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Arsenic species excretion after controlled seafood consumption

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Abstract

Influence of controlled consumption of marine fish on the urinary excretion of arsenite, arsenate, dimethylarsinic and monomethylarsonic acid (DMA, MMA) was investigated in two experiments. Arsenic species were separated by anion-exchange chromatography and detected with hydride-technique atomic absorption spectrometry (detection limit 1, 10, 2, 2 μg/l). Firstly, 13 probands ate different types of seafood after having refrained from any seafood for 1 week. DMA levels rose from 3.4±1.3 μg/g creatinine (n=12; a day before seafood) to a mean peak level of 28.2±20.6 μg/g (n=13; 10–23 h after; P<0.001; max. 77.7 μg/g). No other species were excreted before the meal, but small amounts of arsenite (8.5% positive; max. 1.7 μg/g) and MMA (1.2%; 1.6 μg/g) within 2 days after it (n=82). Consumption of white herring caused the highest DMA levels. Secondly, eight probands ingested white herring (dose 3.5 g/kg; DMA content 32.1±15.3 ng/g wet weight; n=36). No arsenite, arsenate and MMA was found in the urine or in the herring tissues. The mean DMA mass excreted after the meal (65.3±22.0 μg/24 h) was about 6-fold higher than the sum of base DMA excretion (3.0±1.7 μg/24 h) and the ingested DMA mass (7.9±2.7 μg). This indicates that the elevated DMA excretion after herring consumption is not caused by the metabolism of inorganic arsenic but of other arsenic species present in the fish tissue, e.g. arsenobetaine or fat-soluble arsenic species.

Keywords: Seafood consumption; Arsenic compounds

1. Introduction

Arsenic compounds differ distinctly in their acute and chronic toxicity data. Arsenic trioxide has been recognized as a human carcinogen and has a long history as a classical poison because of its high acute toxicity. Industrial exposure to arsenic occurs in the smelting of non-ferrous metals, in arsenic refining, in the glass manufacturing and semiconductor industry among others [1]. Inorganic arsenic oxides are the species of main importance at these work places and inhalation is the predominant route of absorption. For the general population, seafood consumption is the main source of arsenic exposure. In Germany, the daily arsenic intake has been estimated to 0.99 μg/kg body mass in a duplicate diet study [2] and this was mainly caused by fish consumption. Arsenobetaine is the main constituent of marine fish but it is considered as almost non-toxic [3] and not to undergo any metabolism within the human body. Environmental daily intake by inhalation and drinking water was at least one order of magnitude lower (below 0.6 and <10 μg, respectively, for Germany [4]) but water may be a major source of exposure in some regions (e.g. in Argentina) [5,6]. However,

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inorganic arsenic contributes for the greatest part of the arsenic amount in air and water.

Because of this not so simple pattern of different arsenic species, toxicities and exposure routes that are involved, analytical attempts to characterize and quantify the relevant arsenic species independently have started very early. For the field of occupational biomonitoring, the commonly applied analytical procedure within the medical surveillance of workers is based on the direct reaction of arsenic species present in the urine with sodium borohydride with subsequent thermal decomposition of the arsines and atomic absorption spectrometric detection [7]. Because arsenobetaine does not form a volatile arsine, it will not contribute to the analytical results with the hydride technique AAS – in contrast to arsenite, arsenate, dimethylarsinic and monomethylarsonic acid (DMA, MMA). By means of that, the influence of seafood intake seemed to be overcome. But many investigations, e.g. the nation-wide German Environmental Survey demonstrated clearly that consumption of marine fish led to a significant higher arsenic excretion measured by hydride-technique AAS [8]. At this point, it seemed to be necessary to quantify the arsenic species in urine independently and to explore the species pattern after intake of seafood. Therefore, we developed a reliable, robust and sensitive procedure for arsenite, arsenate, DMA and MMA in urine. It is based on the experience of many applications for arsenic species analysis in body fluids [9–14]. The species are submitted to anion-exchange chromatography, online converted to volatile hydrides with flow-injection analysis and detected by AAS. With this analytical methodology, two experiments with volunteers and controlled intake of commercially available seafood were performed. Arsenic species present in seafood were quantified and compared to the species and amount excreted in the urine after the seafood consumption.

2. Experimental

2.1. Study design

2.1.1. Seafood experiment

Thirteen apparently healthy probands (A–M; 11 nonsmokers; 47.2±13.4 years) agreed voluntarily to refrain from any seafood consumption during 14 days except for the eighth day of the experiment when each person had one meal of seafood. Type and mass of the seafood meal was chosen by each person independently. Urine samples, voided on the day before the seafood meal ($n=12$) and up to 48 h after the meal ($n=82$), were collected and stored at −18°C until subsequent analysis of arsenic species and creatinine. Time of urine voiding was recorded. Personal data, time, type and mass of the seafood meal were given in a questionnaire.

2.1.2. White herring experiment

Eight apparently healthy probands (1–8; six nonsmokers) agreed voluntarily to refrain from any seafood consumption during 11 days except for the 8th day of the experiment when each person had a lunch of white herring with a standardized dose of 3.5 g white herring/kg body mass. Herring specimens ($n=36$) were bought in five different local shops. Urine samples were collected as timed 8-h-specimens on the day before and after the lunch up to day 11 and stored at −18°C until subsequent analysis of arsenic species and creatinine. Urine volume of each specimen was recorded and it was used – together with the species concentration in the specimen – for calculation of the excreted DMA amount during the specimen collection period. Personal data were given in a questionnaire. One aliquote of each specimen of white herring ($n=36$) eaten by the probands was separated and stored deep-frozen until subsequent analysis of arsenic species. For each proband, it was recorded which herring specimens and what mass he or she ingested. These data (together with the arsenic species concentration in the white herring specimens) were used for calculation of the ingested DMA mass. Samples of tap-water in the private households of the probands were collected and analyzed for arsenic by hydride-technique flow-injection AAS ($n=8$; <1 μg/l).

2.2. Conditions

2.2.1. Chemicals

Sodium arsenite stock solution (1 g/l, Titrisol, Merck, Darmstadt, Germany), sodium arsenate stock solution (1 g/l, Baker, Phillipsburg, USA), sodium

salt of dimethylarsinic acid trihydrate (>97%, Merck) and monomethylarsonic acid (98%, Tri Chemical Lab., Japan) were used to prepare aqueous solutions containing between 5 and 150 μg/l arsenic for each of the four stated arsenic species. Sodium borohydride (p.a., Fluka), hydrochloric acid (30%, ultrapure, Merck) and sodium hydroxide (ultrapure, Merck) were used to prepare reducing and acidifying solutions for the flow-injection system. Disodium hydrogenphosphate dihydrate and sodium dihydrogenphosphate monohydrate (p.a., Merck) were reagents for preparing the mobile phase in the anion-exchange chromatography. Argon (99.999%, Gerling, Germany) was the carrier gas in the FIAS system.

2.2.2. Equipment

The high-performance liquid chromatography (HPLC) system consisted of a Merck L-6000 solvent delivery system and a Rheodyne 7125 six-port injection valve (Rheodyne, Cotati, USA) with a 100 μl PEEK (poly-ether-ether-ketone) injection loop. The outlet of the HPLC-column was connected via a PEEK capillary to the sample inlet of a Perkin-Elmer FIAS 400 flow injection system. A Perkin-Elmer atomic absorption spectrometer AAS 2100 (detection wavelength 193.7 nm, spectral bandpass 0.7 nm) with an electrodeless discharge lamp (EDL 2 power supply, Perkin-Elmer, lamp current 350 mA) and a quartz cuvette (cell temperature 900°C) was used for arsenic species detection. For quantification of the AAS absorbance signals, a Spectra Physics SP 4270 integrator recorded peak heights. For extraction of the herring tissues, a Sonorex RK 255 sonificator (Bandelin, Berlin, Germany; HF frequency 35 Hz, HF power 250/500 W) was used. All aqueous analytical solution were prepared with water purified in a Seralpure Pro 2C apparatus (0.055 μS cm^{-1}).

2.2.3. Column for chromatographic separation of arsenic species

Anion-exchange colum PRP-X100 (Hamilton, Reno, USA; 10 μm, 250 mm length, 4.1 mm I.D., trimethylammonium polystyrene–divinylbenzene copolymer, column capacity 0.19 meq/g [according to the manufacturer]) with guard column (25 mm length, 2.3 mm I.D.).

2.2.4. Mobile phase

At this stage, 8 mmol of disodium hydrogenphosphate dihydrate and 8 mmol of sodium dihydrogenphosphate monohydrate were dissolved in 1 l of water and the pH was adjusted to 6.0 with phosphoric acid.

2.2.5. Sample treatment for the quanitification of arsenic species in urine

A 2 ml aliquote of the urine specimen (acidified with glacial acetic acid, 1/100 v/v) was diluted 1+1 with the mobile phase and was submitted to membrane filtration (0.45 μm; Chromafil). Then, 100 μl of the diluted and filtered urine was injected into the HPLC system and submitted to anion-exchange chromatography in the isocratic mode at a flow-rate of 2.4 ml/min (room temperature). The HPLC eluate containing the separated arsenic species was online coupled to the FIAS apparatus where sodium borohydride solution (0.5% w/w in 0.05% w/w sodium hydroxide) and hydrochloric acid (4.5% w/w) were added for reduction and generation of volatile arsines. These gaseous arsines were separated from the liquid in the gas–liquid separator of the FIAS system by a stream of argon (50 ml/min) and transferred to the quartz cuvette of the atomic absorption spectrometer for detection.

2.2.6. Sample preparation for white herring tissue and tuna fish reference material

The edible part of the white herring specimen was sliced into small pieces with a disposable plastic knife. The combined pieces were ground to a pasteous mass in a motar with a pestle. To an aliquote of 5 g (wet weight) of the herring tissue or 1 g of the lyophilized tuna fish powder, 10 ml of methanol (90%) was added. After short manual mixing of tissue and methanol, extraction was performed in the sonicator for 15 min. After centrifugation and separation of the supernatant, the residual was again extracted with 10 ml methanol, centrifugated and the supernatant combined with the first extract. This procedure was repeated three further times. All five supernatants were combined and concentrated to a residual volume of approximately 5 ml in a rotatory evaporator under reduced pressure (bath temperature 25°C). The residual volume was transferred quantitatively to a 10-ml flask and made up to the mark with

water. This solution was submitted to the arsenic species determination as described for the urine specimens.

2.2.7. Calibration of the arsenic species determination

Pooled urine (acidified with glacial acetic acid, 1/100 v/v and filtered by a cellulose membrane filter) of persons having refrained from eating seafood for several days and having no occupational exposure to arsenic compounds was spiked with the arsenic species arsenite, arsenate, DMA and MMA (as aqueous standards) to prepare urine standards in the concentration range of 5–150 μg/l. These urine standards were submitted to the same treatment and analysis as described for the urine specimens. The recorded peak heights were plotted against the spiked concentrations to calibration lines (if base levels of species, especially DMA, were found, these have to be taken into account by substraction).

For the calibration of the herring extracts, methanolic standards (90%) of the arsenic species were prepared and submitted to sample treatment and analysis as described for the herring tissues. In about 10% of the herring extracts, standard addition was also applied for calibration leading to results with only minor deviations (max. 5.4%) compared to those with the methanolic standards.

2.2.8. Creatinine

Creatinine was analyzed photometrically after several enzymatically controlled reactions by a commercial kit (Kreatinin PAP Nr. 839434, Boehringer, Ingelheim, Germany). All urine concentrations were corrected for creatinine to adjust for fluctuations in urinary volume.

2.2.9. Quality assessment

Each analytical series consisted of one water blank (in case of herring tissue: methanol, 90%) for contamination control, at least four spiked urine (or methanolic, 90%) standards for calibration, up to ten urine or herring tissue specimens for analysis and, for internal quality control purposes, at least one within-lab-prepared urine or herring tissue control sample. The analytical levels of the quality control specimens were used for calculation of imprecision and recovery rates. Commercially available urine control materials (Lyphocheck 69041, Biorad, Hercules, USA; ClinChek 750, Recipe, Munich, Germany) and urine specimens of the Intercomparison Program of the Institute of Toxicology in Québec, Canada, were analyzed for accuracy testing. External quality assessment for urine was performed by participation in the year 2000 run of the German Quality Assessment Scheme (Institute for Occupational, Social and Environmental Medicine, University of Erlangen/Nuremberg, Germany on behalf of the German Society of Occupational and Environmental Medicine). For the accuracy testing of herring tissue analysis, the CRM 627 tuna fish tissue reference material with a certified DMA concentration was used.

3. Results

3.1. Analysis of arsenite, arsenate, DMA and MMA in urine and white herring specimens

With the described conditions of anion-exchange chromatography and applying hydride-technique atomic absorption spectrometry, four arsenic species that are to be expected in human urine and fish tissue (arsenite, arsenate, dimethylarsinic and monomethylarsonic acid, DMA, MMA) can be separated within 5 min. Examples of chromatograms are given in Fig. 1. With direct hydride-technique AAS detection, arsenobetaine, arsenocholine and according to Ref. [15], also arsenosugars and the tetramethyl arsonium ion will not be detectable.

For arsenic species in urine, anion-exchange chromatography appeared to be much less prone to matrix effects than reversed-phase chromatography which was also tested. The separating anion-exchange column PRP-X100 showed the best results for diluted urine compared to those of other suppliers (e.g. IonPac AS 14 by Dionex, Sunnyvale, USA; alkyl quarternary ammonium on a ethylvinylbenzene/divinylbenzene resin, 65 μeq/column). In combination with a phosphate buffer mobile phase, it has been chosen by several authors for the biological matrices urine [13], algae extracts [16], fish extracts [17] either with AAS or ICP-MS detection. For urine and aqueous standards, we investigated pH variation of the mobile phase between 4.5 and up to 8.0 and

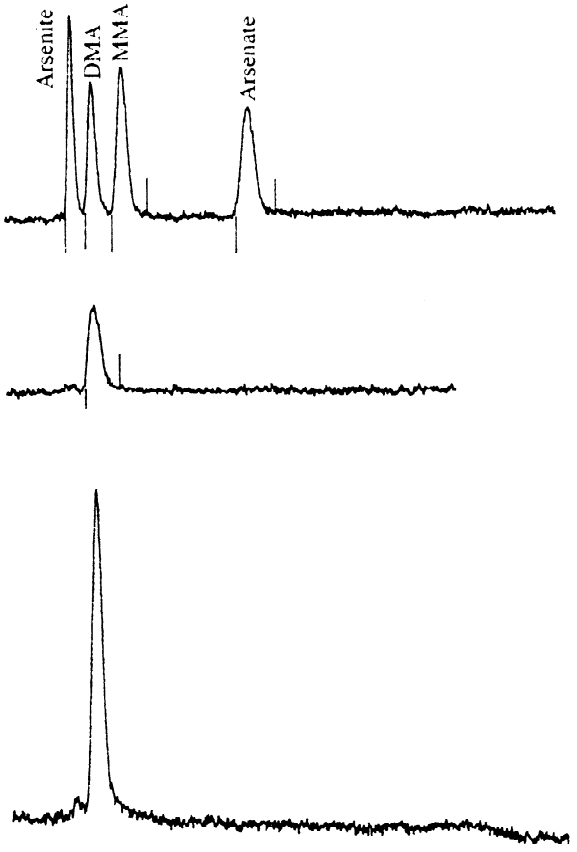

Fig. 1. Chromatograms of arsenite (retention time 1.18 min), DMA (1.55 min), MMA (2.10 min) and arsenate (4.42 min) in (top) a spiked urine standard (10/20/20/50 μg/l arsenite, DMA, MMA, arsenate); (middle) a methanolic extract of a native white herring tissue (28.6 ng/g DMA) and (bottom) a native urine specimen (arsenite 1.1 μg/l, DMA 73.5 μg/l).

found minimal effects for arsenite retention, moderate increase in retention times for DMA but a decrease in retention for arsenate (best choice for urine: pH 6.0–7.0). Molarity of the di/monohydrogenphosphates in the mobile phase was tested between 6 and up to 16 m*M* at pH 7 with no effect on arsenite, a small decrease in DMA retention and a distinct decrease in arsenate retention (best choice for urine: 14–16 m*M*). Column temperature (20–40°C) showed minimal influence on the chromatographic behaviour of the arsenic species. Similar optimization results have been found by Refs. [13,16,17].

Calibration lines turned out to be linear for urine and aqueous standards (Fig. 2). With peak height calculation, the slopes of the urine calibration standards for DMA and MMA were about 20% lower compared to water indicating minor matrix effects that are easily compensated by the calibration with urine standards. We observed an excellent long-term stability of the calibration slopes (variation 16.0–18.2%; $n=26$–33 series within 13 months). These figures indicate the robustness of the analytical procedure.

Extraction of the fish tissue was based on repeated ultrasonic extraction with methanol/water as has been successfully applied for seafood [15], algae [16–18] and in the certification of the tuna fish tissue CRM 627 [19].

Figures of merits of the analytical procedures are summarized in Tables 1 and 2. Recovery experiments for urine revealed recovery rates between 90.3 and 104.8%. Between-day imprecision ranged between 6.7 and 10.2% ($n=30$, urine) and 7.2%, respectively (DMA; $n=10$; fish tissue).

For accuracy testing of arsenic species in urine, no certified reference materials are available up to our knowledge. Because of that, we used control materials with target values for arsenic in urine (by direct hydride-technique AAS) and calculated the percentual deviation of the sum of the four species (Table 2; +7.1 to −8.0%). In the first German external quality assessment run including arsenic species in urine, our results deviated from the target values by −0.4 to +17.3% (with accepted deviations of ±32.7 to 41.4%) and we obtained positive certification for each analyte. Accuracy for the DMA analysis in fish tissue was proven by the good agreement between the certified level in CRM 627 (127–172 ng/g) and our result (149.8 ng/g).

3.2. Seafood experiment

Personal data of the 13 probands within this experiment, their inidividual and group-related results for arsenic species excretion in urine are summarized in Table 3. On average, the persons had 237±66 g of seafood (3.77±0.64 g/kg body mass). After 1 week of refraining from any seafood, DMA was the only arsenic species detected in specimens collected on the day before the seafood meal ($n=12$; 100% positive findings) with a mean level of

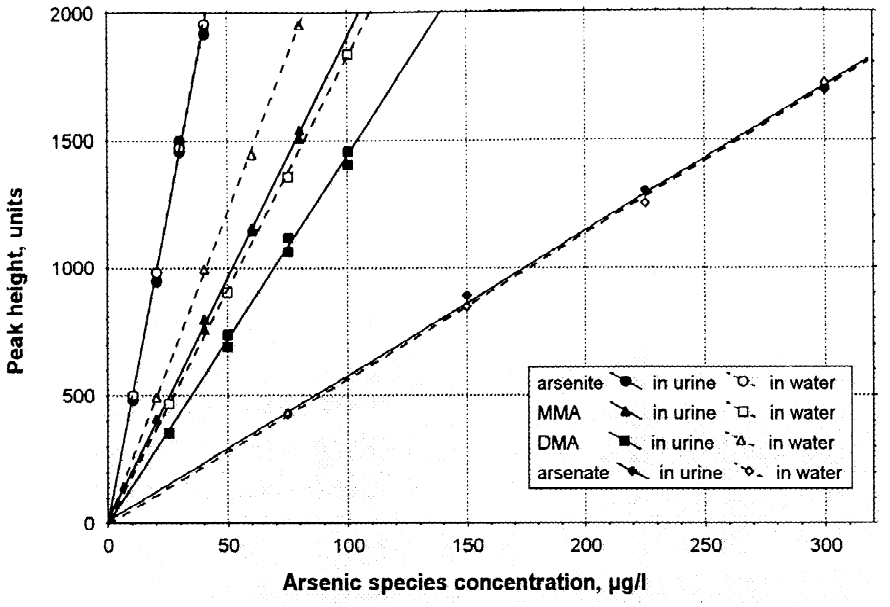

Fig. 2. Calibration lines for arsenite, DMA, MMA and arsenate in urine and water after anion-exchange separation and online hydride-technique atomic absorption spectrometric detection.

Table 1
Figures of merit for the analytical determination of four arsenic species in urine and DMA in fish tissue

Material	Species	No.	Concentration		Between-day imprecision (%)	Detection limit	Recovery rate (%)
			Target	Found			
			µg/l			µg/l	
Urine	Arsenite	30	n.d.[a]+10.0[b]	11.0	6.7	1.0	104.8[c]
	Arsenate	30	n.d.+50.0[b]	50.0	10.2	10.0	90.9[c]
	DMA	30	3.8+20.0[b]	23.0	8.3	2.0	96.6[c]
	MMA	30	n.d.+20.0[b]	20.8	10.1	2.0	99.0[c]
			ng/g			ng/g	
Herring tissue	Arsenite	10	–	n.d.	–	2.0[d]	–
	Arsenate	10	–	n.d.	–	20.0[d]	–
	DMA	10	–	10.1[d]	7.2	4.0[d]	–
	MMA	10	–	n.d.	–	4.0[d]	–
CRM 627 tuna fish tissue	DMA	10	149.8[e]	140.0[e]	7.2	–	93.3
	MMA, arsenite, arsenate		not certified	n.d.	–	–	–

[a] n.d. not detected.
[b] Given as sum of native and spiked concentration.
[c] Basic levels (or half of detection limit if n.d.) taken into account.
[d] Wet weight.
[e] Dry weight.

Table 2
Accuracy of arsenite, arsenate, DMA and MMA determination in urine

Urine control material	Found concentration (μg/l)					Target value (μg/l)	Deviation[a] (%)
	Arsenite	DMA	MMA	Arsenate	Σ Species		
Lyphocheck 69041	n.d.	3.3	n.d.	53.1	56.4	54.0	4.4
ClinChek 1/750	n.d.	46.9	n.d.	19.4	66.3	68.5	−3.2
ClinChek 2/750	n.d.	85.3	n.d.	18.6	103.9	110.8	−6.2
S-97-10[b]	47.3	n.d.	n.d.	15.9	63.2	67.4	−6.2
S-97-16	n.d.	73.0	n.d.	n.d.	73.0	74.9	−2.5
S-98-15	n.d.	107.1	n.d.	n.d.	107.1	108.6	−1.4
S-99-05	22.8	4.1	n.d.	n.d.	26.9	26.2	2.7
S-99-06	31.6	85.7	17.0	n.d.	134.3	134.9	−0.4
S-99-08	n.d.	142.8	n.d.	n.d.	142.8	142.4	0.3
S-00-01	2.7	28.9	3.0	n.d.	34.5	37.5	−8.0
S-00-03	18.4	2.5	n.d.	16.1	37.0	37.5	−1.3
S-00-07	10.0	2.0	n.d.	n.d.	12.0	11.2	7.1
S-00-09	45.6	118.8	12.5	22.3	199.2	187.3	6.4
Run 2000/25 2A[c]	7.9					8.0	−1.0
		19.8				16.9	17.3
			4.4			4.5	−0.4
				11.7		11.5	2.0
Run 2000/25 2B	13.0					12.5	3.9
		36.7				32.7	12.3
			11.9			11.8	0.5
				13.2		13.0	2.2

[a] Percentage of deviation between found and target values.
[b] Specimen of the Intercomparison Program (Institute of Toxicology, Québec, Canada).
[c] Specimen of the German External Quality Assessment Scheme.

3.4±1.3 μg/g creatinine (range 1.3–5.2 μg/g creatinine). One single meal of seafood caused on average a 6-fold rise in the DMA concentration in all 82 urine specimens collected during 48 h after the meal to a mean of 18.6±16.9 μg/g ($P<0.05$). In addition, arsenite and MMA were found in 8.5 and 1.2% of these specimens after the meal at low concentrations not exceeding 1.7 and 1.6 μg/g. No arsenate was found in any specimen. The individual peak excretion of DMA occurred within 1 day (mean: 14.7±4.6 h; range 10.0–23.0 h) and was on average about 8-fold higher than the baseline level ($n=13$; 28.2±20.6 μg/g; $P<0.001$).

The types of seafood chosen by the probands are somehow related to their German market percentage of commercial fish: Herring (four probands) is the most popular fish type in Germany and accounts for about 23% of the fish offered for sale in 1999, followed by pollock (1 proband; 20%), cod (2 probands; 7%), salmon (2 probands; 10%) and Ocean perch (1 proband; 6%) [20].

In Fig. 3, examples are given for the time-course of the DMA concentration in the probands A, B, J with a meal of salmon, Ocean perch and white herring, respectively. While the time course was quite similar for the probands, the amount of the DMA increase differed distinctly: it was less than 6-fold for Ocean perch and salmon whereas white herring led to the most pronounced increase in DMA excretion of about 20-fold.

3.3. White herring experiment

DMA was the only arsenic species in all urine specimens before ($n=32$) and after ($n=88$) the herring lunch. The DMA base level (after 1 week of refraining from any seafood; −32 to 0 h before lunch; $n=32$) was 2.7±2.0 μg/g creatinine with a maximum level at 6.6 μg/g. This baseline DMA excretion (as well as that of 3.4±1.3 μg/g we found in the seafood experiment) is in good agreement with 3.5±3.6 μg/g what we found for 42 persons (out of the total reference group of 101 males) who had no seafood within the last 6 days [30]. In the same

Table 3
Seafood experiment: personal data, mass and type of ingested seafood and individual DMA excretion before and maximum DMA excretion after controlled seafood consumption of 13 probands

Anamnestic data of probands				Ingested seafood Mass and type	DMA excretion in urine		
					Before intake of seafood	Maximum after intake of seafood	
No.	Sex	Age (years)	Body mass (kg)		Concentration (μg/g creatinine)		Time after meal (h)
A	female	46	52	200 g salmon	3.5	17.7[a]	18.8
B	female	34	63	200 g Ocean perch	1.3/1.5	11.6	17.7
C	female	49	42	180 g pike-perch	3.0/5.2/4.6	21.3[a]	17.1
D	female	61	66	280 g *Lengfisch*	4.3	8.5	23.0
E	female	36	60	200 g white herring	2.4	31.7	17.0
F	female	44	57	270 g plaice, white herring	–	50.8[a]	23.0
G	female	67	68	300 g plaice, white herring	–	50.6	23.0
H	female	31	65	200 g pike-perch	3.9	27.0	12.5
I	female	26	59	200 g pollock	–	16.1	10.0
J	male	51	94	400 g herring, white herring	4.6	77.7	10.1
K	male	49	65	250 g salmon	–	18.7	15.0
L	female	55	60	150 g cod	4.1/2.9	8.9	20.0
M	female	29	65	250 g cod	–	22.6[a,b]	11.0

[a] Arsenite was detected at 0.57/0.45 μg/g (proband A); 1.69 μg/g (C); 0.76 μg/g (F); 0.71/0.70/0.47 μg/g (M) in one to three urine specimens after the fish meal.
[b] MMA was detected (1.69 μg/g) in one of the specimens after seafood consumption. –, no specimen collected.

range are the DMA mean values reported by Apostoli [14] and Hakala [10] with a similar online chromatographic separation technique (6.8 and 8.7 μg/g; $n=39$ and 11).

As can be seen in Fig. 4, the DMA concentration started to increase in the first voiding (0–8 h) after the lunch and reached its maximum level in the sampling period 8–16 h after the meal for each of the eight probands (78.9±28.2 μg/g; maximum 134.9 μg/g; $n=8$; $P<0.001$ vs. base level). Even the last specimens (80–88 h after lunch) showed sig-

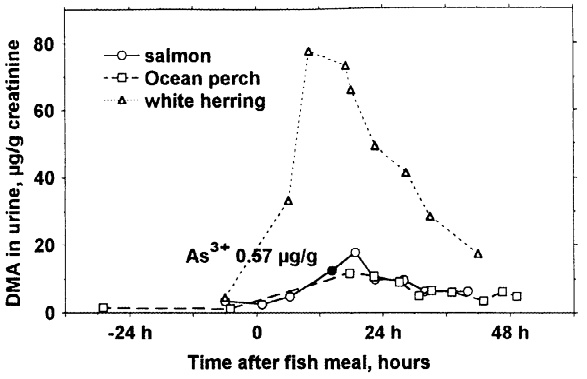

Fig. 3. Time course of DMA excretion in three probands (A, B and J) before and after one single meal of salmon, Ocean perch and white herring, respectively (after 1 week of refraining from any seafood). No MMA, arsenite or arsenate was found in any of these specimens except for 0.57 μg/g arsenite in the marked specimen of proband A.

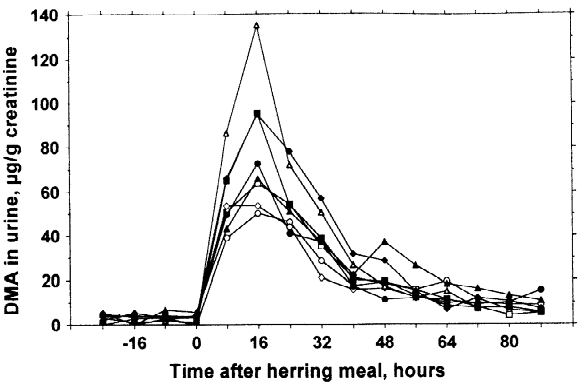

Fig. 4. Time course of DMA excretion in eight probands before and after one single meal of white herring (after 1 week of refraining from any seafood). No arsenite, arsenate or MMA was detected in any of these specimens.

Table 4
White herring experiment: personal data. ingested mass of white herring, ingested DMA mass with the meal and excreted masses of DMA before, within 24 and 48 h after white herring ingestion

Anamnestic data of probands (n=8)				DMA mass (μg)			
Sex	Age (years)	Body mass (kg)	Mass of meal (g)	Ingested with meal at 0 h	Excreted in urine		
					24–0 h before meal	0–24 h after meal	0–48 h after meal
2 males, 6 females	47±13	68±14	237±50	7.9±2.7	3.0±1.7	65.3±22.0	94.3±24. 9

nificantly increased DMA levels compared to the base level (8.4±3.8 μg/g; maximum 15.6 μg/g; n=7; P<0.01).

In the aliquotes of the white herring specimen eaten by the probands, no arsenate or MMA was detected. Arsenite might be seen qualitatively in about 25% of the specimens but it was below the detection limit of 2 ng/g wet weight. DMA ranged between 13.8 and 73.9 ng/g wet weight (mean±SD: 32.1±15.3 ng/g; n=36). For each proband, the ingested amounts of DMA and the excreted DMA masses were calculated (Table 4). On average, the sum of the ingested amount of DMA and the base excretion was at about 11 μg, whereas the mean excreted DMA mass in the first 24 h after the lunch was at about the 6-fold level. That means that the DMA increase in urinary excretion after a herring meal is only to a small extent due to the native DMA level of the seafood (ppb level). By far the larger part must have been caused by metabolic DMA formation in the human body from other arsenic species in seafood. These species are certainly not inorganic arsenic (that we were unable to detect in the herring extracts).

4. Discussion

The predominant arsenic species in most marine fish is arsenobetaine (a trimethylated arsenic species; $(CH_3)_3As^+CH_2COO^-$). It accounts for 97.6–110.0% of total arsenic for cod, herring, plaice and other marine fish species as Ballin et al. [21] found with a gas chromatographic method specific for arsenobetaine. Total arsenic in these fish was mainly in the range of 1–10 μg/g wet weight. Additionally, fat-soluble arsenic species were detected but not characterized between 0.17% (plaice) and a maximum of 4.1% (herring which is a fat fish with about 15% fat content).

Besides the high arsenobetaine content at a ppm level, marine fish also contain small amounts of DMA. For mackerel, herring and crab, this has been demonstrated qualitatively by Arbouine and Wilson [22]. DMA at 0.4 mg/kg dry weight had be measured by Branch et al. [23] in mackerel (a fat fish as herring is) but for six other marine fish species, DMA ranged below their detection limit of 0.3 mg/kg (ppm). For prawns, Yamauchi and Yamamura [24] found inorganic arsenic (0.96% of total arsenic) and DMA (0.14%) beside the trimethylated main compound (98.8%). In our white herring tissues, DMA was quantified at a ng/g (ppb) level but no MMA, arsenite or arsenate were found. Our mean DMA level (32.1 ng/g wet weight) is somewhat lower but in a comparable range to that reported for mackerel [23].

Marine seafood consumption leads to a higher arsenic excretion in urine – that has been demonstrated by numerous investigations either by direct hydride generation AAS (which accounts more or less for the sum of inorganic arsenic besides MMA and DMA) [25–28], or mainly for DMA by species separation techniques such as cryo-trapping of arsines [24], gas or liquid chromatography and hydride-technique AAS [10,22,29,30]. As arsenobetaine cannot be detected with hydride-technique AAS it has to be concluded that this increase in arsenic excretion is due to hydride-forming arsenic species present in the seafood or due to a metabolic degradation of arsenic species in the seafood (e.g. arsenobetaine) in the human body to hydride-forming arsenic species. We showed for controlled intake of different species of marine fish and especially for

white herring that DMA is the predominant excreted species (besides arsenite and MMA on a distinctly lower level). A remarkable influence of the type of fish was observed for the height of the DMA increase. In excellent agreement with the results of Arbouine and Wilson [22], we found herring to be the fish that caused the highest DMA level.

DMA taken orally by humans is not demethylated (to MMA or inorganic arsenic) but is excreted unchanged [31]. DMA is also a main metabolite of arsenite and MMA [31] but these species could not be found in the herring tissues. Arsenosugars have been reported to be metabolised to DMA in humans [32] but marine fish (in contrast to algae) do not contain these species [16,21]. For arsenobetaine, several animal studies gave no evidence for a metabolic alteration [33,34]. In man, arsenobetaine present in prawns (98.8%) was excreted in urine at 87–89% without metabolic transformation while 3–5% appeared in the urine as DMA and MMA besides inorganic arsenic [24] which was interpreted as a partly demethylation of arsenobetaine in the human body. In another human experiment, arsenobetaine in plaice (95%) was excreted in urine at 69–85% and no traces of inorganic arsenic, DMA or MMA could be found with a microwave-induced plasma detector (detection limits not given) [35]. In a study of controlled seafood intake in volunteers [36] DMA in urine increased without any concomitant changes of inorganic arsenic and MMA. Again, a decomposition of organoarsenicals in the seafood was assumed.

So based on our results it seems to be quite convincing that, to a certain extent, organoarsenicals as arsenobetaine or fat-soluble arsenicals might be metabolized to DMA that is excreted in urine after seafood consumption. Taking this hypothesis as given, a DMA increase after fish consumption has to be interpreted in a different way compared to a DMA excretion after occupational exposure to inorganic arsenic: Arsenobetaine is considered as almost untoxic (LD_{50} exceeding 5 g/kg [3]) while tri- and pentavalent inorganic arsenic compounds are classified human carcinogens of high acute toxicity. DMA formation as a metabolite of inorganic arsenic is clearly a detoxification process (less acute toxicity, no carcinogenicity) but this must not be the case for DMA as a potential metabolite of arsenobetaine or not well-characterized fat-soluble arsenicals. We conclude that neither DMA in urine nor the sum of inorganic arsenic and its methylated metabolites adequately reflect the amount of inorganic arsenic (which is related to the risk for cancer) taken up by exposed persons in the case of simultaneous seafood consumption. For an occupational exposure to inorganic arsenic, biomonitoring based on the arsenite excretion in urine, as proposed by Apostoli et al. [14], might be a better choice. However, further biomonitoring field studies applying arsenic species quantification are required to confirm this hypothesis.

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References

[1] A. Aitio, E. Hakala, L. Pyy, in: World Health Organization (Ed.). Biological Monitoring of Chemical Exposure in the Workplace, Vol. 2, 1996, p. 18.
[2] K. Becker, P. Nöllke, E. Hermann-Kunz, C. Krause, D. Schenker, C. Schulz. Umwelt-Survey 1990/91. WaBoLu 3/96, Berlin, 1996.
[3] International Programme on Chemical Safety. Environmental Health Critic 18: Arsenic. Geneva, 1981.
[4] LAI, Länderausschuss für Immissionsschutz. Krebsrisiko durch Luftverunreinigungen. Ministerium für Umwelt, Raumordnung und Landwirtschaft des Landes Nordrhein-Westfahlen. Düsseldorf, 1992.
[5] N. Ishinishi, K. Tsuchiya, M. Vahter, B.A. Fowler, in: L. Friberg, G. Nordber, V.B. Vouk (Eds.), Handbook on the Toxicology of Metals, Vol. 2, Elsevier, Amsterdam, 1986, p. 43.
[6] G. Concha, B. Nermell, M. Vahter, Environm. Health Persp. 106 (1998) 355.
[7] DFG German Science Foundation. Analyses of Hazardous Substances in Biological Materials, in: J. Angerer, K.H. Schaller (Eds.), Wiley–VCH Verlag, Weinheim, Vol. 3, 1991, p. 63.
[8] C. Krause, W. Babisch, K. Becker, W. Bernigau, D. Helm, K. Hoffmann, P. Nöllke, C. Schulz, R. Schwabe, M. Seiwert, W. Thefeld, Umwelt-Survey 1990/92. WaBoLu-Heft 1/96 Berlin, 1996.
[9] B. Chana, N.J. Smith, Anal. Chim. Acta 197 (1987) 177.

[10] E. Hakala, L. Pyy, J. Anal. At. Spectr. 7 (1992) 191.
[11] X. Zhang, R. Cornelis, J. de Kimpe, L. Mees, J. Anal. At. Spectr. 11 (1996) 1075.
[12] J. Lintschinger, P. Schramel, A. Hatalak-Rauscher, I. Wendler, B. Michaelke, Fresenius J. Anal. Chem. 362 (1998) 313.
[13] R. Sur, J. Begerow, L. Dunemann, Fresenius J. Anal. Chem. 363 (1999) 526.
[14] P. Apostoli, D. Bartoli, L. Alessio, J.P. Buchet, Occup. Environm. Med. 56 (1999) 825.
[15] X.-C. Le, W.R. Cullen, K.J. Reimer, Clin. Chem. 40 (1994) 617.
[16] G. Raber, K.A. Francesconi, K.J. Irgolic, W. Goessler, Fresenius J. Anal. Chem. 367 (2000) 181.
[17] K. Falk, Berichte des Forschungszentrums Jülich 3695, D-465 Dissertation Universität-GH-Essen, 1999.
[18] J. Feldmann, K. John, P. Pengprecha, Fresenius J. Anal. Chem. 368 (2000) 116.
[19] F. Lagarde, M.B. Amran, M.J.F. Leroy, C. Demesmay, M. Ollé, A. Lamotte, H. Muntau, P. Michel, P. Thomas, S. Caroli, E. Larsen, P. Bonner, G. Rauret, M. Foulkes, A. Howard, B. Griepink, E.A. Maier, Fresenius J. Anal. Chem. 363 (1999) 18.
[20] Deutsches Fisch-Archiv, Daten und Fakten. Hamburg, 2000.
[21] U. Ballin, R. Kruse, H.-A. Rüssel, Fresenisus J. Anal. Chem. 350 (1994) 54.
[22] M.W. Arbouine, H.K. Wilson, J. Trace Elem. Electrolytes Health Dis. 6 (1992) 153.
[23] S. Branch, L. Ebdon, P. O'Neill, J. Anal. At. Spectrom. 9 (1994) 33.
[24] H. Yamauchi, Y. Yamamura, Bull. Environ. Contam. Toxicol. 32 (1984) 682.
[25] M. Vahter, B. Lind, Sci. Total Environ. 54 (1986) 1.
[26] S. Valkonen, J. Jarvisalo, A. Aitio, in: P. Brätter, P. Schramel (Eds.), Trace Element Analytical Chemistry in Medicine and Biology, Berlin, Walter de Gruyter, 1983, Vol. 2, p. 611.
[27] R. Heinrich-Ramm, R. Wegner, S. Mindt-Prüfert, D. Szadkowski, in: Ph. Collery, P. Brätter (Eds.), Metal Ions in Biology of Medicine, Vol. 5, 1998, 753, J. Libbey Eurotext, Paris.
[28] J.P. Buchet, J. Pauwels, R. Lauwereys, Environ. Res. 66 (1994) 44.
[29] J.-B. Luten, G. Riekwel-Booy, in: P. Brätter, P. Schramel (Eds.), Trace Element Analytical Chemistry in Medicine and Biology, Berlin, 1988, Vol. 2, p. 277.
[30] R. Heinrich-Ramm. S. Mindt-Prüfert, D. Szadkowski, Int. J. Hyg. Enviorn. Health (2000) submitted.
[31] J.P. Buchet, R. Lauwereys, H. Roels, Int. Arch. Occup. Environ. Health 48 (1981) 71.
[32] X.-C. Le, W.R. Cullen, K.J. Reimer, Clin. Chem. 40 (1994) 617.
[33] J.R. Cannon, J.B. Saunders, R.F. Toia, Sci. Total Environ. 31 (1983) 181.
[34] M. Vahter, E. Marafante, L. Dencker, Sci. Total Environ. 30 (1983) 197.
[35] J.B. Luten, G. Riekwel-Booy, A. Rauchbaar, Environ. Health Perspect. 45 (1982) 165.
[36] J.P. Buchet, D. Lison, M. Ruggeri, V. Foa, G. Elia, Arch. Toxicol. 70 (1996) 773.

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