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Arsenic speciation in bodily fluids of harbor seals (*Phoca vitulina*) and harbor porpoises (*Phocoena phocoena*).

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Environmental context. Marine mammals as top predators ingest a lot of arsenic from their food. In this study liver samples and body fluids of marine mammals from the North and Baltic Seas were investigated for arsenic speciation in order to get some information about their feeding habits. Only organic arsenic compounds with low toxicity were detected in all investigated samples. Although a high quantitative variability was observed, the same compounds were determined in all fluids examined.

Abstract. The total arsenic concentrations in whole blood of harbor seals (*Phoca vitulina*) and in liver tissues of harbor porpoises (*Phocoena phocoena*) were determined using TXRF (total-reflection X-ray fluorescence spectrometry). The total arsenic concentration found in whole blood was lower for fish-fed (58-80 µg As L^{-1}, median: 71 µg As L^{-1}) than for free ranging seals (46-780 µg As L^{-1}, median: 190 µg As L^{-1}). In porpoise liver the arsenic concentrations were higher from carcases found in the North Sea (217-899 µg As kg^{-1}, median: 421 µg As kg^{-1}) than from those inhabiting the Baltic Sea or found in the river Elbe (137-392 µg As L^{-1}, median: 250 µg As kg^{-1}).

Furthermore the total arsenic content and arsenic species in urine, plasma, and gastric juice of seals and the urine of porpoises, which were collected from animals at different areas in the North and Baltic Seas, were determined using ICPMS (inductively coupled plasma mass spectrometry). For speciation analysis HPLC (high performance liquid chromatography), running under three chromatographic conditions was coupled to the ICPMS. Arsenobetaine (AB) was the predominat arsenic species in all measured body fluids (seals blood: 17-430 µg As L^{-1}, median: 140 µg As L^{-1}; seals urine: 20-2100 µg As L^{-1}, median: 690 µg As L^{-1}; seals gastric juice: 450 -1100 µg As L^{-1}, median: 670 µg As L^{-1}; porpoise urine: 4.5-820 µg As L^{-1}, median: 140 µg As L^{-1}). Plasma samples of seals contained only one additional species: dimethylarsinic acid (DMA) (2.1 – 23 µg As L^{-1}, median 6.6 µg As L^{-1}). In gastric juice arsenocholine (AC) and trimethylarsine oxide (TMAO) were found with a median concentration of 20 µg As L^{-1} and 5 µg As L^{-1}, respectively. Several arsenic compounds were identified in mammal urine, the major being DMA (seals urine: 7-1600 µg As L^{-1}, median: 68 µg As L^{-1}; porpoise urine: 2-230 µg As L^{-1}, median: 89 µg As L^{-1}) and thio-DMA (seals urine: 15-900 µg As L^{-1}, median: 54 µg As L^{-1}; porpoise urine: 5-400 µg As L^{-1}, median: 36 µg As L^{-1}) but high variability was observed in the relative proportions of each. After oxidation of porpoise urines significantly more DMA was detectable but the corresponding thio-DMA could not be found. From this we can speculate that arsenic is bound as DMA(III) to proteins.

Additional keywords: arsenic speciation, marine mammals, urine, plasma, HPLC-ICPMS

• Introduction

Arsenic levels in sea water are quite constant over the world between a concentration range of 0.5 to 2 µg As L^{-1}. The arsenic is almost entirely present in inorganic forms.[1]

Marine organisms are known to accumulate and convert inorganic arsenic to organic arsenic compounds. Mammals such as porpoises and seals living in the marine environment and feeding exclusively on marine organisms are in an exceptional position. As top predators they could accumulate arsenic due to their position in the food web. Goessler et al., reported in 1997 the arsenic concentrations and speciation in a three organism marine food chain starting with a seaweed followed by two gastropods. They showed that arsenic was accumulated and metabolized within this food chain.[2] These results were in contrast to those of Kubota et al., who found lower arsenic concentrations in marine mammals (seals and porpoises) compared to those of lower tropic level marine organisms from other studies.[3] Furthermore Kubota et al. investigated a possible correlation between arsenic accumulation and gender, age (or body length), or feeding habits in liver tissues of 16 different marine mammal species. The determined arsenic concentrations varied widely among species and individuals ranging from 100 to 7680 µg kg^{-1} dry mass. Marine mammals feeding on fish contained lower arsenic concentrations compared to animals feeding on cephalopods and crustaceans but no trend with gender or age could be seen.[3]

Seals feed with regional and seasonal variations on fish, crustaceans, and cephalopods,[4] in contrast to harbor porpoises, which feed mainly on fish and less frequently on molluscs and squids.[5]

Arsenic contents in tissues of field-collected marine mammals, fish, and invertebrates have been reported in quantities ranges from less than 1000 up to 30000 µg As kg^{-1} dry mass.[6-7] Total arsenic content in whole blood of live harbor seals (*Phoca vitulina*) were determined by Griesel et al. They determined a median arsenic concentration of 174 µg L^{-1} (42 – 592 µg As L^{-1}, n=28).[8]

In fish the arsenic concentration is positively correlated to salinity. Fish from marine waters have arsenic levels up to ten times higher than fish from brackish waters, which themselves have levels up to ten times higher than fish from freshwater lakes and rivers. Differences in total arsenic concentration have been observed for fish taken from the North Sea and Baltic Waters.[9] A recent study by Kunito et al. summarized detailed information about arsenic in marine mammals.[10]

Seals from the North and Baltic Sea are suffering from several diseases. In 1988 and 2002 phocine distemper epidemics killed thousands of them.[11] It is believed that the living place and the industrial and human activities are causing an immunosuppression and as a consequence increased death rates. Understanding the feeding habits is valuable for a better understanding of the mass mortality. Speciation analysis of bodily fluids might help to explain their living circumstances, assuming that the arsenic speciation in bodily fluids reflects the diet.

The aim of our study was the determination of arsenic species in blood of seals to get some information about their feeding habits. At the starting point blood samples of seals living in different areas of the North

Sea and feeding on different prey as well as samples from animals living permanently in the Seal Centre Friedrichskoog were investigated. A clear correlation between feeding habits and arsenic speciation in blood could not be found, so the study was expanded to urine and gastric juice samples from carcases found along the German Wadden Sea coast. For comparison also urine samples of porpoises found dead in the same areas were investigated. Furthermore the arsenic pattern of liver tissue samples from porpoises of the North and Baltic Sea were determined.

• Results and Discussion

Liver tissue

Total arsenic concentrations in liver tissues of porpoises using TXRF were determined as shown in Table 1. Becker et al. reported 1997 a mean arsenic concentration of 340 ± 130 µg As kg^{-1} wet mass (wm) of six liver samples of harbor porpoises, from the North Atlantic.[12] Which is in between the obtained results for North Sea and Baltic Sea samples. The mean arsenic content of three liver samples of harbor porpoises collected in the Northwest Atlantic coast published by Tilbury et al., 580 ± 60 µg As kg^{-1} wet mass, is in a good agreement with our measured results for samples collected in the North Sea.[13] Kubota et al. determined the arsenic content of 14 animals from the Black Sea collected in 1993. They found a concentration range of 200 – 890 µg As kg^{-1} wet mass (dry mass concentration was converted to wet mass concentration assuming a moisture content of 70%).[3] The salinity of the North Sea and the Black Sea are similar as are the arsenic contents found.

Arsenic concentrations in liver of porpoises from the North Sea were significantly higher than in liver from those inhabiting the Baltic Sea and the river Elbe. No significant differences could be found between animals from the Baltic Sea and those from the river Elbe. These results are comparable with investigations on fish by Larsen & Francesconi 2003.[9] They found that fish from marine waters have arsenic levels up to ten times higher than fish from brackish waters, which have still higher levels than fish from freshwater and lakes.

Table 1.
Concentration of total arsenic in liver tissues of porpoises

Region	Number of samples	Range [µg As kg^{-1}wm]	Median [µg As kg^{-1}wm]
North Sea	n = 14	217 - 899	421
Baltic Sea	n = 8	193 - 380	256
River Elbe	n = 4	137 - 392	202

Seals blood and plasma

The total arsenic content in whole blood of three groups of seals, measured using TXRF, is shown in Table 2. The arsenic concentrations in blood of free ranging seals were significantly higher than those of seals living permanently in captivity. While the seals in the Seal Centre Friedrichskoog were fed only with pelagic herring, the seals caught at different areas in the North Sea were opportunistic, feeding on a variety of benthic organisms. Differences in total As content for benthis-feeding and pelagic-schooling species in the North Sea have been previously observed by Larsen et al.[14] They found that plaice (mean

7800 µg As kg^{-1} wm, n=18) contains higher As levels than mackerel (mean 1790 µg As kg^{-1} wm, n=20). From four pups plasma samples were measured when they were found a few days old and brought to the Seal Centre, and again after two months following rehabilitation and feeding on pelagic herring. It can be assumed that before the first blood collection the animals received only breastmilk from their mothers. The arsenic level decreased from a starting median concentration of 193 µg As L^{-1} to 128 µg As L^{-1}. For cetaceans a placental transfer of arsenic to fetus has been previously described.[15]

Table 2.
Concentration of total arsenic in whole blood of seals

Seals	Number of samples	Range [µg As L^{-1}]	Median [µg As L^{-1}]
Free ranging	n = 81	46 - 780	190
Pups	n = 22	49 - 227	99
Captivity	n = 8	58 - 80	71

Arsenic speciation of seals plasma is shown in Table 3. Arsenobetaine (AB) was the main fraction, no inorganic species were found.

Table 3.
Concentration range of total arsenic, AB, DMA, and AC in plasma of seals
nd, not detected

Collection	Number of samples	Age	Total As [µg As L^{-1}]	Total As Median [µg As L^{-1}]	AB [µg As L^{-1}]		DMA [µg As L^{-1}]		AC [µg As L^{-1}]
					Range	Median	Range	Median	
Frisko	n=5	all	65 - 7278	72	17 – 23	20	8.5 - 12	11	7 (n=1)
Lorenz	n=15	all	66 - 502	258	31 – 430	196	nd - 10.7	7.2	-
	n=10	Adults	66 - 352	227	31 – 300	187	nd - 10.7	6.8	-
	n=5	Juvenile[A]	265 - 502	396	196 – 430	310	7.2 - 9.6	7.4	-
Pups	n=4	few days	134 - 302	193	85 – 265	164	-	-	-
	same n=4	2 month	112 - 156	128	53 – 73	72	4 - 23	5.1	-
Romo	n=6	Male adult	73 - 203	160	42 - 161	116	2.5 - 8.8	4.8	-
Lorenz	n=6	Male adult	58 - 446	157	25 – 376	115	3.9 - 6.9	4.5	-
Helgoland	n=4	Male adult	109 - 170	147	73 – 126	101	2.1 - 6.6	4.8	-

[A] Animals not dependent on feeding but not grown up (< 1 year)

Several plasma samples were determined from the seals living in the Seal Centre Friedrichskoog. The blood of abandoned seal pups, which where freshly transferred to the station, contained only AB (85 – 265 µg As L^{-1}). After two months living in the Seal Centre the AB concentrations decreased (53 – 73 µg As L^{-1}) but DMA could now also be found in a concentration range of 4 – 23 µg As L^{-1}.

Just in one plasma sample of a seal living permanently in Friedrichskoog another arsenic compound, AC (~ 7 µg As L^{-1}), could be determined.

Gastric juice

From three seals gastric juice samples were available. Besides the identified arsenic species AB, AC, and TMAO three unknown arsenic compounds were also detected. Two out of these (U_1 and U_2) were retained on the applied cation-exchange column after 5.2 and 7.1 minutes respectively, both less than 5 µg As L^{-1}.The third species (U_3) was seen in all three samples using reversed-phase chromatography. U_3 eluted after 4.2 minutes in a concentration range of 10 –25 µg As L^{-1}. AB was present in a concentration range of 450 – 1100 µg As L^{-1}, whereas AC and TMAO were found in lower concentrations (AC: 10 – 40 µg As L^{-1}; TMAO: 4 – 5 µg As L^{-1}). Ebisuda et al. reported in 2002 arsenic speciation in the stomach contents of ringed seals (*Phoca hispida*). These results are in good agreement with ours; they found that AB (1920 µg As kg^{-1} dry mass) was also the major arsenic compound followed by TMAO (260 µg As kg^{-1} dry mass) and AC (200 µg As kg^{-1} dry mass). They also detected DMA (520 µg As kg^{-1} dry mass).[16]

Arsenic speciation of the corresponding urine samples also showed DMA, thio-DMA, and thio-arsenosugar-glycerol (thio-Gly). Arsenosugars are the major arsenic compounds in marine algae but are usually not detectable in fish; except the silver drummer, where arsenosugars were found as minor arsenic constituents. Traces of sugars were also determined by Maher et al. in stomach, intestine, kidney and gonad tissue of sea mullets.[17] Higher concentrations of arsenosugars are found in molluscs.[18]

There are usually several hours between ingestion and excretion of arsenic taken up via food. For humans an increase in the arsenic concentration - after consumption of a canned cod liver - was observed in the first urine samples collected after 2.5 h and 7 h. The peak arsenic concentrations were recorded between 7 and 15 h. After about two days the arsenic content of the urine decreased to almost background level. More than 85% of the ingested arsenic was excreted within this time frame.[19] Whether the digestion system of marine mammals is comparable to humans is not known to us, but our results clearly indicate metabolism of ingested arsenicals.

Porpoise urine – Arsenic bound to protein?

The total arsenic concentrations determined by flow injection ICPMS in urine samples of porpoises ranged from 100 to 2000 µg As L^{-1} (Table 4). Very low column recoveries were observed for some urine samples. When the samples where oxidized with H_2O_2 the “missing” arsenic was eluted from the applied anion-exchange column. After oxidation a significant signal for DMA (Fig. 1a) was observed, along with other compounds that elute after DMA. H_2O_2 oxidizes thio-arsenicals to their corresponding oxo-forms, which elute much earlier from the anion-exchange column. Surprisingly, no thio-DMA was detectable when reversed-phase chromatography was employed. The presence of unbound DMA(III) can also be excluded considering the instability of this compound and its retention behaviour. DMA(III) is known to be unstable enough to elute from a strong anion-exchange column like PRP-X100. Under our conditions it would be expected to be oxidized before and during the separation and would result in a tailing of the DMA(V)

peak.[20] Šlejkovec et al. observed, using the same anion-exchange column, a loss of arsenite due to specific As(III)-complexing proteins/peptides like cysteine, metallothionein, and reduced GSH, which were stuck on the column during the separation procedure. In their case the "missing" arsenic was retained after lowering the pH via an injection of 100 µL of a 3 mol L^{-1} HCl solution.[21] Naramandura and Suzuki reported recently the identification of the major arsenic binding protein in rat plasma.[22] They oxidized the purified protein (ternary dimethylarsinious hemoglobin haptoglobin complex) with H_2O_2 and detected DMA(V) as only arsenic species. They suggested that arsenic was bound as trivalent DMA(III) to the protein. In our case DMA(V) or due to the higher reactivity more probably DMA(III) bound to a protein could have the same behaviour as observed. Further investigations with special emphasis on sample storage would help to clarify this question.

In Table 4 the arsenic elution between non oxidized and oxidized urine samples on the anion-exchange column and the concentrations of the identified arsenic species are shown. All samples contained AB, nine out of twelve samples contained DMA, eight samples thio-DMA, and only in four samples AC could be detected. Arsenocholine is known to occur in marine animals like plaice, tuna, and jellyfish in low concentration ranges.[18] In 1998 Goessler et al. reported AC in concentration ranges from 2 to 44 µg As kg^{-1} wet mass in liver samples of whales and seals.[23] Additionally AC has been reported to be a significant breakdown product of arsenolipids.[24] Francesconi and Edmonds showed that in fish fed with AC about 40% of the ingested arsenic was bio-transformed into AB and stored in their muscle tissue.[25]

The porpoise urine sample with the highest AB concentration also contained TMAP, which was identified for the first time in fish in the year 2000 where it was discussed to be a common constituent of marine animals.[26] Geiszinger et al. determined two years later TMAP in muscle, liver, kidney, and lung of the sperm whale (*Physeter catodon*).[27] Four urine samples contained DMAP and five contained thio-DMAP. Schmeisser et al.[28] reported in 2006 oxo-DMAP and thio-DMAP as human urinary metabolites after ingestion of arsenolipids present in cod liver. Oxo-DMAP was found as minor constituent in several marine samples like dogfish, salmon, cod, mussels, scallops, and hooded seals by Sloth et al.[29]

Four unknown peaks could be detected in porpoise urine. Employing the anion-exchange column one unidentified compound was detected after 2.1 minutes in a concentration less than 5 µg As L^{-1} (n=1). This compound was stable towards oxidation. But in two urine samples an additional unknown peak, U_4, could be seen after H_2O_2 addition (Fig. 1b). Using reversed-phase chromatography, two unknown species were determined: one arsenic species (U_5) was obtained after 4.5 minutes (n=2, 5 and 10 µg As L^{-1}); and the other, U_6 was eluting after 7.6 minutes (n=1, 10 µg As L^{-1}).

Table 4.
Summary of total arsenic contents in porpoise urine, of chromatographically recorded arsenic before and after oxidation using H_2O_2, and the arsenic compounds identified.

Urine	FI - ICPMS [µg As L^{-1}]	HPLC – ICPMS[A] [µg As L^{-1}]	[%]	HPLC - ICPMS after oxidation[B] [µg As L^{-1}]	[%]	Identified arsenic compounds [µg As L^{-1}]					
						AB	DMA	TMAP	DMAP	Thio-DMA	Thio-DMAP
Pp 1	1410	1360	97	1500	107	820	190	88	9		
Pp 2	190	45	24	200	105	46	2			23	
Pp 3	100	16	16	100	99	17					5
Pp 4	230	120	54	240	107	130	2				
Pp 5	2000	1170	58	1580	78	660	230		10	400	
Pp 6	660	400	60	640	96	460				79	
Pp 7	580	430	75	570	99	160	200		3	39	
Pp 8	280	66	24	230	84	4.5	31		1	75	34
Pp 9	170	200	115	180	106	58	13			10	9
Pp 10	420	420	95	360	87	210	120			5	19
Pp 11	220	160	70	200	88	46	60			33	8
Pp 12	220	150	70	160	72	150					

[A]Arsenic concentration detected with an integration over the whole chromatogram using anion-exchange chromatography (Chromatographic condition I)

[B] Arsenic compounds detected via an integration over the whole chromatogram of the samples oxidized with H_2O_2 running under the same chromatographic conditions as above.

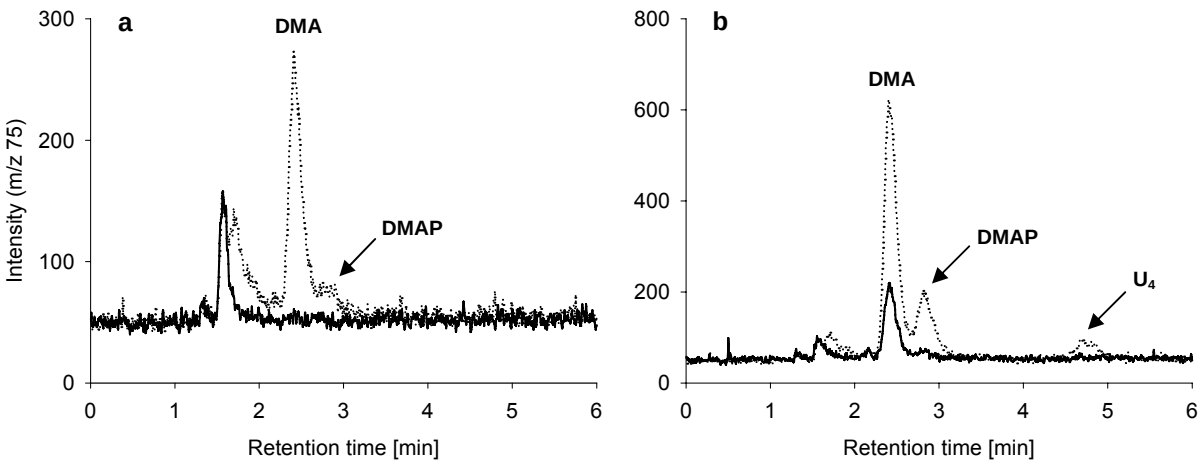

Fig. 1: Anion-exchange HPLC-ICPMS chromatogram of two porpoise urine samples diluted with water (1+9) (solid lines) and the same samples oxidized with H_2O_2 (dotted lines). **a**: sample Pp 3 is shown, after the oxidation a remarkable increase in DMA can be seen but no thio-DMA was found. **b**: sample Pp 8 is shown, in this case there is an increase of DMAP, additionally an unknown peak U_4 that was not found in unoxidized samples was observed. The higher DMA peak is related to thio-DMA present in the sample.
Conditions: PRP-X100 (4.1 x 250 mm), 20 mM $(NH_4)H_2PO_4$, pH 6; flow rate, 1.5 $mLmin^{-1}$; injection volume, 20 µL; and column temperature, 40°C.

Seals urine

The total arsenic contents found in seals' urine ranged from 130 to 3600 µg As L^{-1} with a median concentration of 1100 µg As L^{-1}. The percentage of identified compounds ranged from 86% to 107% with two exceptions at 71% and 74%.

Ten arsenicals could be identified (Table 5), three of which were not found in porpoise urine: TMAO, TETRA, and thio-Gly. AB and DMA were found in all samples. Oxo-DMAP was found in seven samples in a concentration range between 5 and 40 µg As L^{-1} and thio-DMAP was detected in three urines in a range between 4 and 50 µg As L^{-1}. Thio- DMA was detected in seven out of fifteen seal urine samples but it has to be taken into account that thio-DMA is easily oxidized to DMA during storage. Raml et al. reported that thio-DMA decreased to 94% of its initial value after 8 h of storage at 4°C.[30] Eight unidentified peaks were recorded in a concentration range of 3 to 60 µg As L^{-1}. Two unknown peaks, one eluting after 3.2 min (n=1, < 5 µg As L^{-1}) and one after 3.8 min (n=7, 10 – 60 µg As L^{-1}) from the anion-exchange column were stable towards oxidation. Another unknown species, 20 µg As L^{1-} (n=2), could be detected employing the reversed-phase column after 6.6 min. The unknowns U_1, U_2, and U_3, obtained in the gastric juice samples, could also be detected in seals' urine in the same concentration ranges. The unknowns U_5 and U_6, detected in porpoise urine, were also found in seals' urine (n=1, < 5 µg As L^{-1}; n=1, 20 µg As L^{-1}).

Table 5.
Concentration range of arsenic species in urine of seals [µg As L^{-1}]

	AB	DMA	AC	Thio-DMA	TMAP	Thio-Gly	TMAO	TETRA	DMAP	Thio-DMAP
Concentration Range	20 - 2100	7 - 1600	3 - 28	15 - 900	4 - 22	4 - 15	9 - 50	1 - 17	6 - 40	4 - 50
Median	690	68	8	54	6	10	18	2	19	8
Detected in x out of 16 samples	16	16	11	14	5	3	6	3	7	3

Summary

AB is the major arsenic species in urine, blood, and gastric juice of seals, and urine of porpoises. AB is a stable compound which is known to accumulate in marine organism, the reason is unknown. AB is virtually present in all marine animals mostly as major arsenic species.[31]

DMA and thio-DMA were identified in most urine samples independent of the marine mammal. In seals' blood two arsenic compounds could be determined, AB and DMA, whereas the AB/DMA ratio is different from free ranging seals compared to seals living permanently in a Seal Centre.

In summary similar matrices contain similar arsenic species but have a high concentration variability. Altogether ten unknown arsenic compounds were detected in the urine and gastric juice in low concentration ranges. In porpoise urine a high difference between eluting arsenic on an anion-exchange column before and after oxidation was observed. Most probably the "missing" arsenic is bound to a protein and then released and oxidized to the detected DMA(V).

The total arsenic content in seals blood is related to diet and it seems that differences in diet influence the

occurrence of various arsenic species in urine and blood of porpoises and seals, but this has to be confirmed in further investigations.

• Experimental Methods

Instrumentation

As element-selective detector an Agilent 7500ce ICPMS (Agilent, Waldbronn, Germany) equipped either with a PolyPro-ST nebuliser (Elemental Scientific Inc., Omaha, USA) or a Babington type nebuliser, was used. As chromatographic system an Agilent 1100 Series HPLC system (Agilent, Waldbronn, Germany) consisting of a vacuum degasser, a quaternary pump, column oven and an autosampler was employed, connected to the ICPMS via a 100 cm x 0.125 mm PEEK (polyetheretherketone) tubing (Upchurch Scientific, Oak Harbor, USA).

Standards and Reagents

All solutions were prepared with Milli-Q water (18.2 $M\Omega cm^{-1}$). Pyridine (p.a.), aqueous ammonia 25% (suprapure), nitric acid (p.a), and hydrogen peroxide (p.a) were purchased from Merck (Darmstadt, Germany). The nitric acid was further purified in a quartz sub-boiling distillation unit. Formic acid (p.a), ammonium dihydrogen phosphate (p.a.), methanol (p.a.), and sodium dimethylarsinate trihydrate were obtained from Fluka (Buchs, Switzerland). The investigated arsenic species and their abbreviations are shown in Fig. 2. AB, AC, TMAP, and TETRA were prepared according to McShane.[32] TMAO,[33] DMAP,[28] and Gly[34] were synthesized as previously reported. Thio-Gly was prepared from the oxo-Gly as described elsewhere.[35] Thio-DMAP was prepared by purging the headspace of an HPLC vial containing Oxo-DMAP (100 µg As L^{-1}) for 15 seconds. To remove the excess of H_2S the vial was purged with argon for 15 minutes. Thio-dimethylarsinic acid was prepared from DMA according to Raml et al.[36]

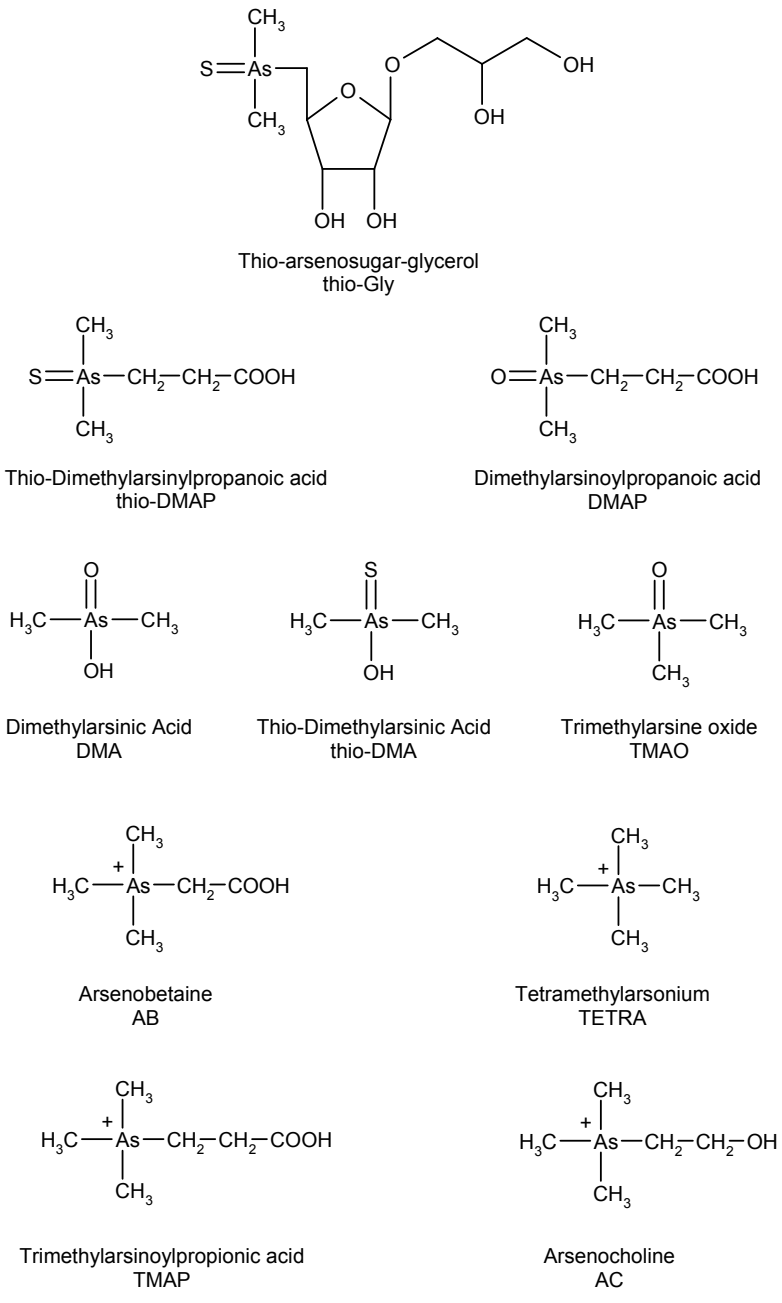

Fig. 2 Structures and abbreviations of the investigated arsenic compounds

Sampling and sample preparation

Fresh whole blood samples were collected from harbor seals of the North Sea between 2004 and 2006. The animals were captured in by net at the Danish and German Wadden Sea Coasts at three different areas (Römö, Lorenzenplate, Helgoland). In 2006 samples from seals living permanently in the Seal

Centre Friedrichskoog (Germany) and from new born seals (pups) which were in the Centre for rehabilitation were additionally collected. From four pups blood was sampled twice (in July and September). For plasma samples the whole blood was centrifuged (3000g, Centrifuge 5804R, Eppendorf AG, Hamburg, Germany). Urine, gastric juice, and liver samples were collected during dissections from carcases found dead along the North and Baltic Sea coasts in 2004-2007. All samples were taken under survey of veterinarians at the Research and Technology Centre (FTZ) in Büsum, Germany. The following abbreviations are used for the sampling stations: Frisko = seals living permanently in the Seal Centre Friedrichskoog, Lorenz = seals caught in the German Wadden Sea at Lorenzenplate, Romo = seals caught at the Danish island Römö, Helgoland = seals caught at the German island Helgoland.

The samples were stored at -20°C until further processing.

For total As analysis in whole blood and liver a microwave digestion system (MarsXpress, CEM GmbH, Kamp-Lintfort, Germany) was applied.[8] The plasma, gastric juice, and urine samples were diluted (1+9) by MilliQ water and filtrated (< 0.2 μm, Nylon ProFill™Plus, MedChrom GmbH, Flörsheim-Dalsheim, Germany).

For speciation analysis the samples were diluted (1+9) and filtered using a syringe (2 mL, Terumo Europe, Leuven, Belgium) and a syringe filter (Profill, 0.2 µm, Markus Bruckner Analysentechnik, Linz, Austria), filled into 1 mL polypropylene vials and closed with Al crimp caps (both Agilent, Waldbronn, Germany). For oxidation to portions of 180 µL sample, 20 µL of water or H_2O_2 solution were added. Before measurement the samples were shaken by hand for about half a minute.

Determination of total arsenic

The determination of the total arsenic content in blood and tissues were performed with a total-reflection X-ray fluorescence spectrometer (Atomika 8030C, CAMECA GmbH, Unterschleissheim, Germany). Mo-Kα radiation was selected for the excitation. The counting time was set to 1000s. With the help of Y as internal standard arsenic could be quantified.

Seronorm ™ trace elements in whole blood L-2 (SERO AS, Billingstad, Norway) and Pygme sperm whale liver homogenate (QC03LH03 from NIST/NOAA, Gaithersburg, USA) were used as reference material with relative standard deviation (RSD) of 8 and 14% and recoveries of 85 and 102% within 3 measurements.

The total arsenic concentration in the urine samples was determined with FI-ICPMS using 0.3% HNO_3 with 10% MeOH as eluent.[37]

Determination of arsenic species by HPLC-ICPMS

Arsenic species identified were quantified with calibration curves established with standard solutions of the corresponding compounds (thio-arsenic and unknown compounds were quantified using the DMA calibration curve). HPLC separations were performed with three chromatographic conditions (summarized in Table 6) using the following columns: a PRP-X100 polymer based anion-exchange column, 4.1 x 250

mm (Hamilton, Reno, USA); a Zorbax 300-SCX silica based cation-exchange column, 4.6 x 250 mm (Agilent, Waldbronn, Germany), and an Atlantis dC18 reversed-phase column (Waters, Milford, USA). For signal enhancement MeOH was pumped to the spray chamber as described elsewhere.[38] The ion intensities at m/z 75 (^{75}As) and m/z 77 ($^{40}Ar^{37}Cl$, ^{77}Se) were monitored using the 'time-resolved' analysis software. For data evaluation the ICPMS chromatographic software version C.01.00 (Agilent, Waldbronn, Germany) was used.

Table 6.
Applied chromatographic conditions for arsenic speciation

	Condition I anion-exchange	Condition II cation-exchange	Condition III reversed-phase
Quantified arsenic species	DMA, DMAP	AB, AC, TMAO, TMAP, TETRA	Thio-arsenic compounds
Column	PRP-X100 (4.1 x 250 mm), 5 µm (Hamilton, Reno, USA)	Zorbax 300 SCX (4.6 x 250 mm), 5 µm (Agilent, Waldbronn, Germany)	Atlantis dC18 (4.6 x 150 mm),5 µm (Waters, Milford,USA)
Mobile Phase	20 mM $(NH_4)H_2PO4$	10 mM pyridine	20 mM $(NH_4)H_2PO4$
pH	6	2.6	3
Column temperature	40°C	30°C	30°C
Flow rate	1.5 $mLmin^{-1}$	1.5 $mLmin^{-1}$	1 $mLmin^{-1}$
Injection volume	20 µL	10 µL	10 µL

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