rajnandgaon (where the arsenic concentration in water is 30–100 μg/L), and 20 hair and ten nail samples from different villages in West Bengal (where the arsenic concentration in water 1–6 mg/L) were used. These samples were surplus samples from a larger epidemiological study in this region.

Species stability test

The stability of the different arsenic species under extraction conditions were tested by spiking an extract of non-contaminated hair with 100 μg As/L as As^{III}, As^{V}, DMA^{V}, DMAS, DMA^{III}, MA^{V} and MA^{III}. The extract was prepared by boiling 3.3 g hair in 50 mL of water for 6 h (concentration of hair after boiling 0.2 g hair/mL solution). To 1 mL of extract, 0.01 mL of the arsenic species (~10 mg As/L) was added. The experiment was carried out in duplicate using the same hair extract. The spiked samples were stored for 24 h at room temperature or boiled for 6 h to resemble the extraction procedure. The exact amount of spike added was determined by measuring the total arsenic in the samples.

Species incubation experiment

To test the ability of the hair matrix to take up different arsenicals, 0.5 g of non-contaminated hair was mixed with 10 mL water containing 0.75 mg/L arsenic in the form of As^{III}, As^{V}, DMA^{V}, DMAS, DMA^{III}, MA^{V}, MA^{III} or 0.5 mg/L as AB. The hair was incubated for 72 h at room temperature. The pH of the incubation solutions was 5. The hair was washed after the incubation using membrane filtration (0.45 μm cellulose nitrate, Satorius, UK) until the arsenic concentration in the wash water reached the blank level (< 0.2 μg As/L), and then dried at room temperature. The experiment was carried out in duplicate. The exact amount of arsenic absorbed was determined by measuring the total arsenic in the digested hair. The accumulation potential (AP) for As^{III} was set to 100 and the APs for the other species were calculated from [(total concentration As in hair×100)/(total As available)/(total concentration As^{III} in hair×100)/(total As^{III} available)]×100.

Experimental set-up for the determination of the origin of DMAS

A DMA^{V} solution was mixed with different sulphur-containing compounds and treated in the same way as hair during the extraction procedure. The sulphur-containing compounds tested were glutathione, cysteine, cystine, sodium thiosulphate, sodium bisulphite, methionine and albumin. The sulphur concentration in human hair and nail was determined by NCS measurements (NA1500, Fisons Instrument, Beverly, USA). The development of H_2S during the extraction procedure was qualitatively followed using lead acetate treated filter paper in the headspace.

Results

Analytical performance of the speciation method

An exemplary separation of standard compounds (As^{III}, As^{V}, DMA^{V}, MA^{III}, MA^{V} and DMAS) under the chromatographic conditions used in all experiments using anion exchange chromatography is shown in Fig. 1. DMAS was identified by ES-MS to be dimethylarsenothioic acid or $Me_2As(S)OH$, as described previously [16]. For quality control, NIST SRM 2670 (elevated) was separated, showing the expected species distribution similar to that published in [18]. The recovery of arsenic in NIST SRM 2670 was 98 ± 5% ($n = 6$) and the species distribution As^{V} 380 ± 40 μg/L, DMA^{V} 60 ± 2 μg/L, AsB 35 ± 4 μg/L and MA^{V} 12 ± 4 μg/L. The mean total arsenic concentration for hair reference material GBW 09101 was 580 ± 20 ng/g (reference value 590 ± 70 ng/g, $n = 5$) and the individual arsenic species As^{V} (93 ± 11 ng/g), As^{III} (236 ± 19 ng/g), DMA^{V} (89 ± 7 ng/g) and MA^{V} (64 ± 1 ng/g); the extraction efficiency was 68 ± 9% ($n = 4$), and the recovery of the extractable arsenic from the column was 92 ± 6% (n = 4). The chromatographic recoveries were calculated from [sum(species)×100]/total arsenic in solution (extract for hair).

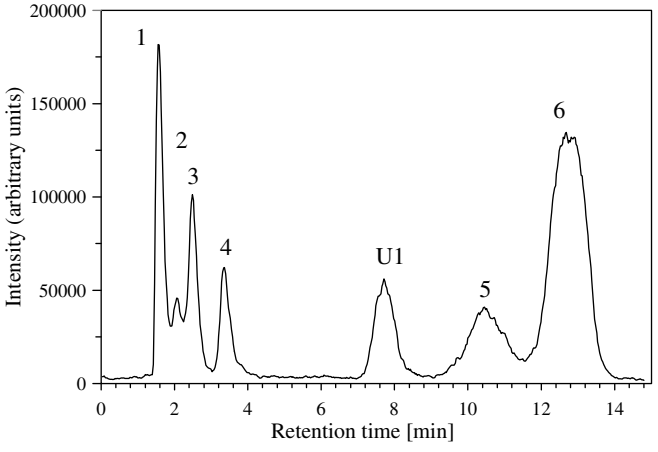

Fig. 1 Separation of standard compounds using anion exchange chromatography (conditions PRP X 100 Hamilton, 20 mM ammonium carbonate, pH 8, 1 mL/min flow); peak identification *1* As^{III}, *2* MA^{III}, *3* DMA^{V}, *4* MA^{V}, *5* DMAS, *6* As^{V}, *U1* by-product of reduction of MA^{V} with Reay and Asher reagent. Concentration range 30–240 ng/mL

Stability of arsenic species during the extraction

The in vitro spiking of hair extract with the different arsenic standards showed that As^{III}, As^{V}, DMA^{V} and MA^{V} were stable at room temperature for at least 24 h and at 100 °C for at least 6 h (Table 1). A species is here defined as "stable" when it does not change its valence and or molecular form and elutes under the chosen chromatographic conditions. At room temperature, DMAS and (in particular) MA^{III} showed a tendency to convert to DMA^{V} and MA^{V} respectively. After boiling they were fully converted to DMA^{V} and MA^{V}. Even at room temperature, DMA^{III} was completely oxidised to DMA^{V} (data not shown). The quantification of the chromatograms and the determination of total arsenic in the extract showed that the recovery from the column was between 67 and 100% after boiling the spiked extract, and between 13 and 70% after storing the spiked extract at room temperature for 24 h. Boiling of the spiked extract improved the chromatographic recovery by up to 40%, depending on the arsenic species, compared to spiking at room temperature. For example, the recovery of spiked As^{V} was 58% after 24 h storage and increased to 93% after 6 h of boiling the extract. As^{III} showed a significantly lower recovery than the other species at both temperatures. It is possible that arsenic thio-compounds are formed in the extract which are not eluting under the chosen conditions from the column [19]. The methylated thioarsenicals are however unstable in sulphide-free solution and some of them can quickly convert to the oxo-species, as suggested by attempts to purify DMAS by HPLC, where about half of the isolated DMAS elutes in the form of DMA^{V} after "re-injection" of the isolate, and the other half elutes as DMAS (using the same chromatographic conditions as for the separation of hair extract).

Incubation of human hair with arsenicals

The in vitro incubation of human hair with different arsenicals showed that inorganic arsenic was much better absorbed than methylated species and that trivalent arsenicals have a higher affinity to the hair matrix than pentavalent arsenicals (Table 2). Extracts of hair incubated with As^{III} or As^{V} contained both As^{III} and As^{V}, where As^{III} was the dominant form with more than 75%. DMA^{V} was the dominant form after incubation with DMA^{V}, DMAS and DMA^{III}. The use of DMAS and DMA^{III} for the incubation resulted in a higher amount of DMAS in the extract. Incubation with MA^{V} and MA^{III} led to dominantly MA^{V} in the extract. The incubation with MA^{V} and MA^{III} also resulted in one unknown species, which might be an analogue to DMAS. The efficiency of extraction from the incubated hair at room temperature was lower for all arsenicals than the extraction efficiency at 100 °C, 34 ± 24 vs. $57 \pm 26\%$. The chromatographic recovery was $80 \pm 7\%$. The extract of hair incubated with AB did not contain any AB.

The ability of DMA^{V} to form DMAS

The sulphur determination for keratinous tissues showed that the sulphur concentration in human hair and nail is between 2.5 and 4% (w/w) (each $n = 3$). Due to the possibility that a series of sulphur-containing compounds may be extracted during boiling, the reaction of DMA^{V} with potential biomolecules was tested. Boiling of DMA^{V} with sodium-thiosulphate and cystine for 6 h results in the transformation of 80–90% of the DMA^{V} to DMAS, whereas at room temperature no DMAS was formed. In the presence of glutathione and cysteine, a small amount of DMAS ($< 2\%$) was created.

Table 1 Stability of arsenic species during storage of spiked hair extract at different temperatures

	As^{III}	As^{V}	DMA^{V}	DMAS	MA^{V}	MA^{III}	U1	Chromatographic recovery (%)
Room temperature for 24 h								
+ blank	1.2 ± 0.3							13 ± 4
+ As^{III}	43 ± 0.3							45 ± 9
+ As^{V}	0.3 ± 0.1	88 ± 3.0						69 ± 2
+ DMA^{V}			105 ± 6.8					85 ± 6
+ DMAS			11 ± 2.1	68 ± 4.6				64 ± 5
+ MA^{V}					96 ± 4.9			71 ± 4
+ MA^{III}					29 ± 3.2	36 ± 4.6	12 ± 0.2	64 ± 8
100 °C for 6 h								
+ blank	2.7 ± 0.4		3.1 ± 0.5		3.8 ± 0.8			84 ± 15
+ As^{III}	45 ± 0.5		5.8 ± 1.7		4.3 ± 1.4			67 ± 4
+ As^{V}	5.8 ± 0.2	102 ± 8.6	6.2 ± 0.2		6.0 ± 0.1			93 ± 7
+ DMA^{V}	3.8 ± 1.1		127 ± 3.2		4.0 ± 0.8			103 ± 4
+ DMAS	4.8 ± 0.5		124 ± 1.8		5.8 ± 0.1			100 ± 2
+ MA^{V}	3.1 ± 0.2		5.8 ± 0.4		116 ± 14			83 ± 10
+ MA^{III}	4.9 ± 0.7		5.3 ± 0.8		128 ± 10			86 ± 7

Values are given as ng As/mL extract. Chromatographic recovery: sum of arsenic species × 100/total As in extract. (*U1* unknown see legend of Fig. 1) ($n = 2$)

Table 2 Arsenic species in hair extract after in vitro incubation with 1.5 mg As/L/g hair (1 mg As/L/g for AB)

	As^{III}	As^{V}	DMA^{V}	DMAS	MA^{V}	MA^{III}	U1	Extraction efficiency (%)	Chromatographic recovery (%)	AP (%)
+DIW	14 ± 3		9 ± 2					65 ± 3	42 ± 4	
$+As^{III}$	1,407 ± 16	153 ± 27	17 ± 2					88 ± 7	80 ± 8	100
$+As^{V}$	548 ± 18	147 ± 8	20 ± 4					80 ± 4	85 ± 5	17
$+DMA^{V}$	19 ± 5		44 ± 9	27 ± 5				42 ± 5	90 ± 5	8
+DMAS	13 ± 3		87 ± 5	159 ± 9				41 ± 6	78 ± 9	16
$+DMA^{III}$	24 ± 2		834 ± 31	721 ± 24				64 ± 7	78 ± 7	79
$+MA^{V}$	29 ± 2		9 ± 4		332 ± 11	56 ± 3	107 ± 10	22 ± 4	85 ± 5	25
$+MA^{III}$	103 ± 8		13 ± 6		854 ± 37	279 ± 18	335 ± 17	33 ± 4	82 ± 4	75
+AB	23 ± 3							35 ± 1	75 ± 1	< 1

Arsenic species in hair extract after in vitro incubation with 1.5 mg As/L/g hair (1 mg As/L/g for AB) with different arsenic species. The acronym +DIW is used for the hair extract used as control. Values of arsenic species in the extract in ng As/g hair, U1 is the unidentified species (see Fig. 1. AP is the accumulation potential of the arsenic species compared to As(III) which was set to 100; ($n = 2$). The extraction was carried out at 100 °C

Table 3 Total arsenic concentrations and the distributions of different arsenic species in hair and nails of people from West Bengal (hair: $n = 20$, nail: $n = 10$) and Central India ($n = 10$)

	Total arsenic (μg/g)	AsIII (%)	DMA^{V} (%)	MA^{V} (%)	U1 (%)	DMAS (%)	As^{V} (%)	sumInorganic As (%)
Hair samples								
West Bengal	4 ± 2	26 ± 20	16 ± 17	2 ± 2	ND	ND	55 ± 19	81 ± 16
Central India	1 ± 1	36 ± 9	1 ± 1	1 ± 1	ND	ND	63 ± 10	99 ± 1
Nail samples								
West Bengal	4 ± 2	56 ± 11	2 ± 1	1 ± 1	ND	1 ± 2	40 ± 11	96 ± 2
Central India	20 ± 27	37 ± 20	6 ± 5	7 ± 4	0 ± 1	1 ± 1	50 ± 17	86 ± 5

ND not determined, below detection limit of < 1 ng/mL in extract

Sodium bisulphite, diethylthiocarbamic acid and albumin did not transform DMA^{V} to DMAS under these conditions. During the boiling of sodium thiosulphate, cystine, glutathione and cysteine, H_2S was liberated from the solution, as shown by the blackening of lead acetate paper.

Arsenic species in hair and nail samples from humans exposed to arsenic

All samples contained dominantly inorganic arsenic in its tri- and pentavalent form. DMA^{V} and MA^{V} were present in most samples but to a far lesser extent. DMAS and an unknown were detectable in a few nail samples, which contained relatively high amounts of the methylated oxo-species (DMA^{V} and MA^{V}). The mean species distribution and the total arsenic is shown in Table 3, while Figs. 2 and 3 show the chromatographic separations of hair and nail extracts of four exemplary samples.

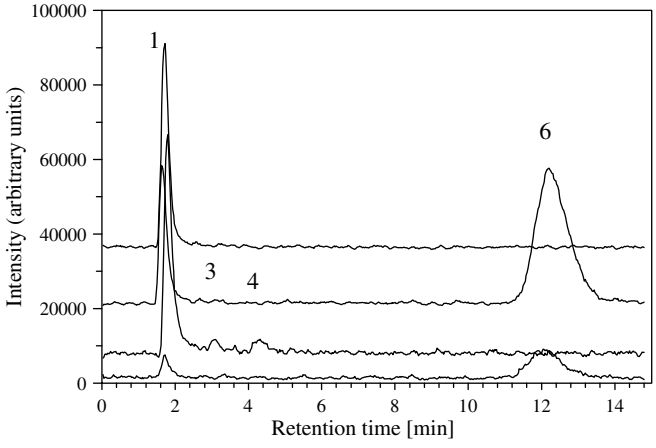

Fig. 2 Separation of hair extracts from people exposed to high levels of arsenic in India using anion exchange chromatography (conditions PRP X 100 Hamilton, 20 mM ammonium carbonate, pH 8, 1 mL/min flow); for peak identification, see legend of Fig. 1

Discussion

Humans excrete most of the inorganic arsenic they are exposed to as dimethylated arsenic in urine [5]. We therefore originally expected that keratinous tissue, like hair, nails and skin, would reflect the species distribution in urine. But, as shown in samples from several humans exposed to arsenic, these tissues accumulate much larger amounts of inorganic arsenic than expected from the species distribution in urine. Inorganic arsenic, which contributes between 10 and 20% of the arsenic in urine, accounts for 85 ± 18% of the total arsenic in keratinous tissue. The species distribution in these matrices

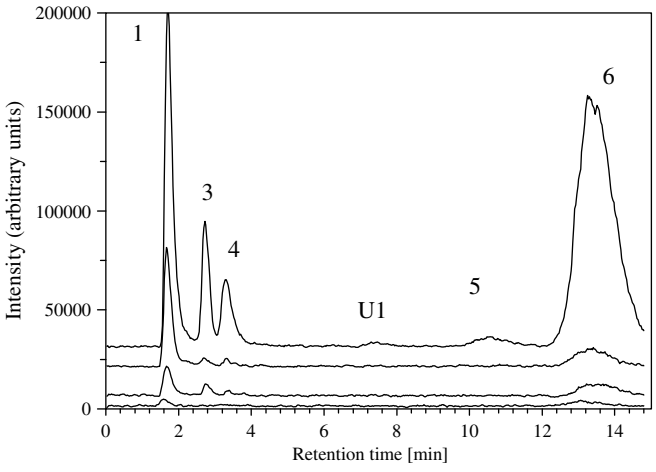

Fig. 3 Separation of nail extracts from people exposed to high levels of arsenic in India using anion exchange chromatography (conditions PRP X 100 Hamilton, 20 mM ammonium carbonate, pH 8, 1 mL/min flow); for peak identification, see legend of Fig. 1

therefore does not, per se, give any information about the methylation capacity of an individual.

In vitro contamination experiments with different arsenic species, which (might) occur as part of the metabolism in the body, showed strong differences in the ability of hair to absorb the species under study. The results showed that trivalent arsenicals are generally better absorbed by hair than pentavalent arsenicals. The higher absorption efficiency of trivalent arsenicals is most likely a result of their tendency to form complexes with molecules with vicinal sulphydryl groups [20]. Pentavalent arsenic species are not per se able to bind to those groups without reduction to their trivalent form. The differences between the uptake ratios of the pentavalent arsenicals are either a result of their different redox potentials or the number of methyl groups attached to the arsenic. In a study with mice, comparing the body retention of As^{III} and As^{V}, Vahter and Nori [21] also found a higher concentration of arsenic in hair/skin after oral administration of As^{III} compared to As^{V}. Different papers were published about the in vitro uptake of As^{III} in hair, and about the factors (like pH and time) that influence it (a good summary can be found in [4]).

Our data clearly show that non-methylated arsenic is absorbed better than methylated, and that this in turn is absorbed better than dimethylated. Tetraalkylated arsenic, tested in the form of arsenobetaine, is not absorbed by hair. This explains why a high intake of fish does not lead to arsenic accumulation in hair. The influence of the number of methyl groups on the amount of bound arsenic suggests that the arsenic is not bound at the outside of the hair, but diffuses into the hair-forming cells. Arsenic is often thought to bind to keratin, but keratin in hair is characterised as low sulphur keratin, whereas there are non-fibrillar proteins with high sulphur content in hair that might bind arsenic [11]. The sulphur groups of keratin in hair form disulphide bridges, necessary to stabilise the molecular structure; it is unlikely that arsenic added in vitro at pH 5 and room temperature can break these disulphide bridges and bind to the sulphur.

The extraction efficiency depends on the duration and the temperature used; boiling the hair for 6 h extracted $57 \pm 26\%$ of the total arsenic, while extraction at room temperature for 24 h gave $34 \pm 24\%$, depending on the species used for incubation. Even when the hair samples are boiled, about 40% of the in vitro bound arsenic is not accessible for species determination, and if the samples are soaked at room temperature it is even less. Others also found low extraction efficiencies even with prolonged soaking at room temperature after in vitro incubation [22]. Pentavalent arsenic species showed a slightly better extraction efficiency at room temperature, where the binding strength to the hair matrix will have a larger influence.

The species used for incubation of the hair were also the main species extracted, but the oxidation state of the species changed somewhat. Although the incubation with inorganic arsenic results in As^{III} as the main form, the ratio of As^{III} to As^{V} depends on which arsenical was used for incubation. DMA^{V} has a very low accumulation potential (only 8% compared to As^{III}); after hot-extraction DMA^{V} and some DMAS was found in the extract, whereas incubation with DMAS and DMA^{III} resulted in extraction of DMAS also at room temperature. The same behaviour was observed for MA^{V} and MA^{III}, forming an unknown species that might be the methyl analogue of DMAS. This leads to the conclusion that during boiling strong reducing agents are extracted from the hair (or activated in the hair), which generate H_2S during boiling and transfer the extracted DMA^{V} into its sulphur-analogue DMAS, allowing at least a partial conversion of DMA^{V} and MA^{V} and stabilising more labile species like DMAS. However, the results from boiling different sulphur compounds with DMA^{V} show that it is not the sulphur-content itself but the kind of organosulphur species that is crucial to the formation of those thioarsenicals in the extract. The boiling of spiked hair extract led to similar changes in the oxidation state, so that it can be assumed that the oxidation states of the arsenic species in the extract are more dependent on the extraction procedure than the oxidation state in the hair itself. In the light of these results, only the occurrence of inorganic arsenicals and the degree of methylation of the accumulated arsenic species in the hair can be deduced from the speciation analysis of hair extracts. The speciation of arsenic in the hair itself can only be determined by direct speciation methods such as XANES and EXAFS. However, the hair/nail sample extracts from people who were exposed to an excess of arsenic in their drinking water showed an enormous variability in the species distribution. Whether these variations reflect differences in the methylation rate of inorganic arsenic or differences in the external accumulation of inorganic arsenic (possibly due to washing hair in water contaminated with arsenic) remains to be seen. Future studies should therefore focus

on the potential of arsenic speciation in keratinous tissue to differentiate between internal and external accumulation of arsenic.

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