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Determination of Arsenic Compounds in Marine Mammals with High-performance Liquid Chromatography and an Inductively Coupled Plasma Mass Spectrometer as Element-specific Detector

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Total arsenic concentrations and the concentrations of individual arsenic compounds were determined in liver samples of pinnipeds [nine ringed seals (*Phoca hispida*), one bearded seal (*Erginathus barbatus*)] and cetaceans [two pilot whales (*Globicephalus melas*), one beluga whale (*Deliphinapterus leucus*)]. Total arsenic concentrations ranged from 0.167 to 2.40 mg As kg^{-1} wet mass. The arsenic compounds extracted from the liver samples with a methanol/water mixture (9:1, v/v) were identified and quantified by anion- and cation-exchange chromatography. An ICP–MS equipped with a hydraulic high-pressure nebulizer served as the arsenic-specific detector. Arsenobetaine (0.052–1.67 mg As kg^{-1} wet mass) was the predominant arsenic compound in all the liver samples. Arsenocholine was present in all livers (0.005–0.044 mg As kg^{-1} wet mass). The tetramethylarsonium cation was detected in all pinnipeds (<0.009 to 0.043 mg As kg^{-1}) but not in any of the cetaceans. The concentration of dimethylarsinic acid ranged from < 0.001 to 0.109 mg As kg^{-1} wet mass. Most of the concentrations for methylarsonic acid (<0.001 to 0.025 mg As kg^{-1} wet mass) were below the detection limit. Arsenous acid and arsenic acid concentrations were below the detection limit of the method (0.001 mg As kg^{-1}). An unknown arsenic compound was present in all liver samples at concentrations from 0.002–0.027 mg As kg^{-1}. © 1998 John Wiley & Sons, Ltd.

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INTRODUCTION

Several review articles summarize the role of arsenic in the environment.[1,2] The toxicity and biological function strongly depend on the chemical form of the arsenic compounds.[2,3] The toxicity of the arsenic compounds decreases in the following order: arsenite > arsenate ≫ methylarsonic acid ≈ arsenic-containing riboses ≈ dimethylarsinic acid ≫ arsenobetaine ≈ arsenocholine ≈ trimethylarsine oxide ≈ the tetramethylarsonium ion.

In 1977 arsenobetaine [$(CH_3)_3As^+CH_2COO^-$] was isolated from the Western rock lobster (*Panulirus cygnus*).[4] Subsequent work proved that arsenobetaine is the major arsenic compound in many marine animals, including fish, crustaceans, molluscs and echinoderms.[2] In 1982 Norin and coworkers detected arsenocholine in addition to arsenobetaine in the shrimp *Pandalus borealis*.[5] In 1987 the tetramethylarsonium ion [$(CH_3)_4As^+$] was identified as the major arsenic compound in gills of the clam *Meretrix lusoria*.[6] Marine algae are

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known to contain arsenic riboses at high concentrations.[2]

For the determination of arsenic compounds an efficient separation step followed by an adequate detection step is necessary. These conditions are met by liquid chromatography coupled to detection systems such as hydride generation–atomic absorption spectrometry (HG–AAS),[10,11] hydride generation–inductively coupled plasma–mass spectrometry (HG–ICP–MS),[12,13] inductively coupled plasma–atomic emission spectrometry (ICP–AES),[14] and inductively coupled plasma–mass spectrometry (ICP–MS).[15,16] The hydride generation technique provides excellent detection limits for arsenic compounds that are reducible to volatile hydrides, but cannot detect arsenobetaine, arsenocholine and the tetramethylarsonium cation without prior chemical conversion. The detection limits for ICP–AES are too high for minor arsenic compounds in biological samples. High-performance liquid chromatography combined with ICP–MS as an arsenic-specific detector provides the low detection limits required and is also capable of detecting 'hidden' arsenic species. High-efficiency nebulizers such as the hydraulic high-pressure nebulizer (HHPN) can lower the detection limits by an order of magnitude.[17]

The ability of marine mammals to accumulate trace elements has been studied intensively. The National Institute of Standards and Technology (NIST) has been banking tissue specimens from marine mammals as part of the National Biomonitoring Specimen Bank since 1987.[18] Subsamples of a portion of these specimens, which are maintained in liquid-nitrogen vapor freezers, are analyzed routinely for trace elements by instrumental neutron activation analysis (INAA) at NIST as part of its efforts to establish a database for baseline concentrations of trace elements in these specimens. Additional analyses of these specimens by other methods are periodically conducted by other researchers as part of specific research projects or as part of quality assurance interlaboratory comparisons administered by NIST. In liver specimens from nine different species of marine mammals archived in this specimen bank, the concentrations of up to 30 elements have been determined.[7,18,19] The hepatic arsenic concentrations for most of these animals (harbor porpoise, *Phocoena phocoena*; white-sided dolphin, *Lagenorhynchus acutus*; beluga whale, *Delphinapterus leucas*; bowhead whale, *Balaena mysticetus*; bearded seal, *Erignatus barbatus*; northern fur seal, *Callorhinus ursinus*; spotted seal, *Phoca largha*) do not exceed 0.62 mg kg^{-1} wet mass. Except for one pilot whale (*Globicephalus melas*), which had a total arsenic concentration in its liver of 1.17 mg kg^{-1} wet mass, the hepatic arsenic in this species was also less than 0.60 mg kg^{-1}.

Positive linear correlations were observed for hepatic concentrations of mercury with selenium ($r = 0.978$), silver with selenium ($r = 0.852$), zinc with cesium ($r = 0.915$) and magnesium with chlorine ($r = 0.850$). Total arsenic concentrations were not correlated with any other element. The hepatic concentrations of selenium, silver and mercury in pilot whales also correlate with animal length, with linear correlation values of 0.866, 0.894 and 0.831, respectively. Body length for this species is related to the age of the animal, indicating that silver, selenium and mercury accumulate as the individual ages. Although vanadium, selenium, cadmium and mercury accumulate in the livers of ringed seals with increasing length of the animal, arsenic concentrations were not correlated with body length, probably indicating that this element does not accumulate in the liver with increasing age.[19]

The animals with the highest arsenic concentrations were ringed seals (*Phoca hispida*) from Norton Sound, Alaska (Bering Sea).[19] For these animals, hepatic total arsenic concentrations up to 2.4 mg kg^{-1} wet mass were measured. Norton Sound is an area of gold-mining activity that began in the 1880s and continues today in the coastal area around Nome, Alaska. Arsenic concentrations are normally elevated near arseniferous deposits and mineralized zones containing gold, silver and sulfides of lead and zinc;[20] therefore, elevated levels of arsenic are not surprising in food webs leading up to ringed seals that feed in the region. Liver, as well as other portions of this animal, are consumed as food by local communities. Because the relative toxicity of arsenic depends on its valence and molecular form, the determination of the arsenic compounds that contribute to the total arsenic concentrations in marine mammal livers is important. No information about the arsenic compounds in marine mammal livers is available except the work of Bratton *et al*., who reported that 98% of the total arsenic in the livers of bowhead whales is arsenobetaine.[9]

Liver tissues archived in the National Biomonitoring Specimen Bank that had been collected from nine ringed seals in Norton Sound were selected for the determination of arsenic compounds. These samples were chosen because they have relatively high concentrations of total arsenic as determined

Table 1. Detailed information on the animals sampled

Species	Animal identification no.	Sampling site[a]	Sex	Length (cm)	Mass (kg)
Pilot whale	PW599	Hyannis, MA	F	413	
Pilot whale	PW600	Hyannis, MA	F	439	
Ringed seal	RGSL008	Barrow, AK	M	104.0	23.1
Ringed seal	RGSL011	Nome, AK	M	119.5	33.6
Ringed seal	RGSL012	Nome, AK	F	124.5	36.3
Ringed seal	RGSL013	Nome, AK	M	103.5	31.8
Ringed seal	RGSL014	Nome, AK	M	101.0	29.5
Ringed seal	RGSL015	Nome, AK	F	83.2	20.0
Ringed seal	RGSL017	Nome, AK	F	113	40.8
Ringed seal	RGSL018	Nome, AK	F	87.0	21.8
Ringed seal	RGSL029	Nome, AK	M	106.7	
Ringed seal	RGSL032	Nome, AK	M	79.0	29.0
Beluga whale	BLKA011	Point Lay, AK	M	433	
Bearded seal	BDSL001	Nome, AK	M	166.7	

[a] Hyannis, MA: Hyannis, Massachusetts, North Atlantic coast of the United States; Barrow, AK: Barrow, Alaska, located at the boundary of the Chukchi Sea and Beaufort Seas. Arctic Ocean; Nome, AK: Nome, Alaska, located on Norton Sound, which is part of the Bering Sea; Point Lay, AK: Point Lay, Alaska located on the Chukchi Sea, Arctic Ocean.

previously by INAA. For comparison, liver samples from a ringed seal from another region of Alaska, one bearded seal, one beluga whale and two pilot whales were also included. Detailed information on these animals is given in Table 1. As part of an interlaboratory quality assurance comparison, total arsenic concentrations were determined for each sample by both ICP–MS and by INAA. Arsenic compounds were identified and quantified in methanol/water extracts by HPLC–ICP–MS.

MATERIALS AND METHODS

Instrumentation

Digestions were performed with an MLS-1200 Mega microwave system (MLS, Leutkirch, Germany). Total arsenic was determined in the digests with a VG PlasmaQuad 2 Turbo Plus inductively coupled argon–plasma mass spectrometer (ICP–MS, VG Elemental, Winsford, UK) equipped with a Meinhard concentric glass nebulizer, type TR-30-A3.

The high-performance liquid chromatography system consisted of a Hewlett-Packard 1050 solvent delivery unit (Hewlett-Packard, Waldbronn, Germany) and a Rheodyne 9125 six-port injection valve (Rheodyne, Cotati, USA) with a 100-mm^3 injection loop. The separations were performed on a Hamilton (Reno, USA) PRP-X100 anion-exchange column (25 cm × 4.1 mm i.d., 10-µm styrene–divinylbenzene particles with trimethylammonium exchange sites) and a Supelcosil LC-SCX (Supelco, Bellefonte, USA) cation-exchange column (25 cm × 4.6 mm i.d., 5-µm silica-based particles with propylsulfonic acid exchange sites).

The outlet of the HPLC column was connected via 60-cm, 1/16-inch (1.6 mm) PEEK (polyether-ether-ketone) capillary tubing (0.25 mm i.d.) to a hydraulic high-pressure nebulizer (Knauer, Berlin, Germany). The VG PlasmaQuad 2 Turbo Plus ICP–MS served as arsenic-specific detector. The ion intensity at *m/z* 75 (^{75}As) was monitored using 'time-resolved' analysis software© Version 1a (Fisons Scientific Equipment Division, Middlesex, UK). Additionally, the ion intensity at *m/z* 77 (^{40}Ar^{37}Cl, ^{77}Se) was monitored to detect possible argon-chloride (^{40}Ar^{35}Cl) interferences on *m/z* 75. Before each HPLC–ICP–MS experiment the ion intensity at *m/z* 87 (Rb added to the mobile phases) was optimized at the rate meter of the instrument. Instrumental settings used throughout this work are summarized in Table 2. The chromatograms were exported and the peak areas were determined with software written in-house. The arsenic compounds were quantified with external calibration curves established with each of the eight arsenic compounds.

Reagents, standards and mobile phases

All solutions were prepared with MilliQ (18.2 MΩ cm) water. Concentrated nitric acid (Merck, p.a.) was purified in a quartz subboiling distillation unit. The mobile phase for the anion-exchange

Table 2. Operating conditions for the HPLC–HHPN–ICP–MS system

High-performance liquid chromatography	
Columns:	
Hamilton PRP-X100 anion-exchange	Mobile phase: 20 mM $NH_4H_2PO_4$, pH 6.0
Supelcosil LC-SCX cation-exchange	Mobile phase: 20 mM Pyridine, pH 2.6
Hydraulic high-pressure nebulizer	
Desolvation	Heating: 150°C Cooling: 2.0°C
Nebulizer gas	$1.00\ l\ min^{-1}$
Back pressure	~200–250 bar
Inductively coupled plasma–mass spectrometry	
Plasma:	
Rf power	Forward: 1.35 kW Reflected: <1 W
Argon gas flow:	
Cooling gas	$13.5\ l\ min^{-1}$
Auxiliary gas	$1.1\ l\ min^{-1}$
Vacuum:	
Expansion	2.4 mbar
Intermediate	$<1.0 \times 10^{-4}$ mbar
Analyzer	5.4×10^{-6} mbar
Ion sampling:	
Sample cone	Nickel, orifice 1.00 mm diameter
Skimmer cone	Nickel, orifice 0.75 mm diameter
Measuring parameters:	
Monitored signal	^{75}As, $^{40}Ar^{37}Cl$ or ^{77}Se
Time/slice	0.53 s
Total analysis time	~400 s (column-dependent)

HPLC was prepared by dissolving 2.30 g $NH_4H_2PO_4$ to 1000 cm^3 and adjusting the pH of this solution to 6.0 by addition of NH_3 (Merck, p.a.), and the mobile phase for the cation-exchange HPLC by dissolving 1.58 g pyridine to 1000 cm^3 and adjusting the pH to 2.6 by addition of formic acid (98%, Fluka, puriss. p.a.). Rubidium (RbCl) was added to all mobile phases to achieve a concentration of 50 ng cm^{-3}.

Calibration curves for the HPLC–ICP–MS measurements were obtained by chromatographing aliquots (100 mm^3) of solutions containing 5.00, 10.0 or 50.0 ng As cm^{-3} of arsenous acid (arsenite), arsenic acid (arsenate), methylarsonic acid (MA) and dimethylarsinic acid (DMA) on the Hamilton PRP-X100 anion-exchange column, and 1.00, 5.00 or 10.0 ng As cm^{-3} of arsenobetaine (AB), arsenocholine (AC), tetramethylarsonium iodide (TETRA) and trimethylarsine oxide (TMAO) on the Supelcosil LC-SCX cation-exchange column.

Samples

Liver samples were obtained from the NIST National Biomonitoring Specimen Bank in Gaithersburg, MD, USA. Specimens collected for this specimen bank were frozen in liquid-nitrogen vapor in the field and maintained at liquid-nitrogen vapor temperatures −120 °C until analysis. The methods and procedures for the collection of these specimens have been described elsewhere.[7,8]

In preparation for analysis, the liver specimens were homogenized cryogenically by a procedure described in detail by Zeisler *et al.*[21] consisting of fracturing the frozen tissue into smaller pieces, weighing pieces of a few grams each, and placing these pieces in a Teflon ® mill that has been cooled to liquid-nitrogen vapor temperature. The pieces of frozen tissue are ground for about 3 min to pulverize the material completely into a frozen powder. Pre-weighed aliquots of this frozen powder were lyophilized at a pressure of 1 Pa for five days, after which dry weights were recorded. During this period the temperature gradually increased from −20 °C to 5 °C. The freeze-dried, homogenized tissues were used for the analyses. Detailed information about the animals included in this study is given in Table 1.

Determination of total arsenic

Total arsenic concentrations were determined by two independent methods: instrumental neutron activation analysis (INAA) and microwave digestion followed by ICP–MS determination. INAA was performed at NIST. Subsamples of the freeze-dried, homogenized tissues were formed into disk-shaped pellets. These disks were doubly encapsulated in bags formed from acid-washed polyethylene film. Portions of Standard Reference Material (SRM) 1577a Bovine Liver, included in the analysis scheme for quality control, were formed into disks and packaged in the same manner. Standards were prepared by pipetting solutions containing known amounts of the elements of interest onto Whatman filter papers. After being dried, these papers were formed into disks for analysis. Samples, standards and SRMs were irradiated together in the NIST research reactor at a reactor power of 20 MW, which corresponds to a neutron flux rate of 3×10^{13} cm^{-2} s^{-1}, for 16 h. After approximately one week of decay, γ-ray spectrometry was performed to determine the concentrations of As in each sample. Quantification was based on comparison with standards, using the 559-keV γ-ray line from ^{76}As. Results of analyses of SRMs agreed with certified values within the uncertainty of the method.[8]

The microwave digestion–ICP–MS method, conduced at the Karl-Franzens University, used aliquots of the freeze-dried, homogenized tissues (≈ 0.2 g) weighed to 0.1 mg into Teflon digestion vessels. Concentrated nitric acid (5.0 cm^3) and 30% hydrogen peroxide (1.00 cm^3, Merck p.a.) were added to each vessel. The vessels were closed, secured in the rotor, and placed in the microwave oven. The samples were digested using the following program: 2 min 250 W, 0.5 min 0 W, 5 min 300 W, 0.5 min 0 W, 5 min 450 W, 0.5 min 0 W, 5 min 600 W, 7 min 500 W, 2 min 0 W (ventilation). The digests were transferred quantitatively into 50-cm^3 volumetric flasks. An aliquot (0.250 cm^3) of a solution containing 10 µg In cm^{-3} was added to each flask. The flasks were filled to the mark. Total arsenic concentrations were determined in these solutions by ICP–MS with an external calibration curve established with arsenate solutions. All concentrations of arsenic are reported on a wet mass basis.

Extraction of the arsenic compounds

Aliquots (≈ 0.4 g) of the powders of whale and seal livers were weighed to 0.1 mg into 50-cm^3 polyethylene tubes. A mixture of methanol/water (9:1 v/v, 20 cm^3) was added, and the tubes were shaken for 20 h. The samples were centrifuged at 2500 rpm and washed three times with methanol/water (9:1 v/v, 20 cm^3 each). The supernatants were transferred into round-bottomed flasks and the methanol was evaporated on a Rotavapor (Büchi, Swizerland) at room temperature under an aspirator vacuum. To each of the evaporation residues water was added to a total mass of 10.0 g. The redissolved extraction residues were centrifuged at 3000 rpm

Table 3. Total arsenic concentrations in livers from whales and seals

	Total arsenic concentration (mg kg^{-1} wet mass)			
Animal identification no.	INAA[a,b]	ICP-MS[c]	Sum of arsenic species	Extractable arsenic[d] (%)
PW599	1.17 ± 0.04	1.27 ± 0.03	0.915	72.0
PW600	0.29 ± 0.03	0.267 ± 0.005	0.159	59.6
RGSL008	0.61 ± 0.04	0.566 ± 0.006	0.389	68.7
RGSL011	2.42 ± 0.06	2.40 ± 0.02	1.85	77.3
RGSL012	1.62 ± 0.14	1.55 ± 0.04	1.11	71.7
RGSL013	2.11 ± 0.06	2.11 ± 0.04	1.59	75.5
RGSL014	1.33 ± 0.08	1.27 ± 0.02	0.822	64.7
RGSL015	1.75 ± 0.14	1.75 ± 0.003	1.26	71.7
RGSL017	1.15 ± 0.08	1.11 ± 0.01	0.777	70.0
RGSL018	0.81 ± 0.02	0.765 ± 0.008	0.515	67.3
RGSL029	0.70 ± 0.02	0.776 ± 0.02	0.495	63.8
RGSL032	0.93 ± 0.02	1.05 ± 0.05	0.770	73.3
BLKA011	0.07 ± 0.04	0.167 ± 0.007	0.074	44.3
BDSL001	0.56 ± 0.02	0.483 ± 0.003	0.229	47.4

[a] Refs 7, 8, 19.
[b] Values represent the average from duplicate portions and uncertainties represent the 95% confidence level.
[c] Values represent the average from triplicate portions and uncertainties represent one standard deviation.
[d] Extractable arsenic calculated from the sum of arsenic species and the total arsenic concentrations in livers determined by ICP–MS.

and the supernatants filtered through 0.22-µm Millex-GS cellulose ester filters (Millipore, Bedford, USA). Aliquots of the filtered supernatants were chromatographed on the HPLC–HHPN–ICP–MS system.

RESULTS AND DISCUSSION

Total arsenic concentrations

The total arsenic concentrations determined by ICP–MS in the liver samples from marine mammals ranged from 0.167 to 2.40 mg kg^{-1} (wet mass) (Table 3). The lowest arsenic concentration (0.167 ± 0.007 mg kg^{-1}) was found in the liver of the beluga whale from Point Lay, Alaska. The range of the total arsenic concentrations in the livers of 15 beluga whales from Alaska was 0.0475–0.62 mg kg^{-1} (wet mass).[8]

The arsenic concentration in the liver of the bearded seal was 0.483 ± 0.003 mg kg^{-1}. Liver samples from two pilot whales from Hyannis, Massachusetts (North Atlantic Coast of the United States) had arsenic concentrations of 0.267 and 1.27 mg kg^{-1} (wet mass). These arsenic concentrations are in the same range as those found in livers from 10 pilot whales caught near the Faroe Islands.[22]

The arsenic concentrations in livers from ten ringed seals from Alaska ranged from 0.566 to 2.40 mg kg^{-1} wet mass. The average arsenic concentration in livers of nine ringed seals that were caught in Nome, Alaska was 1.42 ± 0.56 mg kg^{-1} (wet mass). The lowest arsenic concentration (0.566 ± 0.006 mg kg^{-1}) in the liver of a ringed seal was found for the animal (RGSL008) caught near Barrow, Alaska. Different sources of prey may be the cause for the divergent concentrations of arsenic. The total arsenic concentrations in liver samples of ringed seals did not correlate with the length or mass of these animals.

The arsenic concentrations determined by INAA and ICP–MS agree within two standard deviations, except for the sample BLKA011 (Table 3). In the liver of this animal, the concentration determined by ICP–MS is about 2.5 times higher than the concentration determined by INAA.

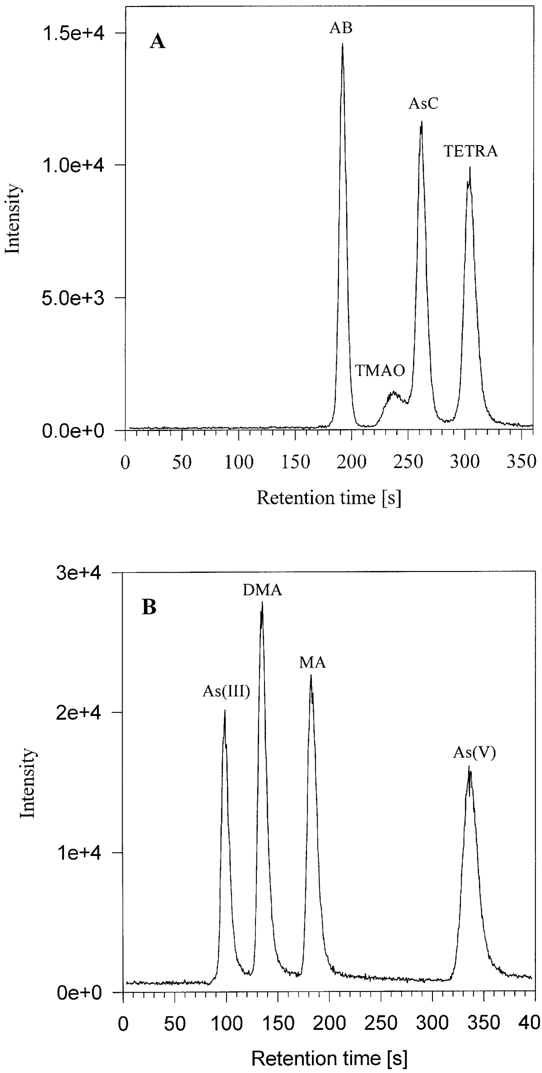

Figure 1. HPLC–HHPN–ICP–MS chromatograms: (A) of aqueous standard solution containing arsenobetaine, trimethylarsine oxide, arsenocholine and tetramethylarsonium iodide (10 ng As g^{-1}, each species) obtained with a Supelcosil LC-SCX cation-exchange column (mobile phase 20 mM aqueous pyridine at pH 2.6, injection volume 100 mm^3, flow rate 1.5 cm^3 min^{-1}). (B) of aqueous standard solution containing arsenous acid, dimethylarsinic acid, methylarsonic acid, and arsenic acid (10 ng As g^{-1}, each species) obtained with a Hamilton PRP-X100 anion-exchange column (mobile phase 20 mM $NH_4H_2PO_4$ at pH 6.0, injection volume 100 mm^3, flow rate 1.5 cm^3 min^{-1}).

Arsenic compounds

The Supelcosil LC-SCX cation-exchange column is capable of separating arsenobetaine, trimethylarsine oxide, arsenocholine and tetramethylarsonium iodide (Fig. 1A) with an aqueous pyridine solution (20 mM) at pH 2.6 as mobile phase. Under these conditions the anionic arsenic compounds arsenic acid, arsenous acid, methylarsonic acid and dimethylarsinic acid elute before arsenobetaine and are not well separated. For this reason the extracts of the liver samples were also chromatographed on a Hamilton PRP-X100 anion-exchange column. This column allows the separation of arsenous acid (in the absence of cationic arsenic compounds), dimethylarsinic acid, methylarsonic acid and arsenic acid (Fig. 1B).

The freeze-dried liver samples were extracted with a mixture of methanol/water (9:1, v/v). This mixture was found to be very suitable for the extraction of arsenic compounds from biological samples. The extractable arsenic, defined as the sum of the concentrations of arsenic compounds (expressed as mg As kg^{-1} wet mass), obtained from HPLC–ICP–MS experiments of the different liver samples accounted for 66.2 ± 9.5% of the total arsenic concentrations in the liver samples. The lowest values for the percentage of extractable arsenic were found in the bearded seal liver (44.3%) and the beluga whale liver (47.4%); see Table 3. These two liver samples had very low concentrations of total arsenic.

Arsenobetaine

Most of the pinnipeds and many of the cetaceans feed in the upper level of the food web and are pelagic fish feeders, except for the bearded seal, which is a benthic feeder. The ringed seals represent animals that, depending on age, feed from the mid-level of the food web, on pelagic crustaceans (shrimps and amphipods), to the upper level, on finfish.[23] Arsenic in the marine ecosystems has been intensively reviewed by Francesconi and Edmonds.[2] The concentrations varies greatly among species but no clear trends have emerged. Although arsenic concentrations may be higher in benthic than in pelagic animals, the total arsenic concentration in a particular animal is not correlated with its trophic position. Total arsenic concentrations are also not correlated with the size or age of an animal.[2] In the muscles of crustaceans the total arsenic concentrations ranged from 3.5 to 26 mg As kg^{-1} (wet mass). At least 50% of total arsenic is present as arsenobetaine. The total arsenic concentration in the muscle of the Alaskan king crab (*Paralithodes camtschatica*) was found to be 8.6 mg As kg^{-1} (wet mass) of which 82% was arsenobetaine.[24] In fish, total arsenic concentrations up to 166 mg As kg^{-1} have been reported.[2] Arsenobetaine was the major arsenic compound in muscles of octopus[25] and squids.[26]

The arsenic compounds identified in the liver samples were dominated by arsenobetaine. The concentrations in the liver extracts ranged from 0.052 to 1.67 mg As kg^{-1} (wet mass) (Table 4), corresponding to 68–98% of the extractable arsenic. The lowest arsenobetaine concentration was found in the liver of the beluga whale (0.052 mg As kg^{-1}). The livers of the two pilot whales had concentrations of 0.887 and 0.147 mg As kg^{-1}. The livers of the ringed seal contained arsenobetaine concentrations between 0.337 and 1.67 mg As kg^{-1}. The total arsenic

Table 4. Concentrations of arsenic compounds in liver samples of whales and seals determined by HPLC–HHPN–ICP–MS

Animal identification no.	Concentration of arsenic compounds[a] (mg As kg^{-1} wet mass)							
	AB	AC	TETRA	TMAO	Unknown[b]	DMA	MA	Arsenate
PW599	0.887	0.005	<0.001	<0.01	0.016	0.004	0.003	<0.001
PW600	0.147	0.005	<0.001	<0.01	0.007	<0.001	<0.001	<0.001
RGSL008	0.337	0.005	0.010	<0.01	0.016	0.021	<0.001	<0.001
RGSL011	1.67	0.020	0.041	<0.01	0.014	0.109	<0.001	<0.001
RGSL012	1.02	0.012	0.026	<0.01	0.013	0.040	<0.001	<0.001
RGSL013	1.46	0.015	0.038	<0.01	0.013	0.067	<0.001	<0.001
RGSL014	0.764	0.008	0.009	<0.01	0.009	0.032	<0.001	<0.001
RGSL015	1.17	0.011	0.043	<0.01	0.013	0.018	<0.001	<0.001
RGSL017	0.702	0.025	0.015	<0.01	0.007	0.028	<0.001	<0.001
RGSL018	0.417	0.021	0.021	<0.01	0.010	0.036	0.010	<0.001
RGSL029	0.386	0.044	0.017	<0.01	0.027	0.021	<0.001	<0.001
RGSL032	0.684	0.018	0.016	<0.01	0.023	0.004	0.025	<0.001
BLKA011	0.052	0.002	<0.001	<0.01	0.002	0.016	0.002	<0.001
BDSL001	0.156	0.016	0.024	<0.01	0.008	0.021	0.004	<0.001

[a] Mean of two different extracts.

[b] Same unknown compound detected in DORM reference material.

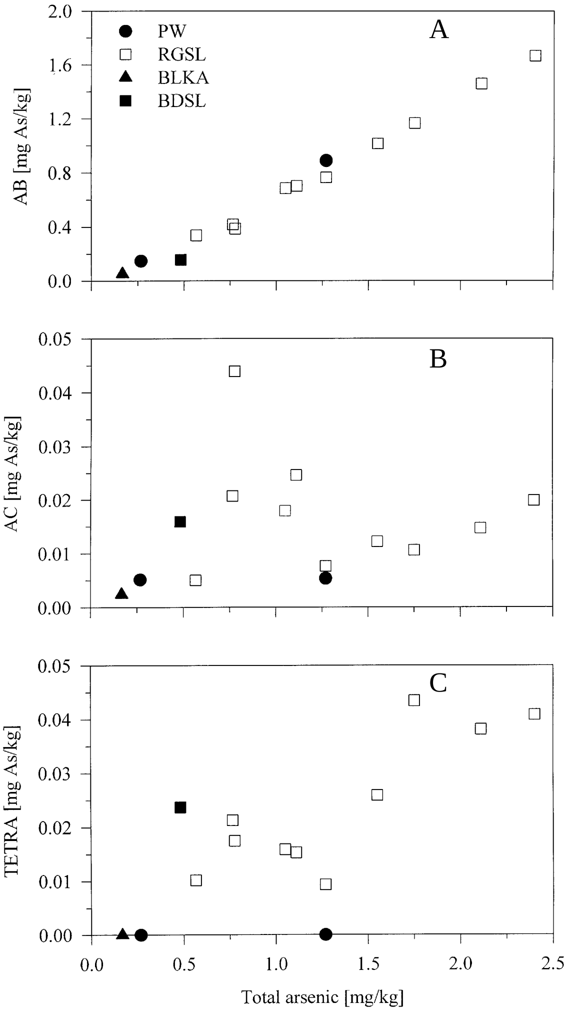

Figure 2. Hepatic concentrations of individual arsenic compounds (mg As kg^{-1} wet mass) in marine mammals, plotted versus the total arsenic concentration (mg As kg^{-1} wet mass): (A) arsenobetaine (B) arsenocholine C) tetramethylarsonium ion. The mammals are identified in Table 1.

concentration is highly correlated with the arsenobetaine concentration (Fig. 2A).

Arsenocholine

Arsenocholine, a rather elusive member of the marine arsenic cycle, was reported to be present in shrimp,[5,27] lobsters,[28] mussels[27,29] and fish.[2,27] Arsenocholine represented 0.5–9% of the extractable arsenic in the livers of marine mammals. The concentrations ranged from 0.002 to 0.044 mg As kg^{-1} (wet mass) (Table 4). The lowest arsenocholine concentration was found in the liver of the beluga whale (0.002 mg As kg^{-1}). The livers of the two pilot whales had arsenocholine concentrations of 0.005 mg As kg^{-1}, of the ringed seals between 0.005 and 0.044 mg As kg^{-1}. The concentrations in the livers of the cetaceans were generally lower than those of pinnipeds. The total arsenic concentrations did not correlate with the arsenocholine concentrations (Fig. 2B).

The tetramethylarsonium cation

The tetramethylarsonium cation was first isolated from the clam *Meretrix lusoria*[6] and was later identified in clams,[30] in the herbivorous gastropod *Austrochochlea constricta*,[31] in the sea cucumber *Stichopus* sp.[26] and as a trace constituent in crabs.[26] The concentrations of the tetramethylarsonium ion in the livers of the three cetaceans were below the detection limit, <0.001 mg As kg^{-1} (wet mass). For the seals, the tetramethylarsonium ion contributed 1–10% of the total extractable arsenic and concentrations ranged from 0.009 to 0.043 mg As kg^{-1} (wet mass) (Table 4). The tetramethylarsonium ion concentration increased with the total arsenic concentration (Fig 2C). Surprisingly, no tetramethylarsonium ion was found in the livers of the cetaceans (Fig. 3). The origin of the tetramethylarsonium ion is still not clear. A reduction and subsequent methylation of trimethylarsine oxide or the biosynthesis within the animals from still-unknown arsenic precursors was discussed.[2]

Whether food differences between the pinnipeds and cetaceans, different biotransformations of the arsenic compounds in these animals, or some other factors are responsible for the differences in the concentrations of the tetramethylarsonium ion in the livers of these two groups of marine mammals, is not known. Exact knowledge about trophic relationships, principal prey, the arsenic compounds associated with the prey and arsenic metabolism in the cetaceans and pinnipeds is needed to resolve the open questions. Generally, pilot whales are pelagic fish feeders. Beluga whales feed extensively on pelagic fish, but also consume other marine organisms in near-shore coastal areas. Bearded seals feed extensively on benthic organisms and ringed seals feed at several trophic levels, depending on the age of the animal and season of the year. Thus, different arsenic sources and different metabolic patterns in these species must be considered.

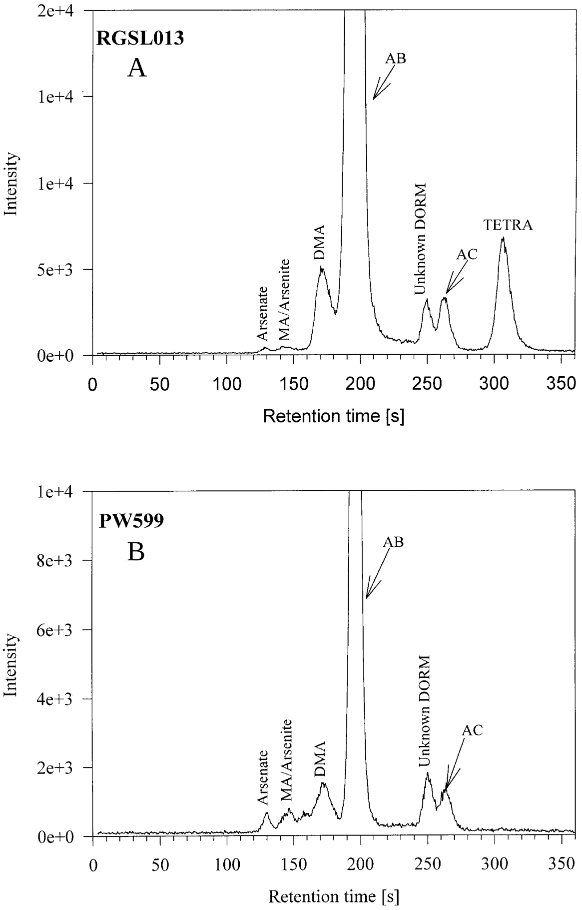

Figure 3. HPLC-HHPN-ICP-MS chromatograms of aqueous extracts of marine mammals, obtained with a Supelcosil LC-SCX cation-exchange column (mobile phase 20 mM aqueous pyridine at pH 2.6, injection volume 100 mm^3, flow rate 1.5 cm^3 min^{-1}): (A) *Phoca hispida* (animal identification number RGSL 013; (B) *Globicephalus melas* (animal identification number PW599).

Dimethylarsinic acid, methylarsonic acid, arsenic acid and arsenous acid

The hepatic dimethylarsinic acid concentrations ranged from <0.001 to 0.109 mg As kg^{-1} (wet mass). The lowest concentrations were found in the two pilot whales (<0.001, 0.004 mg As kg^{-1}). For the beluga whale 0.016 mg As kg^{-1} was found. This value represented 22% of the total extractable arsenic. In the livers of the other marine mammals only 0.4–9% of the total extractable arsenic was dimethylarsinic acid (Table 4). The hepatic dimethylarsinic acid concentrations increased with the total arsenic concentrations (Fig. 4A).

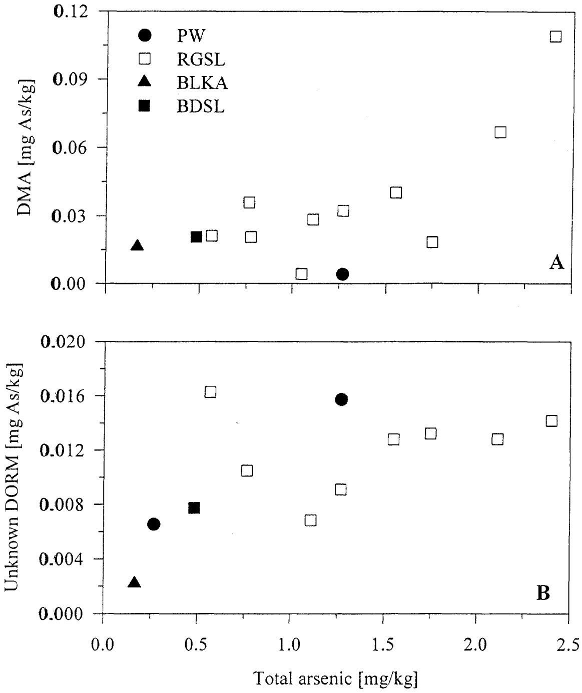

Figure 4. Hepatic concentrations (mg As kg^{-1} wt mass) in marine mammals plotted versus the total arsenic concentration (mg As kg^{-1} wt mass): (A) dimethylarsinic acid; (B) an unknown arsenic compound found also in the NRCC dogfish reference material DORM1 and DORM2. The mammals are identified in Table 1.

Methylarsonic acid was present above the detection limit of the method in only five livers. The concentrations were between 0.002 and 0.025 mg As kg^{-1}. Surprisingly, the ringed seal, RGSL032, the individual with the lowest dimethylarsinic acid concentration (0.004 mg As kg^{-1}) had the highest concentration for methylarsonic acid (0.025 mg As kg^{-1}). In human urine the arsenic concentration ratio of dimethylarsinic acid to methylarsonic acid is about 8:1. The high methylarsonic acid concentration in RGSL032 may indicate a methylation problem within this animal.

The concentration of arsenic acid in the livers of all the marine mammals analyzed was below 0.001 mg As kg^{-1} (wet mass). Arsenous acid could not be detected with anion-exchange chromatography because of the high concentrations of arsenobetaine. In the cation-exchange chromatography arsenous acid has the same retention time as methylarsonic acid but is separated from arsenobetaine. Methylarsonic acid can be separated from arsenous acid/arsenobetaine on the anion-exchange column. Thus, the difference between the concentrations from the signals of methylarsonic acid/arsenous acid from cation-exchange chromatography and the concentration from the signals for methylarsonic acid from anion-exchange chromatography should reflect the concentrations of arsenous acid. Because the concentrations of methylarsonic acid in most of the samples were very small, the concentrations of arsenous acid must be less than 0.001 mg As kg^{-1} (wet mass).

Other arsenic compounds

A signal for an unknown arsenic compound was detected in all of the liver extracts (Fig. 3). The calibration curve for arsenobetaine was used to quantify this compound. The concentrations in the range from 0.002 to 0.027 mg As kg^{-1} (wet mass) (Table 4) account for 1–5% of the total extractable arsenic. The concentration of this unknown arsenic compound increased with the total arsenic concentration (Fig. 4B). This compound, also detected in extracts of the NRCC dogfish reference materials DORM1 and DORM2,[27,32] cannot be one of the dimethyl(ribosyl)arsine oxides that have been identified so far.[2] Under the chromatographic conditions used for the separation of the arsenic compounds in the liver extracts, dimethyl(ribosyl)arsine oxides are clearly separated from this unknown compound (Figs 3A, 3B).

CONCLUSIONS

Among the arsenic compounds identified in the extracts of livers from marine mammals, arsenobetaine is predominant. Dimethylarsinic acid, methylarsonic acid, arsenocholine, tetramethylarsonium ion and an unknown arsenic compound were minor arsenic-containing constituents. These arsenic compounds are frequently detected in marine organisms. Whether they are formed in the body of the marine mammals, or taken up with the food, cannot be deduced from the available data. Identification of arsenic compounds in members of the food web supporting these marine mammals may help to identify the source(s) of these compounds.

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