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Arsenic concentrations and speciation in the tissues of ringed seals (*Phoca hispida*) from Pangnirtung, Canada†

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Concentrations of total arsenic and arsenic compounds were determined in tissues of ringed seals (*Phoca hispida*) using hydride generation–atomic absorption spectrometry and high-performance liquid chromatography–inductively coupled plasma mass spectrometry. Arsenic was accumulated at high concentrations in the blubber as lipid-soluble arsenic compounds (arsenolipids). Arsenobetaine (AB) was the most predominant arsenical in the liver, kidney, muscle and gonad, accounting for about 70% of total arsenic in these tissues. Significant positive correlations were observed for AB concentrations among liver, kidney, muscle and gonad, suggesting that the distribution of AB among these tissues was at equilibrium. Concentrations of AB in the tissues were elevated up to 1–3 years of age, and then fell to a lower level. In contrast, dimethylarsinic acid concentration was significantly increased with age in the liver and kidney. Arsenocholine was also detected in the liver, kidney and gonad, but was not detected in the muscle. Among the arsenic compounds, only AB showed a considerable decrease of the concentration in the gastrointestinal contents with their passing through the gastrointestinal tract, which might indicate that the absorption rate of AB was higher than the absorption rates of other arsenic compounds in ringed seals. To our knowledge, this is the first report of arsenic speciation in several tissues and gastrointestinal contents in marine mammals.

KEYWORDS: arsenic; speciation; ringed seal; HPLC–ICP–MS; arsenobetaine; dimethylarsinic acid; arsenocholine; arsenolipids; body distribution; marine mammals

INTRODUCTION

Arsenic is a ubiquitous element in the marine environment, where it exists in various chemical forms.[1–4] The toxicity and physiological function of arsenic differs greatly with its chemical form.[1,5] Concentrations of total arsenic and several water-soluble arsenic compounds, such as arsenobetaine (AB), arsenocholine (AC), methylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), tetramethylarsonium ion (TETRA), arsenic-containing ribofuranosides, arsenate (As(V)) and arsenite (As(III)) have been determined in lower trophic marine organisms.[2,3] Marine animals such as fish, crustaceans, mollusks and echinoderms contain arsenic mainly as AB, and arsenic-containing ribosides are the predominant forms in marine algae.[1–4] In contrast, knowledge of lipid-soluble arsenic compounds in marine organisms is limited. Until now, two kinds of lipid-soluble arsenic have been isolated and identified as phospholipids in brown alga (*Undaria pinnatifida*)[6] and western rock lobster (*Panulirus cygnus*).[7] Recently, two alkali-labile arsenolipids (a dimethylated arsenic-containing lipid and an arsenocholine-containing lipid) were found as major lipid-soluble arsenic compounds in the tissues of the starspotted shark (*Musterus manazo*).[8]

Determination of arsenic compounds in marine mammals is necessary for a comprehensive understanding of the biogeochemical cycling of arsenic in marine ecosystems, because marine mammals are the top predators in the marine food web. Total arsenic concentrations in the tissues of marine mammals have been determined in several

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studies.[9–11] However, arsenic speciation in the tissues of marine mammals has not been examined, except for a few studies on the liver of pinnipeds and cetaceans.[12–15]

The present study determined total arsenic concentrations in the tissues (liver, kidney, muscle, gonad, blubber, hair and gastrointestinal contents) of ringed seals (*Phoca hispida*). Arsenic speciation was also conducted for liver, kidney, muscle, gonad and gastrointestinal contents to elucidate the accumulation pattern of arsenic compounds in ringed seals.

MATERIALS AND METHODS

Samples

A total of 18 (male, $n = 12$; female, $n = 6$) ringed seals (*P. hispida*) were collected from Pangnirtung, Canada, from 4 to 9 June 1999. Six tissues (liver, kidney, muscle, blubber, gonad and hair) were dissected, and the gastrointestinal contents were also collected from the seals. Tissues and gastrointestinal contents were stored in a deep freezer at −20 °C until analysis. These samples were provided by the indigenous people. Ages of seals were determined by counting growth layers in canine teeth. Moisture contents of liver, kidney, muscle and gonad averaged 68.3%, 75.3%, 69.3% and 77.2% respectively.

Determination of total arsenic

Analysis of total arsenic was based on a procedure described previously.[11] Liver, kidney, muscle gonad, and gastrointestinal contents were freeze-dried and homogenized before digestion. Hair samples were used for analysis after washing with 3% polyoxyethylene lauryl ether, rinsed with MilliQ water (18.2 MΩ cm) and dried. The samples were accurately weighed directly into a Kjeldahl flask, and an acid mixture (HNO_3, H_2SO_4 and $HClO_4$) was added, followed by digestion over 300 °C. Total arsenic concentrations in the solution were measured using a Shimadzu HVG-1 hydride generator coupled to a Shimadzu AA680 atomic absorption spectrometer. In the present study, total arsenic concentrations were expressed on a wet weight basis (μg g^{-1} wet wt) except for hair samples. The accuracy of the method was assessed using the certified reference material (CRM) DORM-2 (National Research Council of Canada); the concentration obtained was in good agreement with the certified value.[16]

Arsenic speciation

Chemical speciation of arsenic was performed according to the method of Goessler *et al.*[17] with slight modifications.[14] Briefly, arsenic compounds were extracted from freeze-dried tissue (*ca* 0.1 g) with a mixture of methanol/MilliQ water (9:1 v/v). After evaporation, MilliQ water was added, and the extracts were kept frozen at −80 °C for later use. Concentrations of arsenic compounds were determined by high-performance liquid chromatography (HPLC; Shimadzu, LC-10A Series) combined with inductively coupled plasma mass spectrometry (ICP—MS; Hewlett-Packard 4500) as an arsenic-specific detector. A Hamilton PRP-X100 anion-exchange column (250 mm × 4.1 mm i.d.) and a Supelcosil LC-SCX cation-exchange column (250 mm × 4.6 mm i.d.) were used for the separation of arsenic compounds. The mobile phase was 6.7 mM $NH_4H_2PO_4$ buffer (pH 6.0) for the anion-exchange column and 6.7 mM pyridine buffer (pH 2.6) for the cation-exchange column. As(III), As(V), MMA, DMA, AB, AC, TMAO and tetramethylarsonium iodide were used as standard substances for qualitative and quantitative analysis. Concentrations of arsenic compounds were expressed on a wet weight basis (μg As g^{-1} wet wt).

In the absence of an appropriate CRM with certified values of organoarsenic compounds, DORM-2 was used for the analysis and the values obtained were compared with those of other studies[16–18] to validate the speciation procedure; the results obtained were satisfactory. To examine the mass balance of arsenic in the entire speciation procedure, the total arsenic remaining in the sample following extraction (residue arsenic) was also measured.

Separation of lipid-soluble arsenic compounds and alkaline hydrolysis

Lipid-soluble arsenic was extracted from freeze-dried samples (liver, kidney, muscle, gonad and stomach contents) and fresh blubber with chloroform/methanol (2:1) according to Hanaoka *et al.*[8] Briefly, water was added to reach a water/chloroform–methanol ratio of 1:4 and the extracts were partitioned into a chloroform phase and a methanol–water phase. The arsenic compounds that separated into the chloroform phase and methanol–water phase were referred to as lipid-soluble and water-soluble arsenic respectively. The chloroform layer was evaporated and then dissolved in ether. Tetraethylammonium hydroxide in methanol was added to the ether solution. A gummy precipitate formed, which was the base-hydrolyzed lipids.

Statistical analyses

Statistical analyses were executed by the program SYSTAT version 9 (SPSS Inc.). Data were tested for goodness of fit to a normal distribution using Kolmogorov–Smirnov's one-sample test. Because some variables were not normally distributed, non-parametric tests were used. The difference between the two groups was examined by the Mann–Whitney's *U*-test. Correlation was expressed by Spearman's rank correlation coefficient.

RESULTS AND DISCUSSION

Body distribution of arsenic

Total arsenic concentrations in the liver, kidney, muscle, gonad, blubber and hair of ringed seals are shown in Table 1. Arsenic concentrations ranged from 0.11 ± 0.04 μg g^{-1} dry wt in the hair to 1.14 ± 0.37 μg g^{-1} wet wt in the blubber (Table 1). A high accumulation of arsenic in the blubber was

Table 1. Concentrations of arsenic compounds and total arsenic in the tissues of ringed seals (range given in parentheses)

Tissue	[As] mean ± SD (μg g^{-1} wet wt)					
	AB	DMA	MMA	AC	Residue	Total As
Liver (n = 18)	0.352 ± 0.175 (0.042–0.819)	0.027 ± 0.008 (0.013–0.046)	0.055 ± 0.040 (n = 2)[a] (<0.02–0.083)	0.041 ± 0.016 (0.020–0.083)	0.046 ± 0.016 (0.020–0.083)	0.48 ± 0.15 (0.19–0.74)
Kidney (n = 18)	0.272 ± 0.152 (0.040–0.619)	0.026 ± 0.006 (0.016–0.037)	0.020 (n = 1)[a] (<0.02–0.020)	0.035 ± 0.010 (n = 17)[a] (<0.015–0.060)	0.022 ± 0.007 (0.011–0.032)	0.35 ± 0.15 (0.11–0.65)
Muscle (n = 18)	0.128 ± 0.063 (0.020–0.251)	0.026 ± 0.006 (0.017–0.036)	0.022 ± 0.002 (n = 4)[a] (0.020–0.024)	<0.015	0.021 ± 0.013 (0.005–0.052)	0.18 ± 0.09 (0.06–0.46)
Gonad (n = 7)	0.165 ± 0.105 (0.074–0.350)	0.014 ± 0.004 (0.010–0.020)	<0.02	0.029 ± 0.017 (0.018–0.068)	0.012 ± 0.010 (0.003 ± 0.029)	0.22 ± 0.09 (0.07–0.35)
Blubber (n = 18)	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	1.14 ± 0.37 (0.60–1.76)
Hair (n = 2)	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	0.11 ± 0.04[c] (0.07–0.14)

[a] The values were calculated using only detected data.
[b] NA: not analyzed.
[c] The value was expressed on a dry weight basis (μg g^{-1} dry wt).

notable for the ringed seal. Similarly, a relatively high concentration of arsenic in blubber was reported by other studies on cetaceans.[9,10] Assuming that the tissue weight ratio as liver:kidney:muscle:blubber:hair is 10:1:100:200:5, it is estimated that about 90% of the arsenic burden in these five tissues is concentrated in the blubber of the ringed seal. Significant positive correlation was observed between total arsenic concentrations in the liver, kidney, muscle and gonad (data not shown). However, this tendency was not observed between blubber and other tissues. This is likely due to the accumulation of different forms of arsenic compounds in the blubber, as discussed below.

The average arsenic concentration in the liver of 18 ringed seals from Pangnirtung (0.48 ± 0.15 μg g^{-1} wet wt; Table 1) was lower than that from Nome, Alaska (1.42 ± 0.56 μg g^{-1} wet wt).[13] In the coastal area around Nome, gold-mining activity began in the 1880s and arsenic concentrations increased. The higher level of arsenic found in ringed seals around Nome is explained by the gold-mining activities.[13] In contrast, anthropogenic input of arsenic seems to be low in the area around Pangnirtung.

AB

AB, DMA, AC and MMA were detected in the tissues of ringed seals (Table 1 and Fig. 1). Among these arsenic compounds, AB was the most predominant arsenic compound, accounting for about 70% of the total arsenic in all the tissue samples analyzed (Table 1). Concentrations of AB in both the liver and kidney were significantly higher than those in the muscle (Mann–Whitney's U-test, $p < 0.001$ and $p < 0.01$ respectively).

Because arsenic intake is mainly via diet[19] and AB is not biosynthesized in the body of marine animals,[2] AB accumulated in the tissues was likely to be taken from the food. The diet of ringed seals was thought to be crustaceans and fish, which varied with habitats and age.[20] In several studies, a high concentration of AB was reported in crustaceans and fish.[1,2] In fact, the major arsenic compound in the stomach contents of ringed seal was AB (Table 2). It has been generally accepted that mammals absorb AB efficiently in the gastrointestinal tract and that it is rapidly excreted into the urine without being biotransformed.[21] Therefore, the concentration of AB in the tissues of ringed seals might reflect recent intake from diet.

Significant positive correlations were found for AB concentrations among liver, kidney, muscle and gonad (Fig. 2). These results suggest that AB was readily transported through blood in the body and the distribution of AB among tissues was at equilibrium. The equilibrium concentration ratio of AB in the liver:kidney:muscle:gonad was about 3:2:1:1.

The concentration of AB tends to be highest at the ages of 1–3 years (Fig. 3). This might be due to the high intake of arsenic by the young ringed seals because of their higher feeding rate. Furthermore, the arsenic concentration in the diet of young seals might also be high.

DMA

DMA was detected in all the tissues analyzed (Table 1). No significant difference was observed among the DMA concentrations in liver, kidney and muscle. On the other hand, the DMA concentration in the gonad was significantly lower than in other tissues (Mann–Whitney's U-test, $p < 0.01$ for liver, $p < 0.001$ for kidney and $p < 0.01$ for muscle). The concentration of DMA was significantly increased with age in the liver (Spearman's rank correlation, $r = 0.533$, $p < 0.05$) and kidney ($r = 0.555$, $p < 0.05$) (Fig. 3). This age-dependent accumulation of DMA was notable because of the genotoxi-

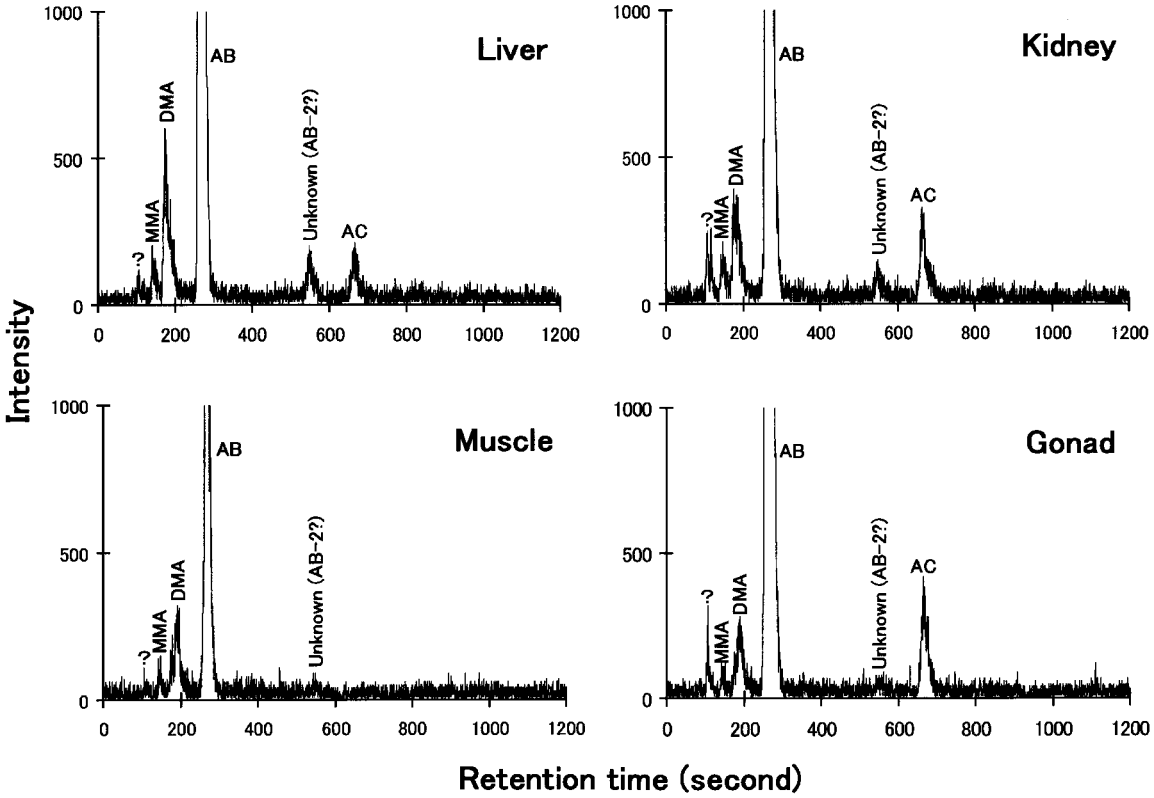

Figure 1. HPLC–ICP–MS chromatograms of aqueous extracts of a ringed seal (male; age, 1 year; length, 103 cm), obtained with Supelcosil LC-SCX cation-exchange column (mobile phase, 6.7 mM aqueous pyridine at pH 2.6; injection volume, 100 μl; flow rate, 1.5 ml min^{-1}).

city of DMA. Recently, some studies have indicated the carcinogenic potential of DMA.[22]

There was a significant positive correlation between DMA concentrations in the liver and in the kidney (Spearman's rank correlation, $r = 0.536$, $p < 0.05$). Goessler *et al.*[13] reported that hepatic DMA concentrations increased with total arsenic concentrations in marine mammals. However, this trend was not observed in the present study.

AC

AC was found in the liver, kidney and gonad of ringed seals (Table 1). In contrast, AC was not detected in any of the muscle samples examined (<0.015 μg g^{-1} wet wt). Significant positive correlation was found for AC concentration between liver and kidney ($r = 0.554$, $p < 0.05$).

MMA

The concentration of MMA in most of the samples was below the limit of detection (0.02 μg g^{-1} wet wt; Table 1). However, up to 0.083 μg g^{-1} wet wt of MMA was observed in one liver sample (male; age, 2 years).

Other arsenic compounds and residue arsenic

The concentrations of TMAO, TETRA, As (V) and As (III) were below the detection limits (<0.08 μg g^{-1}, <0.02 μg g^{-1}, <0.012 μg g^{-1} and <0.008 μg g^{-1} wet wt respectively) in all the samples analyzed. In contrast, TETRA was detected in the liver of ringed seals from Alaska in the range 0.010–0.043 μg g^{-1} wet wt.[13] The residual arsenic accounted for about 10% of total arsenic in the tissue (Table 1). Lipid-soluble or trivalent arsenic compounds would occur as

Table 2. Concentrations of arsenic compounds in the gastrointestinal contents of ringed seal

Position	[As] (μg g^{-1} dry wt)					
	AB	DMA	MMA	AC	TMAO	Residue
Stomach	1.92	0.52	<0.05	0.20	0.26	0.52
Intestine						
front	1.31	0.28	<0.05	0.19	<0.25	0.25
rear	0.68	0.21	<0.05	0.31	<0.25	0.90

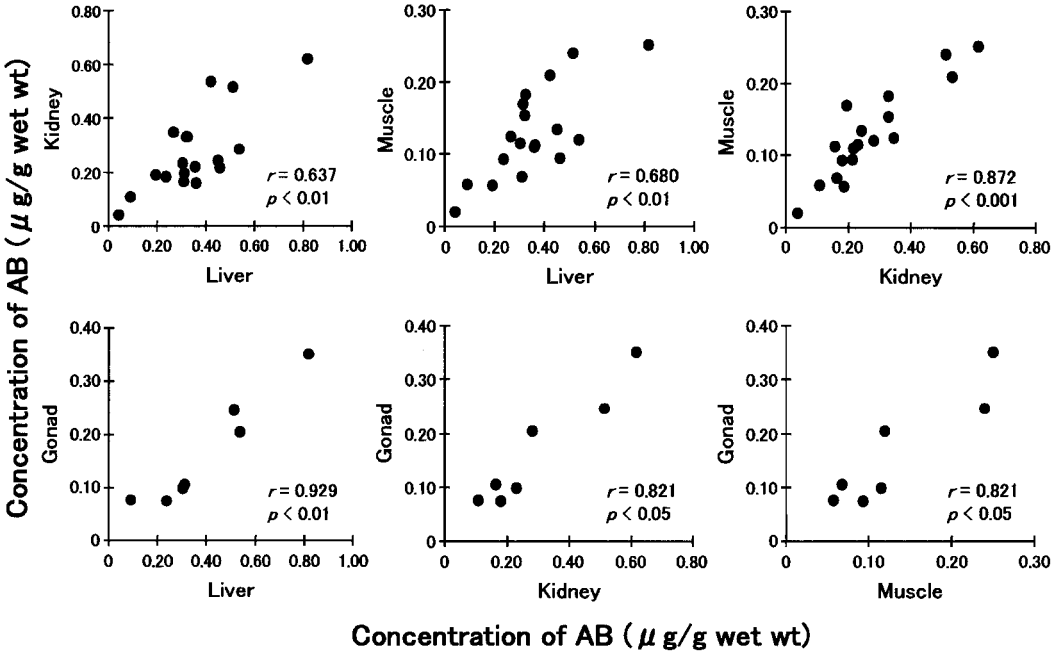

Figure 2. Relationship between AB concentrations (as arsenic) in tissues of ringed seals.

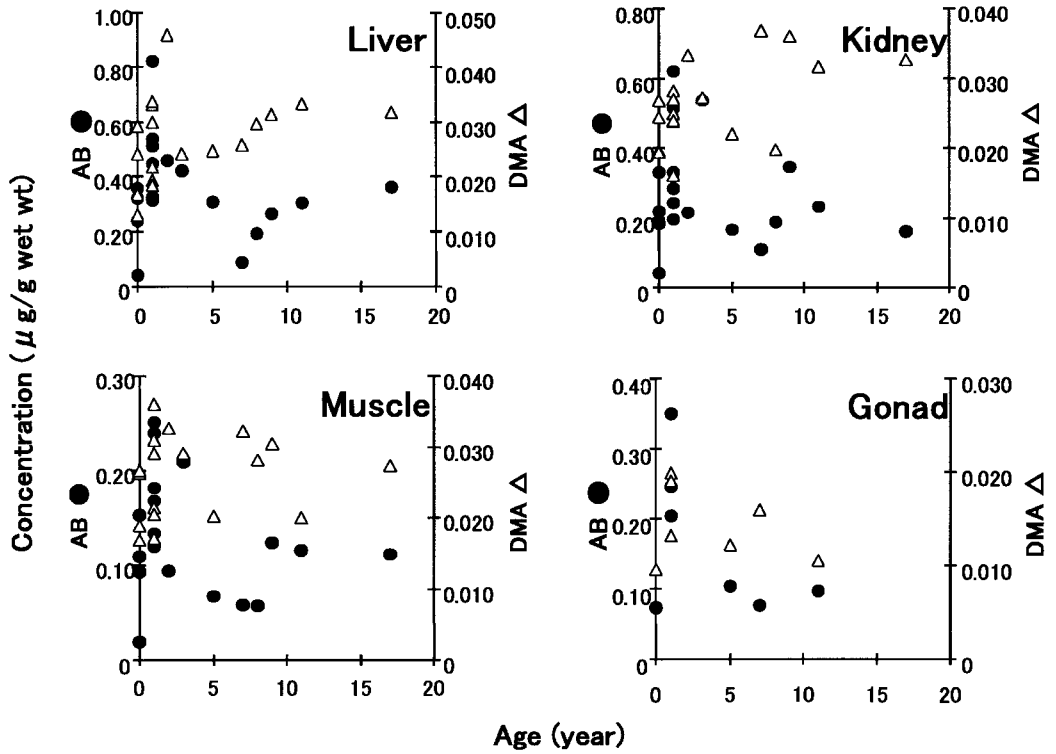

Figure 3. Variations of AB and DMA concentrations (as arsenic) with age in tissues of ringed seals.

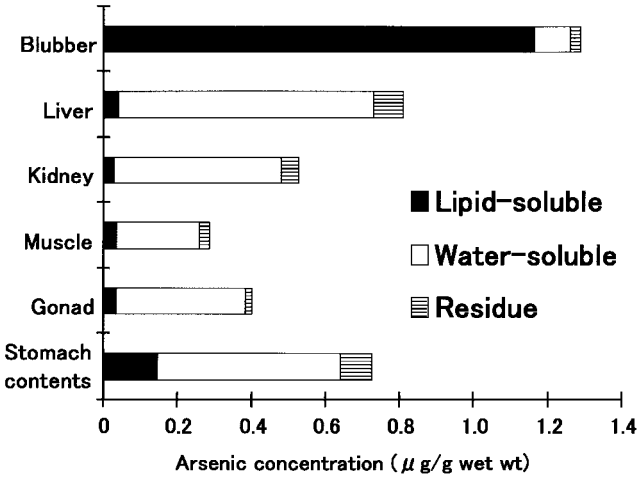

Figure 4. Concentrations of lipid-soluble, water-soluble and residue arsenic in tissues and stomach contents of ringed seals.

residue because these compounds could not be extracted by methanol–MilliQ water from the samples.[23,24]

Trimethyl(2-carboxyethyl)arsonium ion (AB-2) might be present in some samples (Fig. 1). This compound is an arsenic-containing betaine, recently identified in a coral reef fish (*Abudefduf vaigiensis*) by LC–MS.[25] The AB-2 also occurred in DORM-2 and the liver of four species of marine mammals.[25] We assume that the unknown compound (retention time 548 s) in Fig. 1 is AB-2 because of the similarity in the retention time of an unknown compound in DORM-2 (probably AB-2)[25] and the unknown compound in the tissue of ringed seal (Fig. 1).

Gastrointestinal contents

AB, DMA, AC and TMAO were found in the gastrointestinal contents of ringed seal (Table 2), which may indicate that these arsenic compounds are taken from the diet by the ringed seal. It is known that the organic arsenic compounds are absorbed efficiently in the gastrointestinal tract.[21] Notably, the AB concentration in gastrointestinal contents decreased with their passing through the gastrointestinal tract (Table 2). In mammals, AB is excreted without biotransformation,[21] whereas absorbed AC and DMA could be metabolized. It is known that AC is oxidized to AB in mammals.[21] It was also reported that DMA was, to some extent, biotransformed to TMAO and other unknown compounds in rats.[26] However, TMAO was not detected in the tissues of ringed seals, although some intake of DMA was expected (Table 2) and DMA was present in the tissues of ringed seal (Table 1).

An unknown arsenic compound, whose chromatographic properties were similar to those of the unknown arsenic compound in the tissues as described above, was also found in the stomach contents of ringed seal (not shown). Hence, this unknown compound, assumed to be AB-2, in the tissues of ringed seals might have come from their diet.

Lipid-soluble arsenic compounds

Lipid-soluble arsenic compounds were minor in the liver, kidney, muscle and gonad of ringed seals (Fig. 4). However, high concentrations of lipid-soluble arsenic were observed in the blubber, accounting for about 90% of total arsenic. This lipid-soluble arsenic was partly digested by tetraethylammonium hydroxide (not shown). Hanaoka *et al.* suggested the presence of DMA- and AC-containing lipids as alkali-labile compounds in the tissues of *M. manazo*.[8] Hence, similar arsenic compounds might be present in the blubber of ringed seals. Identification of these arsenic compounds would help in understanding the metabolism of arsenic in ringed seals.

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