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The quantitation of butyltin and cyclohexyltin compounds in the marine environment of British Columbia

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A HPLC/GF AA procedure based on the use of C-18 columns is described for the quantitation of butyltin species in marine samples. When a mass spectrometer was used as detector (HPLC MS), evidence was obtained for the presence of other tin compounds in the samples. Extracts of samples were treated with CH_3MgBr and examined by using GC MS; the presence of Bu_nSnMe_{4-n} (n = 3–1, Bu = butyl) and Cy_nSnMe_{4-n} (n = 3, 2, Cy = cyclohexyl) was confirmed in the derivatized seawater, bivalve flesh, and bivalve shell samples. Quantitative data are given for butyl- and cyclohexyl-tin species in seawater and the surface microlayer, and oyster flesh.

Keywords: Butyltin species, cyclohexyltin species, Plictran, Cyhextin, oysters, seawater, surface microlayer, HPLC AA, GC MS, HPLC MS

INTRODUCTION

Much has been written about the problems associated with the use of tributyltin derivatives in antifouling paint[1–6] and there seems little doubt that high concentrations of tributyltin compounds and their metabolites and degradation products can be found in water, sediments, and biota sampled from or near marinas. There also seems little doubt that high concentrations of tributyltin compounds are environmentally unacceptable. However, there do seem to be some doubts about the impact of low concentrations (ppt, ng dm^{-3}) of these compounds, and in order to make proper decisions regarding the regulation of use of tin-based antifouling paints these doubts need to be resolved.[6]

The method most commonly used for the analysis of butyltin compounds involves treatment of the sample with sodium borohydride ($NaBH_4$) to yield hydrides that are volatile enough to be separated either on the basis of relative volatility or by gas chromatography. Specific compounds are usually identified and quantitated by measuring retention times and by using tin-selective detectors. There are reports of the presence of unidentified tin compounds in samples that have been examined by this procedure.[7,8] A more satisfactory analytical procedure, from the point of view of compound identification, is to react the sample with a Grignard reagent (RMgX) to afford the butyltin compounds Bu_nSnR_{4-n}, n = 3–1. These stable alkyl derivatives are best separated, identified, and quantitated by using GC MS (GC FID, GC AA, etc. can be used, but this involves the loss of structural information).

The methods described above require the preparation of a volatile derivative and it would be desirable to develop a separation technique that would eliminate this step.

The first part of this communication describes an attempt to develop an HPLC/GF AA procedure to quantitate butyltin compounds in samples of marine biota. Because these studies, when extended to the use of HPLC MS, revealed the presence of organotin compounds other than butyl derivatives, it became necessary to employ the Grignard derivative/GC MS procedure to identify these compounds. As a result, the identification and quantitation of cyclohexyltin compounds in marine samples is described in the second part.

EXPERIMENTAL

The HPLC system consisted of Waters Associates' Models M45 and M510 pumps, and an automated gradient controller. This was fitted

with a C-18 reversed-phase steel column (μ-Bondapak, 3.9 mm × 30 cm) and a silica guard column. The effluent from the HPLC column was collected with the aid of a Gilson microfractionator and transferred manually to the automatic sample delivery system of the graphite furnace atomic absorption spectrometer (GF AA). HPLC MS measurements were made by using a Kratos MS 80 RFA mass spectrometer equipped with a Vestec Kratos thermospray interface. GC MS measurements were made by using a Carlo Erba 4160 gas chromatograph. The capillary column (J and W DB-1, 0.32 mm × 15 m) was directly coupled to the ion source of the Kratos mass spectrometer. The atomic absorption instrument was a Varian 1275 spectrophotometer equipped with a deuterium background corrector and a Varian GTA-95 graphite-tube atomizer. The spectrophotometer was operated at 1 nm spectral-slit width. The hollow cathode lamp was supplied by Hamamatsu Photonics of Japan, and operated at a current of 8 mA. The 224.6 nm tin spectral line was used for all analyses. The pyrolytically coated graphite tubes were obtained from Varian Techtron. A Sorvall Superspeed RC 2-B automatic refrigerated centrifuge operating at 2500 rpm was employed in the extraction procedure. Tetrapropyltin (Pr_4Sn) was synthesized from PrMgBr. Other organotin compounds were purchased from Alfa Inorganics and the Aldrich Chemical Company.

Seawater samples were collected by using Niskin bottles. The water was filtered immediately after collection and stored frozen in polyethylene bottles. Some surface microlayer samples were collected by deploying a sheet of glass 75 cm × 150 cm × 8 mm from the ship's deck with the aid of a winch, in a similar manner to that described for 'hand dipping' of smaller glass sheets.[9] This hand-dipping technique was used in Vancouver Harbour. The microlayer was collected from the glass with the aid of Teflon squeegies[7] and stored frozen, unfiltered, in polyethylene bottles. Samples of biota were frozen immediately after collection.

HPLC separation of organotin compounds

Initial studies were conducted by injecting acetone solutions of Bu_3SnCl and Bu_2SnCl_2 onto the C-18 HPLC column and monitoring the effluent by using GF AA. A wide variety of eluents were investigated. The most satisfactory proved to be 2% tetrahydrofuran/98% acetone containing 2% acetic acid. This solvent system does not separate Bu_2SnCl_2 from $BuSnCl_3$; however, we were unable to find one that does.

Recovery studies

Aliquots (0.5–1.5 cm^3) of Bu_3SnCl and Bu_2SnCl_2 working solutions (10 $\mu g\ cm^{-3}$ in acetone) corresponding to 5–15 μg of the tin compounds were added to test-tubes so that each tube contained the same amount of the two compounds. The solutions were reduced in volume to ~0.2 cm^3 by gentle warming on a water bath. Standard dogfish liver (0.1 g) was added to each tube and the contents were vortex-mixed. The samples were worked up and analysed as described for tissue samples; one 50-μl injection onto the HPLC column provided enough sample for analysis.

Tissue samples: HPLC analysis

Frozen marine bivalves were allowed to thaw and then gutted. Tissue samples (34–220 g, wet weight), were placed in a blender with water (100 cm^3), homogenized for 3 min, and the resulting slurry transferred to an Erlenmeyer flask (1 dm^3) Sodium chloride (20 g), concentrated hydrochloric acid (HCl 12 M; 50 cm^3) and dichloromethane (CH_2Cl_2; 100 cm^3) were added to the slurry and mixed on a mechanical shaker (30 min). The mixture was centrifuged for 20 min and the CH_2Cl_2 layer separated. The remaining aqueous slurry was re-extracted with CH_2Cl_2 (50 cm^3, ×2). The CH_2Cl_2 fractions were pooled and evaporated to dryness. The residue was dissolved in hexane, filtered, and made up to volume (50 cm^3) with hexane. This solution was used for the HPLC analysis (50-μl injection). Fractions of the eluate were collected (30 s intervals) for analysis where appropriate. In order to bring the tin concentration to a value convenient for HPLC analysis, fractions from replicate injections (up to 20) were pooled and evaporated to about one-third the original volume. This solution was analysed by GF AA (40-μl injection). The modifier was 20 ppm palladium in 2% citric acid (5 μl).

Shell samples: HPLC analysis

Dry shells (12–36 g) were crushed with the aid of a mortar and pestle; the powder was transferred to a 250 cm^3 beaker and dissolved in 50 cm^3 of concentrated HCl. The resulting solution was filtered if necessary and diluted to 100 cm^3 with water. This solution was used for the HPLC analysis (50-μl injection) as described above for tissue samples.

Tissue samples: GC analysis

The procedure was essentially the same as that described for HPLC analysis, except that diethyl ether was used for the extraction in place of CH_2Cl_2. After pooling the extracts, the organic phase was evaporated to dryness, and, for quantitation, an aliquot of a heptane solution of tetrapropyltin was added at this stage. Methylmagnesium bromide (0.5–1 cm^3 of a 3 mol dm^{-3} solution in diethyl ether) was added to the sample, which was then washed with dilute sulphuric acid (H_2SO_4) to remove excess Grignard reagent. For clean-up purposes, the organic phase was passed through a small silica-gel column prepared in a Pasteur pipette. The organotin compounds were eluted with pentane ($\sim$3 cm^3). The eluate was reduced in volume to $\sim$0.4 cm^3 with the aid of an air stream and 0.2 cm^3 heptane was added. About 1 μl of the sample was injected (splitless injection) onto a fused-silica capillary column. The column temperature was programmed from 50 to 240 °C at the rate of 20 °C min^{-1}. The mass spectrometer was operated in the electron impact (EI) mode, scanning from *m/z* 50 to 500. For quantitation the selected ion-monitoring technique was adopted. Monitored masses were *m/z* 161, 163, 165 [$(CH_3)_3Sn^+$] for monobutyltrimethyltin, *m/z* 203, 205, 207 [$Bu(CH_3)_2Sn^+$] for dibutyldimethyltin, *m/z* 245, 247, 249 (Pr_3Sn^+) for tetrapropyltin (internal standard), *m/z* 189, 191, 193 [$Bu(CH_3)HSn^+$] for tributylmethyltin, *m/z* 229, 231, 233 [$Cy(CH_3)_2Sn^+$] for dicyclohexyldimethyltin, and *m/z* 215, 217, 219 [$Cy(CH_3)HSn^+$] for tricyclohexylmethyltin. All MS measurements were repeated three times; the total additive ion currents of the three masses was used (peak area measurement provided better precision than peak height). Linear calibration curves were established as follows (RI = relative intensity and C = relative concentration with respect to the standard):

$BuSn(CH_3)_3$: $RI = 0.78094 \times C - 0.001901$ ($r = 0.963$)

Bu_2SnMe_2: $RI = 1.17976 \times C + 0.000109$ ($r = 0.999$)

$Bu_3Sn(CH_3)_2$: $RI = 2.75006 \times C + 0.004477$ ($r = 0.991$)

$Cy_2Sn(CH_3)_2$: $RI = 1.28906 \times C + 0.001066$ ($r = 0.998$)

Cy_3SnCH_3: $RI = 1.93407 \times C + 0.00084$ ($r = 0.998$)

The detection limit was less than 10 pg.

Shell samples: GC method

An aliquot of the acid solution (100 cm^3) prepared as for the HPLC method was extracted with a solution of tropolone in pentane (0.05%, w/v). The organic phase was dried ($MgSO_4$) and treated with CH_3MgBr as described above.

Seawater and microlayer: GC method

Seawater (250 cm^3) was spiked with the internal standard (tetrapropyltin in CH_3OH) so that the concentration of tin was about 1 ng cm^{-3}. The sample was acidified with HBr (5 cm^3) and extracted ($\times$2) with a solution of tropolone in pentane (0.1%, w/v). The combined extracts were dried and taken with care nearly to dryness with the aid of an air stream (Pr_4Sn can be lost at this stage, so care is necessary). Heptane ($\sim$0.5 cm^3) was added, followed by CH_3MgBr as described above.

HPLC/MS analysis

The eluent used for this analysis was the same as that described above. The flow rate through the HPLC column was 0.6 cm^3 min^{-1} and the ionizing

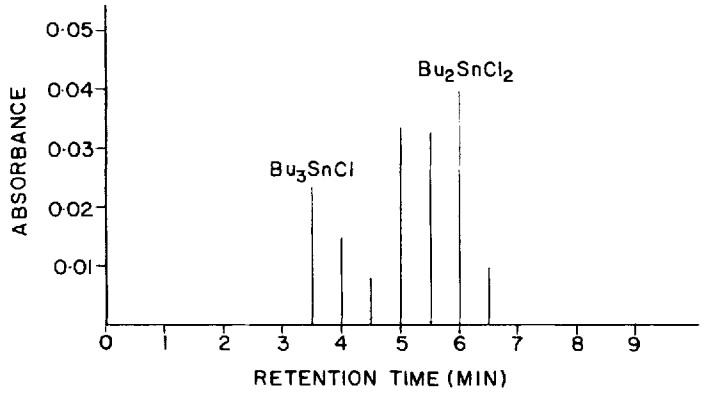

Figure 1 HPLC/GF AA chromatogram of a mixture of Bu_3SnCl and Bu_2SnCl_2 on a C-18 column.

medium, 0.2% trifluoroacetic acid in water, was added post-column at a flow rate of 0.4 $cm^3\ min^{-1}$ before entering the thermospray interface. Standard solutions (100 ppm) of Bu_nSnCl_{4-n} ($n=3, 2$) and Ph_2SnCl_2 (as internal standard) were made up in acetone and the retention time and mass spectrum of each were determined (10-μl injection). The thermospray conditions were optimized by using Bu_3SnCl as the analyte, and measuring the fragment ion at *m/z* 349. The following conditions were established: vaporization temperature, 182 °C; probe temperature, 117 °C; ion source, 225 °C; jet temperature, 213–215 °C.

Necause hydrochloric acid solutions are not desirable in this procedure, the solution obtained from shells was extracted into dichloromethane; the organic layer was evaporated to dryness and the residue reconstituted in acetone/THF (9:1) containing Ph_2SnCl_2 (34.5 $\mu g\ cm^{-3}$ as Sn). The mixture (20 μl) was used for the HPLC MS investigation. The flesh extracts were treated in an analogous way, but omitting the dichloromethane extraction step.

RESULTS AND DISCUSSION

One of the initial objectives of this work was to develop a method for the quantitation of organotin compounds in marine samples that would not involve a derivatization step. The use of HPLC has been studied for this purpose and separation of compounds of the class R_nSnX_{4-n} ($n=1$–3; X = some functionality other than a hydrocarbon bound directly to tin through a Sn–C bond) has been achieved on cation-exchange[10–14] Styrogel[15] and cyanopropyl[16] columns, although most separations were achieved on compounds differing in R rather than *n*. Because we ultimately wish to develop a method that might be sensitive to the nature of X, we investigated the use of a C-18 reverse-phase column. The separation of Bu_3SnCl and Bu_2SnCl_2 on such a column, as monitored by GF AA, is shown in Fig. 1. The mobile phase for achieving this separation, 2% THF/98% acetone containing 2% acetic acid, was found after many trials, and even this system does not separate Bu_2SnCl_2 from $BuSnCl_3$. Nevertheless, the separation seemed satisfactory enough to develop into an analytical method and recovery studies were made to this end. Some results are shown in Table 1. Quantitation of Bu_3SnCl was effected by normal calibration procedures and Bu_2SnCl_2 by standard additions. At the level of study (5–15 $\mu g\ 0.1\ g^{-1}$ of standard dogfish liver) reasonable recoveries are found. The detection limit was estimated to be 0.3 ng cm^{-3} as tin and the relative standard deviation was 3%.

Application of the method to marine samples was next attempted and some results follow (all values as tin):

Table 1 Recovery studies on butyltin chloride

Amount added ($\mu g\ 0.1\ g^{-1}$ std dogfish liver)	Compound	Recovery (%)
5	Bu_2SnCl_2	89 ± 1
	Bu_3SnCl	97 ± 1
10	Bu_2SnCl_2	86 ± 1
	Bu_3SnCl	83 ± 1
15	Bu_2SnCl_2	92 ± 1
	Bu_3SnCl	90 ± 1

Table 2 Major fragment ions of standard organotin compounds

Compound[a]	Major fragment ion	Assignment	Relative intensity (%)
$(C_4H_9)_3SnCl$	349	$[(C_4H_9)_3SnCO(CH_3)_2]^+$	100
(MW = 325.19)	327	$[(C_4H_9)_3SnCl]^+$	10
	291	$[(C_4H_9)_3Sn]^+$	57
$(C_4H_9)_2SnCl_2$	351	$[(C_4H_9)_2Sn[CO(CH_3)_2]_2+H]^+$	57
(MW = 303.69)	327	$[(C_4H_9)_2SnClCO(CH_3)_2-H]^+$	81
	293	$[(C_4H_9)_2SnOOCCH_3]^+$	100
	269	$[(C_4H_9)_2SnCl]^+$	28
$C_4H_9SnCl_3$	363	$[C_4H_9SnOOCCF_3C_4H_8O+H]^+$	46
(MW = 282.19)	327	$[C_4H_9SnOOCCF_3Cl]^+$	28
	305	$[C_4H_9SnCl(OH)_2OOCCH_3]^+$	100
	268	$[C_4H_9Sn(OH)_2CO(CH_3)_2-H]^+$	15
$(C_6H_5)_2SnCl_2$	391	$[(C_6H_5)_2SnOOCCH_3OC(CH_3)_2]^+$	11
(MW = 343.69)	367	$[(C_6H_5)_2SnClOC(CH_3)_2-H]^+$	40
	333	$[(C_6H_5)_2SnOOCCH_3]^+$	22
	309	$[(C_6H_5)_2SnCl]^+$	13
	175		100

[a] The concentration of the standard solution was usually 100 ppm. A 1000-ppm solution was required for $BuSnCl_3$. See text for experimental details.

Butter clam tissue:
Bu_3Sn^+, 22 ± 4 ng g^{-1}; Bu_2Sn^{2+} 19 ± 2 ng g^{-1}

Oyster shell:
Bu_3Sn^+, 2.8 ± 0.6 μg g^{-1}; Bu_2Sn^{2+}, 20 ± 1 μg g^{-1}.

The concentration of tin compounds in the shell are high enough to permit quantitation by using the effluent from one HPLC injection. Those in the flesh are lower and require multiple injections in order to accumulate enough sample for analysis. This is a tedious procedure and is a definite disadvanage of the method as developed. We also find that the separation of the tin species is not complete on the C-18 column; thus application of the Grignard/GC MS procedure (see below) to the isolated fractions revealed the presence of Bu_3Sn^+ species in fractions that should contain only Bu_2Sn^{2+}. Because of these problems we sought an alternative procedure.

HPLC MS offers a means of detection that is capable of providing information on speciation on a continuous basis and this was next explored for use with our samples. The thermospray technique was used as the interface. The principal peaks in the thermospray mass spectra of Bu_nSnCl_{4-n} ($n = 1$–3) and Ph_2SnCl_2, a possible internal standard, are listed in Table 2. The phenyltin compound elutes between the butyltin compounds. Satisfactory spectra were obtained from

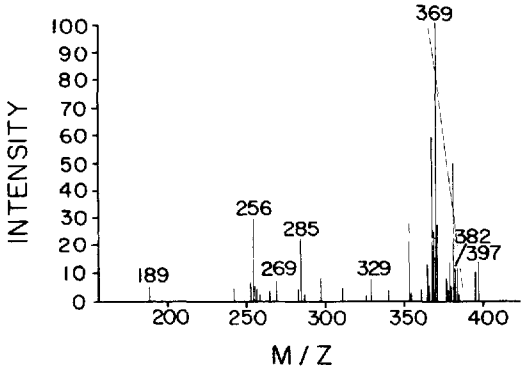

Figure 2 Thermospray mass spectrum of the HPLC fraction containing Bu_2SnCl_2 extracted from oyster flesh. The butyltin compound is not evident.

solution of these compounds at a concentration of ~ 36 μg cm^{-3} (20-μl injections), although the sensitivity towards $BuSnCl_3$ was much less than the others; a 10 μg cm^{-3} solution was not detected.

When solutions of the tissue extracts or shells were examined by HPLC MS, spectra being recorded at the appropriate retention times, we were unable to verify the presence of any butyltin compounds. A typical spectrum obtained from clam tissues extracts is shown in Fig. 2. This fraction should contain Bu_2SnCl_2, as judged by the HPLC/GF AA result, yet none is observed.

Thus it seems that the HPLC/thermospray MS technique is either not sensitive enough to detect the tin compounds, or is very affected by matrix effects. In either case the use of the technique for the quantitation of tin compounds seems limited.

Confirmation of butyltin compounds in clam shells was made by determining the electron impact spectrum of the solid residue obtained by evaporating to dryness a dichloromethane extract of the hydrochloric acid solution of the shell.[17]

One unexpected feature of the thermospray mass spectra of the flesh extracts and shell solutions is the presence of clusters and peaks that show a pattern similar to that of the isotopes of tin. An example is seen in the cluster around *m/z* 369 in Fig. 2; clusters at *m/z* 303 in other spectra show this same pattern. These features prompted a search for other possible tin derivatives in the samples, but by using a technique that would allow the unequivocal identification of the compounds. (The compounds responsible for these ion clusters at *m/z* 369 and 303 remain unidentified.) This constraint led us to use the Grignard derivative/GM MS procedure in which the R_nSnX_{4-n} compounds in extracts are treated with CH_3MgBr to afford R_nSnMe_{4-n} prior to separation and quantitation. The methyl derivatives were chosen for chemical convenience even though methyltin compounds have been reported in low concentration in the Canadian environment (the identification is not unequivical).[18] The distribution of these environmentally observed methyl species is narrow and it seems unlikely that any would be encountered in the samples chosen for study. The greater reactivity of the methyl Grignard reagent was the overriding factor in its choice for use in the present investigation.

GC MS analyses

In order to develop a GC MS procedure for the quantitation of the methyl derivatized species it was necessary to establish both an internal standard and a sensitive ionization mode. It has been reported that butyltin derivatives give the greatest response in the chemical ionization (CI) mode.[19,20] However, we were unable to find any significant difference in the intensity of the monitored ions (see the Experimental section) of the compounds Bu_nSnMe_{4-n} ($n = 3$–1) when using CI or electron ionization (EI). We chose, therefore, to use EI because of fewer problems with data reproducibility. We also chose to use tetrapropyltin (Pr_4Sn) as an internal standard because of its suitable retention time. Tripentyltin chloride (Pl_3SnCl) was initially tried, but once it was derivatized, the product, methyltripentyltin

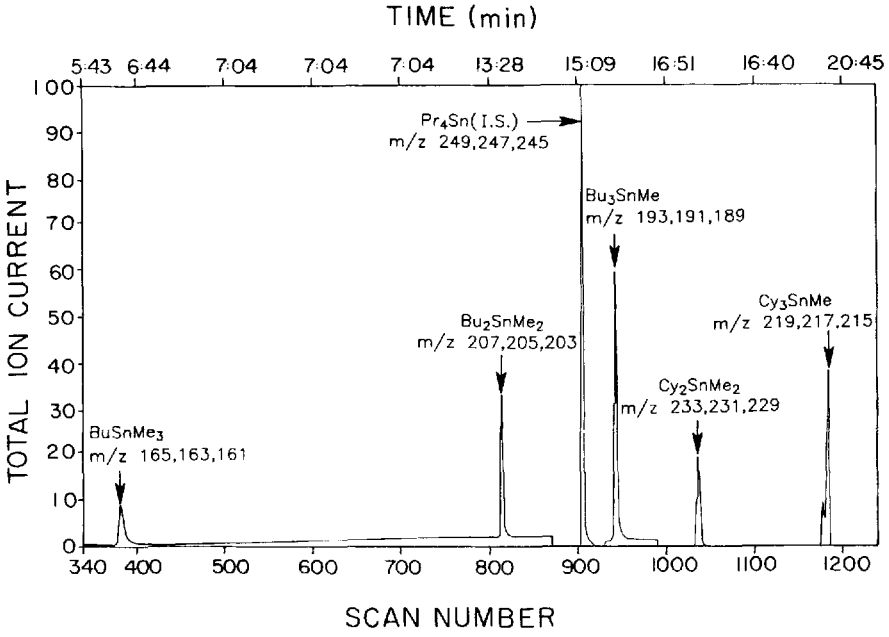

Figure 3 GC MS chromatogram of a mixture of butyl- and cyclohexyl-tin derivatives. The *m/z* values correspond to the selected ions monitored.

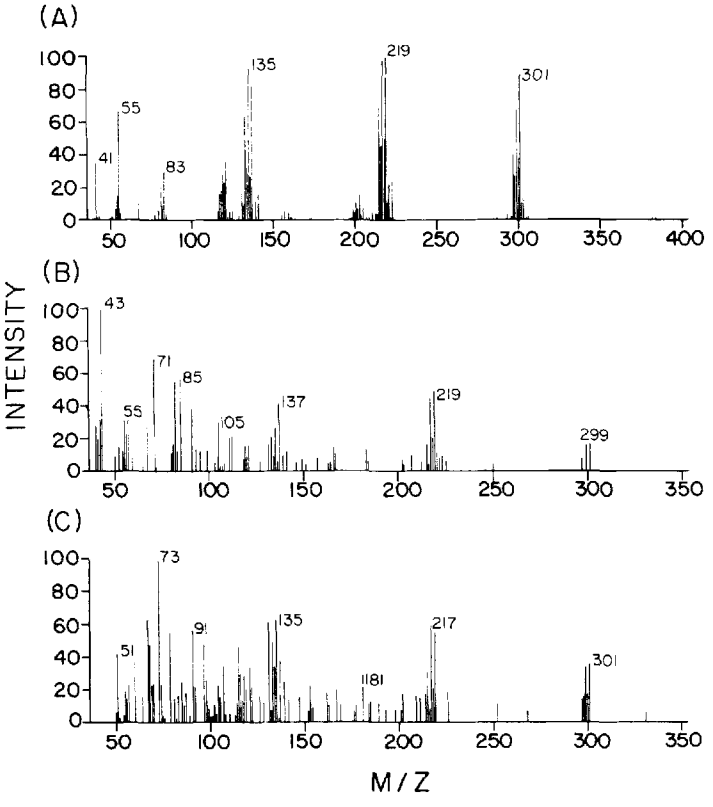

Figure 4 GC MS scans of the Cy_3SnCH_3 fractions derived from (A) Cy_3SnCl standard; (B) oyster flesh; (C) oyster shell.

Table 3 Analytical results for butyltin and cyclohexyltin species in oyster flesh (*Crassostrea gigas*)[a]

Location[c]	Sampling date	Concentration(ng Sn g^{-1} wet weight)[b]			
		BuSn	Bu_2Sn	Bu_3Sn	Cy_2Sn[b]
Fanny Bay, Vancouver Island	Nov. 1988	26 ± 9	68 ± 22	25 ± 9	12 ± 4
Wreck Beach, Vancouver	May 1989	34 ± 9	178 ± 30	51 ± 15	34 ± 4
Jervis Inlet	April 1989	7 ± 9	17 ± 5	15 ± 3	1 ± 0.5

[a] Cy_3Sn species were not detected in these samples because of masking. The Grignard method was used for these analyses. [b] Average values of four determinations from a pooled sample of at least six oysters. [c] Station coordinates available from the authors.

(Pl_3SnCH_3), eluted with dicyclohexyldimethyltin [$Cy_2Sn(CH_3)_2$] and similar problems arose with using tripropyltin chloride (Pr_3SnCl) and triphenyltin chloride (Ph_3SnCl).

Figure 3 shows a chromatogram produced by using selected ion monitoring of a synthetic mixture of the compounds being studied. Good separation is achieved; the elution time between $BuSn(CH_3)_3$ and Cy_3SnCH_3 is 14 min. The cyclohexyltin compounds were included because, as will be outlined below, they were likely to be found in the British Columbia environment because of their use in agriculture.

When the GC MS procedure was applied to oyster tissue extracts and solutions of their shells, unequivocal qualitative evidence for the presence of $BuSn^{3+}$, Bu_2Sn^{2+}, Bu_3Sn^{+}, Cy_2Sn^{2+} and Cy_3Sn^{+} species was readily obtained. Thus, components in the derivatized samples had both the retention times of the appropriate methylated derivatives and the corresponding mass spectra. For example, some results for Cy_3SnCH_3 derived from

Table 4 Analytical results for butyltin and cyclohexyltin species in seawater and the surface microlayer[a]

Location	Sampling station[b]	Sampling date	Depth (m)	Concentration (ng Sn dm^{-3})[a,c]			
				BuSn	Bu_2Sn	Bu_3Sn	Cy_2Sn
Saanich Inlet	(SI)	3 Apr. 1989	0	—	13	4	7
			5	7	26	5	4
			10	19	35	3	4
			20	—	—	—	—
		21 Apr. 1989	20	—	—	—	—
			30	—	—	—	—
			50	—	—	—	—
	(PBI)	Nov. 1989	Microlayer	—	28	24	—
Jervis Inlet	(JV1)	4 Apr. 1989	5	—	—	—	—
			10	—	—	—	—
			20	—	—	—	—
			30	—	—	—	—
			50	—	—	—	—
		20 Apr. 1989	Microlayer	—	66	16	—
Desolation Sound	(PS1)	5 Apr. 1989	0	—	20	3	6
			5	—	12	3	6
			10	—	25	5	4
			20	—	—	—	—
			30	—	—	—	—
			50	—	—	—	—
			Microlayer	—	116	—	—
Vancouver Harbour	(CH1)	Nov. 1989	Microlayer	—	29	11	—
	(CH2)		Microlayer	—	53	35	—
	(CH3)		Microlayer	—	30	27	—
	(CH4)		Microlayer	—	41	29	—
	(VH 22B)	Nov. 1989	0	—	49	36	—
			2.5	—	49	25	—
			5	—	49	14	—
			10	—	38	21	—
			Microlayer	—	74	31	—
	(RT1)	Nov. 1989	0	—	45	35	—
			2.5	—	56	20	—
			5	—	58	25	—
			Microlayer	—	50	35	—

—, Not detected.

[a] Cy_3Sn and Cy_2Sn species were masked in Vancouver Harbour samples; elsewhere, Cy_3Sn was not detected. The Grignard method was used here. [b] Station coordinates are available from the authors. [c] The error is approximately ±30%.

oyster flesh and shells are shown in Fig. 4. In these oyster samples the concentrations of the organotin compounds are high enough to allow the recording of the whole spectrum. In the case of clam tissue and shell the butyltin species are readily identifiable by complete mass spectra, but the results from fractions that should contain the cyclohexyltin species are not totally convincing as to the presence of these compounds.

Some quantitative results for oyster flesh are listed in Table 3. These values were obtained as outlined in the Experimental section by comparing the intensities of selected peaks of the standard with the intensities of selected peaks of the unknowns. Peaks due to Cy_3SnCH_3 are masked by other compounds, so quantitative results cannot be obtained by using the present methodology.

The quantitative analysis of these tin compounds in water (Table 4) is a more difficult task because of the lower concentrations encountered and also because the compounds of interest can

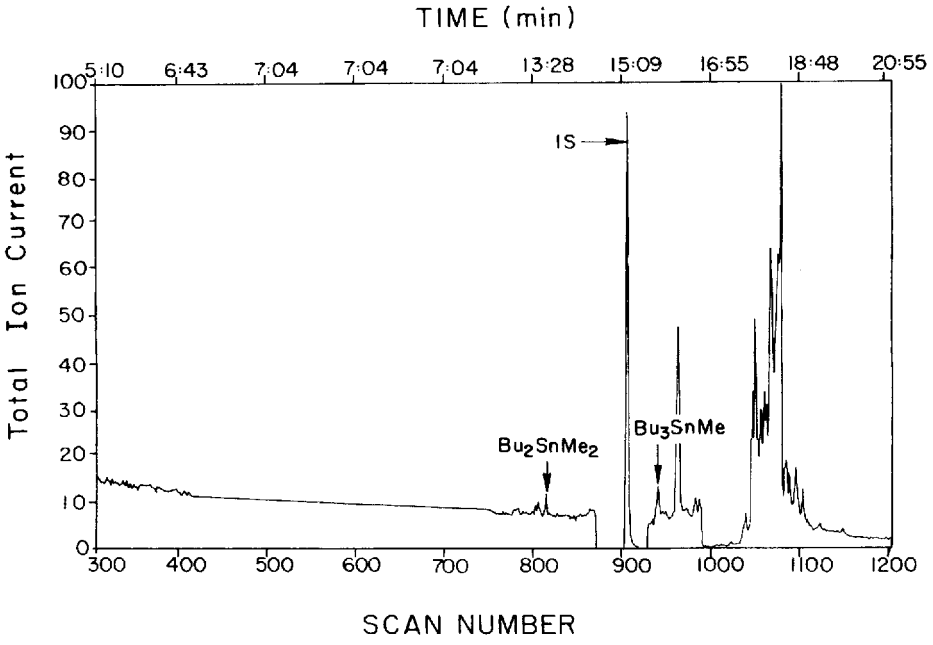

Figure 5 GC MS chromatogram selected ion monitoring of extracted and derivatized Vancouver Harbour water. IS = internal standard.

be more easily masked by other compounds, especially if the sample is particularly polluted, as is the water of Vancouver Harbour. This phenomenon is shown in Fig. 5, where any response due to the presence of Cy_3SnCH_3 would be masked by the presence of other compounds, in spite of the use of selective ion monitoring. (The use of other GC columns and/or conditions as yet unexplored might alleviate this problem).

The principal features of interest in the data of Tables 3 and 4 are the presence and distribution of the cyclohexyltin derivatives. The source of these compounds in the marine environment probably is the agrochemical[21] Plictran (also known as Cyhextin), $(C_6H_{11})_3SnOH$, which was used in Canada until 1988, when it was withdrawn from the market because of problems associated with human exposure. (Cyclohexyltin compounds could also enter the environment in other parts of the world through the use of a related substance, Peropal, $(C_6H_{11})_3Sn—N—N=CH—N=CH$).) It is estimated that between 1977 and 1988 about 10 000 kg of Plictran was used in the Fraser Valley.[22] This probably accounts for the high concentrations found in the oysters on Wreck Beach, which is at the mouth of the Fraser river. The presence of these compounds in oysters taken from Vancouver Island and Jervis Inlet, a site remote from agricultural input (as is Desolation Sound; Table 4), points to the persistence of these species in the environment. It seems likely that the Cy_3Sn^+ moiety, like Bu_3Sn^+, degrades by stepwise loss of alkyl groups. This would account for the presence of Cy_2Sn^{2+} species in oysters and water.

The relative concentrations of butyltin species in the water are much as would be expected from the locations sampled, with Vancouver Harbor being highest and Jervis Inlet, a region of low boating activity, lowest. It is significant that the organotin compounds are not found at depth; none is detected at or below 20 m depth. As a result, seawater taken at 50 m depth can be used as an operational blank for these measurements.

With regard to the results, Maguire *et al.*[18] have reported a value of 10 ng dm^{-3} Bu_3Sn^+ (as Sn) for a Vancouver Harbour site and have detected organotin compounds in Saanich Inlet. The highest concentrations of Bu_3Sn^+ in Canadian waters were found in Port Hope, Ontario (2340 ng dm^{-3}) and in 21 of the 43 locations at which Bu_3Sn was determined, the concentration was greater than 70 ng dm^{-3}, which was taken as a toxicity threshold barrier.[18] Apart from one Vancouver Harbour result, all the present values including those from the microlayer, whch can have greatly enhanced concentrations,[7,23] are below this arbitrary level. It should be noted, however, that the tributyltin

derivatives are present in concentrations above the 20 ng dm^{-3} initially suggested as an environmental quality target for the UK and that even this value is now considered to be too high.[24]

The concentrations of the organotin compounds in oysters (Table 3) are considerably lower than have been reported from other parts of the world, e.g. for Bu_3Sn (as Sn) 1.5 mg kg^{-1} ($\mu g\ g^{-1}$)[25] and 135 $\mu g\ kg^{-1}$ ($ng\ g^{-1}$)[3] in the UK.

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