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Occurrence of Butyltin Compounds in Beluga Whales (*Delphinapterus leucas*)

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Fifty-two beluga whale samples (Six liver samples, and 46 blubber samples including ones from different depths of the fat layer) from the St Lawrence River, Canada, were analyzed for butyltin compounds (mono-, di-, and tri-butyltin) with a view to investigating the occurrence and contamination of butyltin in these animals. A special procedure was also developed for the determination of butyltin compounds in blubber samples with high lipid content (up to 95%). Total-butyltin concentrations in liver samples were found to be much higher than those in the blubber samples. The concentrations of butyltin compounds in blubber samples were observed to be related to their lipid content. Concentration levels of butyltin species in beluga whale were compared with those in other marine vertebrates in other parts of the world. The presence of butyltin compounds in liver and blubber samples suggests the accumulation of these toxicants by beluga whale. © 1998 John Wiley & Sons, Ltd.

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INTRODUCTION

The occurrence of butyltin compounds in the aquatic environment[1–3] and aquatic organisms[4,5] has been reported in the literature. Recently, the occurrence of tributyltin and its degradation products has been reported in marine bottlenose dolphins (*Tursiops truncatus*), pygmy sperm whale (*Kogia breviceps*) and Atlantic spotted dolphin (*Stenella frontalis*) along the US Atlantic and Gulf coasts.[6] It was suggested that the high accumulation of TBT and DBT, which are potential immunosuppressants, might have contributed to bottlenose dolphin mortality events in these areas. Accumulation of butyltin compounds was also found in dolphins (*Platanista gangetica*) in freshwater systems.[7]

The beluga whales (*Delphinapterus leucas*) in the estuary of the St Lawrence River have been the subject of intensive investigation. Studies included toxic compounds and health and reproductive effects,[8] as well as the presence of lipophilic organochlorine compounds such as polychlorinated biphenyls (PCBs) and chlorinated pesticides.[9,10]

The objective of this study was to determine concentrations and accumulation of butyltin compounds in blubber and liver of beluga whale collected in the St Lawrence River estuary. To our knowledge, this is the first report on the occurrence of butyltin compounds in beluga whales.

The lipid content in blubber samples from beluga whales is high, ranging from 68 to 96%. Normal digestion methods are not suitable for this type of sample. A special procedure had to be developed for fat removal before the analysis. The present study describes an efficient clean-up procedure for fat removal prior to speciation analysis of the derivatized butyltin compounds by the gas chromatography – plasma atomic emission spectrometry (GC–AED) technique.

MATERIALS AND METHODS

The organotin compounds and special reagents were obtained from either Alfa AESAR (Ward Hill, MA, USA) or Aldrich (Milwaukee, WI, USA). The carrier gas for the chromatography and make-up gas for the plasma (helium, high-purity, 99.999%) and the spectrometer purge gas (nitrogen, ultra-high

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purity) were supplied by Canox (Canada). Other solvents, acids and common laboratory reagents were of analytical grade. Distilled water further purified by the Milli-Q system (Millipore, USA) was used throughout the experiments. Individual stock solutions of organotin compounds (1000 μg ml^{-1} as Sn) were prepared by dissolving the equivalent amounts of organotin in methanol or toluene. Speciation analysis of the butyltin compounds was carried out by the GC–AED technique. A description of the GC–AED system and operational parameters have been given in a previous publication.[3]

Samples of blubber and liver from beluga whale (*Delphinapterus leucas*), found dead in the St Lawrence River estuary, were obtained frozen from Dr R. Bailey of the Institute Maurice Lamontagne, Department of Fisheries and Oceans, Mont-Joli, Quebec. All samples were frozen, and stored in amber glass jars. They were freeze-dried and homogenized in our laboratory and stored in the freezer before analysis. Water samples were not available, so it was not possible to estimate bioaccumulation factors.

Sample digestion and clean-up

Difficulties in speciation analysis of biological samples include dissolution of samples without destruction of the chemical structures of the analytes, and the isolation of the analytes from the complex sample matrices for analysis. The conventional acid digestion or ashing techniques with oxidizing acids are not suitable. This problem has been overcome by the use of a tissue solubilizer, such as tetramethylammonium hydroxide (TMAH), which dissolves the sample and liberates the analytes of interest.[11]

Liver samples

Samples (1 g) containing tripentyltin (TPeT) as an internal standard (0.5ml of a 1μg Sn ml^{-1} solution; absolute amount, 0.5 μg as Sn) were digested in 5 ml of 25% aqueous solution of TMAH in a 50 ml Erlenmeyer flask at 60°C on a hot plate for 60 min with occasional shaking. After the addition of 10 ml of water, 5 ml of acetic acid, 6 g of NaCl and 4 ml of 0.2% tropolone–toluene solution, the mixture was stirred magnetically for 60 min, after which 2 ml of toluene was removed and dried using a stream of nitrogen. The volume was brought back to 1 ml with hexane and the mixture was allowed to react with 0.5 ml of ethylmagnesium bromide (1.0M in THF) for 5 min. After the destruction of the excess ethylmagnesium bromide by 3 ml of 0.5M H_2SO_4, the organic layer was removed quantitatively and cleaned by silica gel in a micro-column using a Pasteur pipette (packed with 5 cm of silica gel, topped with 1 cm of anhydrous Na_2SO_4) and eluted with 5 ml of hexane. After reduction of the eluate volume to 1 ml by nitrogen, 1 μl of the eluate was injected into the GC–AED system for analysis.

Blubber samples

Blubber samples contain mostly fat and lipids which cause difficulties in sample digestion and clean-up. The use of a tissue solubilizer, such as tetramethylammonium hydroxide (TMAH), was investigated in breaking down the complex matrices before the clean-up procedures. A single stage clean-up with silica gel was found insufficient to remove the fat. Double-stage clean-up with a gel-permeation chromatography (GPC) technique and silica gel was used.

A 1g blubber sample was weighed in a 50 ml Erlenmeyer flask, 5 ml of 25% aqueous TMAH solution was added and the sample was digested for 60 min at 60°C. Then 5 ml of acetic acid, 10 ml of H_2O, 6 g of NaCl and 3 ml of 0.4% tropolone–toluene solution were added and stirred magnetically for 60 min; the organic layer was removed quantitatively by adding water to the flask to bring the solvent up to the neck of the flask, and evaporated to 2 ml in a test tube by a stream of nitrogen. The extract was allowed to react with 1 ml of ethylmagnesium bromide (1.0 M in THF) for 5 min. The extraction and derivatization procedures were slightly modified by increasing the amount of reagents to counteract the large amounts of lipids and other organic compounds resulting from the destruction of the samples. After destruction of the excess ethylmagnesium bromide by addition of 4 ml of 0.5 M H_2SO_4, the organic layer was quantitatively removed and loaded into a gel-permeation chromatography column (GPC), 2.3 cm (i.d.) × 50 cm (height), filled with pre-washed Biobeads, S-X3, 200–400 mesh. The GPC column was eluted with a hexane–dichloromethane solution (50:50, v/v) to separate the butyltin compounds from the co-extracted lipids. The fraction (100–140 ml) which had been shown to contain the butyltin compounds and some co-eluted lipids (less than 5%) was collected. A further clean-up step was required to remove the residual impurities present in the eluate. After evaporation of the eluate to 1–2 ml, it was loaded onto a silica-

Table 1 Recoveries of butyltin compounds from spiked beluga blubber sample[a]

	MBT	DBT	TBT	TPeT
Recovery (%)	93.2 ± 7.9	91.0 ± 4.4	81.0 ± 5.2	81.0 ± 3.3

[a] $n = 3$. Spike: 0.1 μg Sn/g blubber sample.

gel column (15 cm × 1.5 cm i.d.) containing about 6 g of silica gel packed from bottom to top in the following order: glass wool, silica-gel bed, 1 cm anhydrous Na_2SO_4. The butyltin compounds could be eluted quantitatively by 25 ml of hexane. After reduction of the eluate volume to 1–2 ml under reduced pressure in a rotary evaporator at 30°C, the eluate was transferred to a test-tube and further evaporated under a stream of nitrogen to 1 ml, from which 1 μl was injected to the GC–AED system for analysis.

Lipid content was determined by mixing a 1 g blubber sample with anhydrous sodium sulfate, and milling with 10 ml of hexane. After centrifuging, the solution was removed and another 10 ml of hexane was added to repeat the lipid extraction. The hexane extracts were combined and evaporated. The weight of the residue was used for calculation of the lipid content.

Recoveries

The experiment on the recoveries was performed by spiking 100 μl of a mixed MBT, DBT, TBT and TPeT standard solution (1 μg Sn ml^{-1}) into 1 g of butyltin-free blubber sample. The sample was dried in the air for 1 h and carried through the whole extraction procedure mentioned above. The recoveries were evaluated by comparing the peak areas with those of the corresponding butyltin compounds of a mixed standard solution directly derivatized with ethylmagnesium bromide without the sample and without going through the extraction and the clean-up processes. The recoveries of the individual butyltin compounds are given in Table 1, representing the absolute recovery of the method. In spite of the multiple steps in sample dissolution and clean-up procedure, the recoveries for individual butyltin compounds are satisfactory. The concentrations of butyltin compounds in beluga tissues were not corrected for the recoveries.

RESULTS AND DISCUSSION

Analysis of biological samples normally requires a digestion step for breaking down the tissue to release the analytes of interest before any analytical procedures. For element speciation analysis, the common digestion procedures involving the use of strong acids or oxidizing agents are not suitable. Two techniques have been used successfully for the digestion of biological samples without altering the chemical forms (speciation) of the analytes, namely enzyme hydrolysis with mixed enzymes (lipase and protease)[12] and use of tissue solubilizers, such as tetramethylammonium hydroxide (TMAH).[13] The tissue solubilizer was preferred because of its effectiveness in digesting the samples.[11]

Although gel permeation chromatography (GPC) is effective in removing lipids from biological

Table 2 Concentrations of butyltin compounds in beluga liver samples[a]

			Concentration (ng Sn g^{-1} dry wt)				
Sample no.	Sex[b]	Age (y)	MBT	DBT	TBT	BTs[c]	TBT/BTs
DL-143-88	f	0.1	— [d]	—	—	—	—
DL-10-88	f	1.3	196.2	—	2.0	198.2	0.010
DL-1-88	m	3.5	106.4	203.2	87.1	396.7	0.220
DL-12-88	f	16	303.1	396.6	101.2	800.9	0.126
DL-7-88	m	19+	372.1	72.2	72.3	516.6	0.140
DL-8-88	m	20+	845.7	515.3	84.1	1445.1	0.058

[a] $n = 3$, RSD = 2.5–18.3
[b] f, female; m, male.
[c] Total butyltin BTs = MBT + DBT + TBT.
[d] — , Not detected (< 0.5 ng Sn/g dry weight).

samples, it cannot totally separate the lipid components and derivatized organotin species in beluga blubber samples. A second silica-gel column was necessary to clean the residual impurities. After the two-stage clean-up procedure, the interference due to lipids was eliminated for chromatography.

All three species of butyltin (TBT, DBT, MBT) were present in four of the six liver samples (Table 2). In each sample, the concentrations of both degradation products, DBT and MBT, were higher than that of TBT, suggesting degradation of TBT in the sample matrix. In one sample, the MBT species was present in high concentration without the presence of DBT and TBT.

That the total butyltin (TBT + DBT + MBT) content in the liver increases with the age of the whale suggests high accumulation factors for butyltin in the beluga liver. In an independent study of accumulation of tributyltin and its degradation products in dolphins and whales,[6] the high total butyltin concentrations were also found in liver, indicating high concentration factors in that organ.

Most of the 28 beluga blubber samples were taken from the middle of the fat layer. TBT was present in 27 of them whereas DBT was found in 16 samples and MBT in only six samples (Table 3). The pattern of distribution of butyltin compounds in blubber samples was quite different from that in the liver samples. The overall total butyltin concentrations in liver tissue were generally higher than those in the blubber tissue. Since the concentrations of DBT and MBT in liver tissue were higher than that of TBT, high degradation rates of TBT in liver were suggested. The lack of degradation products in the blubber tissue may indicate that the degradation rates are lower in fat matrix, or, if there was

Table 3 The concentrations of butyltin compounds in beluga blubber samples[a]

Sample no.	Sex[b]	Age(y)	Lipid (% wet wt)[c]	Depth	Concentration (ng Sn g^{-1} dry wt)		
					MBT	DBT	TBT
DL-10-88	f	1.3	86	Middle	—[d]	5.4 ± 1.3	10.7 ± 0.4
DL-102-87	f	2.5	86	Top	d[e]	d	d
DL-1-88	m	3.5	87	Middle	—	—	d
DL-101-91	m	11+	68	nr[f]	3.2 ± 0.0	6.6 ± 1.0	17.6 ± 1.4
DL-126-86	m	14+	96	nr	—	—	—
DL-12-88	f	16	91	Top	—	d	10.5 ± 0.5
DL-1-87	f	18+	88	Middle	—	9.4 ± 1.3	6.3 ± 1.3
DL-3-87	f	19+	86	Middle	—	—	10.2 ± 3.4
DL-7-88	m	19+	77	Middle	d	7.8 ± 1.9	18.8 ± 0.9
DL-109-87	f	19+	86	Middle	—	—	9.1 ± 1.0
DL-1-91	f	19+	81	Middle	—	3.3 ± 0.4	17.8 ± 1.4
DL-8-88	m	20+	82	Middle	d	d	8.6 ± 0.1
DL-163-88	f	20+	89	Middle	—	6.0 ± 0.3	21.9 ± 0.9
DL-6-91	m	20+	78	Middle	—	d	7.3 ± 0.3
DL-127-86	m	20+	86	Middle	—	—	4.9 ± 0.4
DL-4-88	f	21+	85	Middle	—	4.3 ± 0.3	10.7 ± 0.6
DL-13-88	f	21+	86	Middle	—	—	7.4 ± 1.9
DL-9-88	f	22+	91	Middle	—	2.8 ± 0.4	10.0 ± 0.1
DL-130-87	m	22+	87	Middle	—	—	9.7 ± 0.4
DL-3-91	m	22+	85	Middle	—	d	11.6 ± 0.4
DL-14-88	f	23+	88	Middle	—	—	6.9 ± 0.9
DL-106-88	f	23+	89	Middle	—	—	8.1 ± 0.9
DL-125-87	m	23+	79	nr	d	d	16.9 ± 0.6
DL-2-87	f	25+	86	Middle	—	—	4.3 ± 0.5
DL-11-88	f	25+	87	Middle	—	—	5.9 ± 0.3
DL-2-91	f	27+	85	Middle	—	8.3 ± 0.5	19.0 ± 1.0
DL-133-87	f	29+	85	Middle	—	—	7.8 ± 0.9
DL-139-88	f	31+	88	Middle	d	d	23.9 ± 0.9

[a] $n = 2$.
[b] f, Female, m, male.
[c] Hexane-extractable lipids.
[d] — , Not detected (< 0.5 ng Sn g^{-1} dry weight).
[e] d, Detected (< 2 ng Sng^{-1} dry weight).
[f] nr, No record.

Table 4 The concentrations of butyltin compounds in different fat layers of beluga whales[a]

Sample no.	Depth	Concentration (ng Sn g^{-1} dry wt) Lipid (% wet wt)[b]	MBT	DBT	TBT
DL-4-88	Top	94	— [c]	—	6.1 ± 0.3
	Middle	85	—	4.3 ± 0.3	10.7 ± 0.6
	Bottom	81	d[d]	22.3 ± 0.7	30.1 ± 0.5
DL-7-88	Top	87	—	6.4 ± 0.1	11.1 ± 1.5
	Middle	77	d	7.8 ± 1.9	18.8 ± 0.9
	Bottom	72	5.4 ± 0.2	7.8 ± 0.3	20.6 ± 0.3
DL-8-88	Top	89	—	d	4.3 ± 0.9
	Middle	82	d	d	8.6 ± 0.1
	Bottom	77	3.9 ± 0.4	6.6 ± 0.8	21.3 ± 3.7
DL-9-88	Top	91	—	—	d
	Middle	91	—	2.8 ± 0.4	10.0 ± 0.1
	Bottom	87	—	7.4 ± 0.5	25.7 ± 3.4
DL-10-88	Top	94	—	—	d
	Middle	86	—	5.4 ± 1.3	10.7 ± 0.4
	Bottom	80	7.0 ± 0.1	5.3 ± 0.3	17.1 ± 0.5
DL-12-88	Top	95	—	—	d
	Middle	84	—	d	10.5 ± 0.5
	Bottom	86	d	d	16.2 ± 0.1

[a] $n = 2$.
[b] Hexane-extractable lipids.
[c] — , Not detected (< 0.5 ng Sng^{-1} dry weight).
[d] d, Detected (< 2 ng Sng^{-1} dry weight).

degradation, the products DBT and MBT may not have been retained in the fat medium due to the hydrophilicity of these molecules (Table 3). In a few selected blubber samples where samples were taken at different depths of the fat layer, it was observed that concentrations of butyltin compounds

Table 5 Butyltin compounds in marine vertebrates

Animal	*n*	MBT	DBT	TBT	Reference
US Atlantic and Gulf coasts					
Liver (Bottlenose dolphins)	17	22–1537	28–4233	2.4–316	6[a]
Blubber (Bottlenose dolphins)	1	53	158	98	6[a]
Liver (Atlantic spotted dolphins)	2	11–116	29–194	5–31	6[a]
Blubber (Atlantic spotted dolphins)	1	75	6	33	6[a]
Liver (Pygmy sperm whale)	3	59–109	122–148	2–5	6[a]
Mediterranean Sea					
Blue shark Liver (Blue shark)	5	3–9	2.2–3	5–9	14[a]
Blubber (Blue shark)	5	< 3.4	< 0.5	0.4–3.7	14[a]
St Lawrence River, Canada					
Blubber (Beluga whales)	28	0–2.9	0–8.3	0–20.8	This work[b]
Liver (Beluga whales)	6	0–68.2	0–41.6	0–8.2	This work[b]

[a] Original concentrations ng/g wet wt. (as the cation) were converted to ng/g wet wt. (as Sn) for comparison;
[b] Original data in ng Sn/g dry wt. were converted to ng Sn/g wet wt. for comparison;
n = number of samples.

varied with the depth of the fat layer (Table 4). The concentrations of butyltin compounds in blubber samples were observed to be related to lipid content. Higher butyltin concentrations were always found in the bottom fat layer, which comprised a relatively lower lipid content and visually observable blood and muscle tissue. The butyltin compounds are polar in different degrees, with the MBT being the most polar species; their concentrations at the various depths of the fat layer with different lipid contents are expected. All three butyltin species were detected in most samples containing less than 80% lipids, whereas they were barely present in the blubber samples having lipid content greater than 90%. It is believed that the lipid content affects the bioaccumulation of butyltins in blubber of beluga whale, so it becomes difficult to interpret the concentrations of butyltin compounds in fat layers from beluga whales of different ages in Table 3.

The ratio of TBT concentration to total butyltins [TBT + DBT + MBT] is often used as an indication of degradability of TBT in different media.[5] The ratios for beluga liver samples ($n = 6$) ranged from 0.01 to 0.22, (average = 0.11), which might indicate that TBT was metabolized at faster rates in liver than in the fat tissue, where the concentration of TBT was dominant in most samples. Despite the small number of samples investigated, the concentrations of butyltin compounds in beluga whale were comparable with those reported in dolphins[6] and other marine vertebrates[14] caught in other locations (Table 5). The butyltin concentrations in liver and blubber tissues of beluga whale were in general comparable with those in the Atlantic spotted dolphins, but quite different from those of the Mediterranean blue sharks and of the pygmy sperm whales. In other words, both these marine mammals may exhibit a similar physiology in accumulating and degrading tributyltin compounds in their ambient environment.

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