Applied Organometallic Chemistry (1988) 2 151–157

0268-2605/88/02208151/$03.50

Speciation of butyltin compounds in oyster samples

S. Rapsomanikis and Roy M Harrison*
Institute of Aerosol Science, Department of Chemistry and Biological Chemistry, University of Essex, Colchester CO4 3SQ, UK

Received 15 January 1988 Accepted 1 February 1988

Experimental instrumentation for the speciation of butyltin compounds by hydride generation and gas chromatography–quartz furnace atomic absorption spectrometry is described. Evaluation of a number of extraction methods revealed that simple acid leaching of oyster samples gave adequate recoveries. The levels of each butyltin species in oyster flesh and gill are reported together with the tentative identification of a mixed methylbutyltin compound.

Keywords: Speciation, gas chromatography, quartz furnace atomic absorption spectrometry, butyltin compounds, oyster samples, mixed methylbutyltin compounds

INTRODUCTION

Organotin compounds have been utilised substantially in industrial applications in recent years. Their wide range of use includes plastic stabilizers, fire retardants, industrial catalysts and agricultural pesticides and fungicides.[1] They are generally more toxic than their inorganic counterparts and toxicity increases with the number of organic groups bonded to tin. Conversely, toxicity decreases with alkyl chain length but triphenyl-, tricyclohexyl- and trineophyl-tin compounds are effective fungicides and acaricides.[2]

Only in recent years has attention been focussed on the use of bis-tributyltin oxide ($[Bu_3Sn]_2O$; TBT) as antifouling paint in pleasure craft. It was first suggested by Alzieu *et al.*[3] that the growth of shellfish was adversely affected by the presence of TBT in the waters of oyster cultures. The debate that followed led to a ban on the use of TBT in boats of less than 25 m length in France. Manufacture of marine paints has been restrained in the UK and the Royal Yachting Association has urged its members to avoid the use of TBT-containing paints.[4] There is now a complete ban on retail sales of paint formulations containing TBT in the UK.[5]

Analytical methods for determining the concentrations of butyltin compounds in biological samples need to be sensitive and species-specific. A large amount of data of butyltin levels in oysters, waters and sediments have been obtained using non-species-specific solvent extraction procedures.[6] A variety of organotin compounds can be extracted by this method but it is not possible to establish with certainty that organotin concentrations reported are due to TBT.[7,8] A proportion of organotin compounds occurring in the natural environment may exist as $Me_nSn^{(4-n)+}$,[9–12] and recent publications have reported the presence of mixed methylbutyltins in sediments.[12,13] Two recent conferences[14] have summarized the present state of knowledge on environmental organotin chemistry.

Speciation of organotins in environmental samples may be effected in a number of different ways. Samples may be leached with acid or alkali, extracted with an organic solvent containing a chelating agent, preconcentrated, derivatized with a Grignard reagent with an alkyl group different from the one being determined and then analysed by gas chromatography with flame photometric, mass spectrometric or atomic absorption detection.[15–17] Alternatively, after acid or alkali leaching, volatile organotin hydrides can be formed by reaction with $NaBH_4$ and concentrated in a cold trap containing chromatographic packing. Analysis can be effected by desorbing and eluting the hydrides according to their boiling point into the quartz furnace atomizer of an atomic absorption spectrometer.[9,11,18]

*Author to whom correspondence should be addressed.

The latter method presents distinct advantages over the previously described derivatization gas chromatographic methods. Sample handling is kept to a minimum, and organic solvent extractions with layer-separation problems and chromatographic-column fouling are avoided. Possible contamination due to impurities in the Grignard reagents are also avoided.[13] The whole sample (not an aliquot) is analysed by the hydride generation method, giving rise to lower relative limits of detection. Furthermore, the absolute limits of detection are comparable with the flame photometric detector without the inconvenience, limitations and interferences of the latter.[19,20]

Hydride generation, trapping and classical chromatography–quartz furnace atomic absorption spectroscopy were used in the present study to analyse oyster samples for butyltins. A number of different extraction procedures have also been evaluated.

EXPERIMENTAL

Materials

All chemicals were purchased commercially and used without further purification (Aldrich Chemical Co. Ltd, Gillingham, Dorset, UK; BDH Chemicals, Poole, Dorset, UK). High-purity distilled deionized (Milli-Q) water was used throughout the study. Stock solutions of 1000 μg (as Sn) cm^{-3} of each butyltin compound were prepared in ethanol. They were kept refrigerated (4°C) in the dark and working solutions were made by appropriate dilutions in distilled deionized water (DDH_2O). The apparatus used is shown in Fig. 1 and is based on those described previously.[11]

Preconcentration of the hydrides was carried out on a GN Concentrator (GN Instrumentation Consultancy Ltd, Wimbledon, London, UK), which was connected to the injection port of a Hewlett Packard 5750 Research Chromatograph. The trap of the GN Concentrator was a glass-lined stainless-steel U-tube, packed with glass beads (60–80 mesh), silanized with 5% dichlorodimethylsilane [$(CH_3)_2SiCl_2$]. The PTFE gas chromatographic column was 1.8 m long, 4 mm o.d. and packed with 10% OV-101 on Chromosorb 750 (60–80 mesh). Other solid supports including Chromosorb W AW-DMCS caused peak tailing and splitting for Bu_3SnH. A PTFE sleeve was inserted at the injection port of the gas chromatograph (GC) to minimize contact with metal surfaces. All pressure connections were made with stainless-steel Swagelok

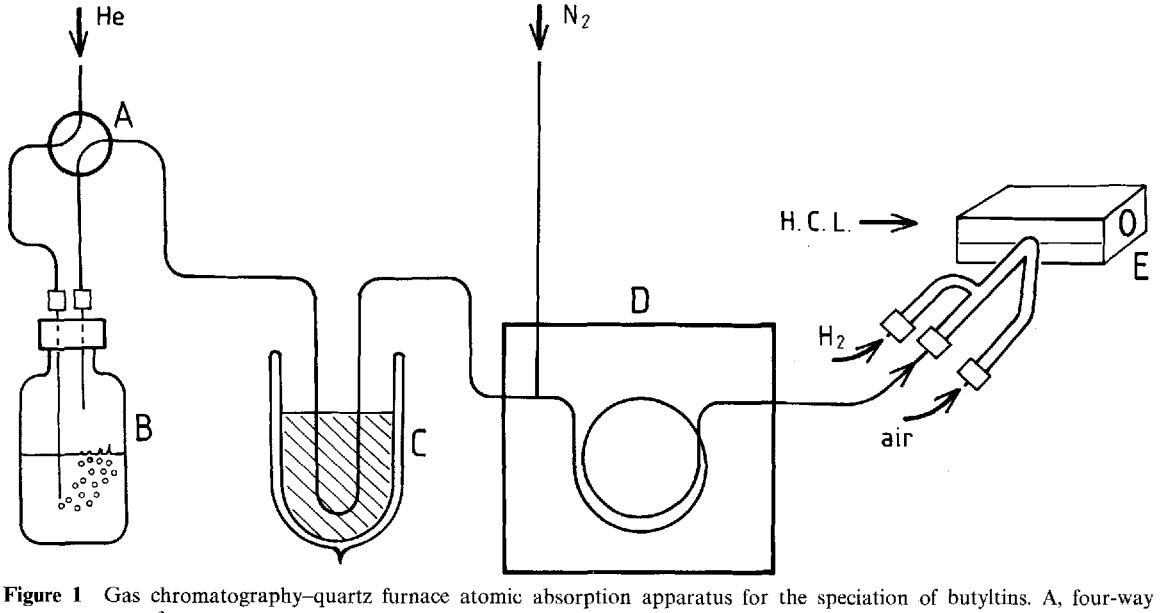

Figure 1 Gas chromatography–quartz furnace atomic absorption apparatus for the speciation of butyltins. A, four-way valve; B, 120 cm^3 hypovial with PTFE-lined septum; C, GN Concentrator at liquid-nitrogen temperatures, desorption at 170°C; D, gas chromatograph; E, quartz atomization cell electrically heated to 950°C, insulated with firebrick; H.C.L., hollow cathode lamp.

connectors, sometimes drilled through to minimize dead volume. The atomic absorption spectrometer (AA) was a Perkin–Elmer 305 equipped with a tin hollow cathode lamp operated at 10 mA, a wavelength of 235.5 nm and a slit width of 1 nm. A low-pass 0.5 Hz passive filter was used for signal processing. The GC was interfaced to the AA by means of a PTFE transference line. Atomization of the eluting organotins was effected in an electrically heated quartz furnace which was kept at 950°C. The cell was 16 cm long, 1.2 cm o.d. and had three gas inlets. It was mounted on the unfuelled burner of the AA and insulated with firebrick. All transference lines and gas lines were PTFE of 3 mm o.d. The interface line between the GC and AA, the line between the injection port and the GN Concentrator and the lines out of the reaction vessel and from the four-way valve to the GN Concentrator were heated. A protecting layer of PTFE tape was applied to them followed by windings of 28-gauge Nichrome wire. They were then threaded through PTFE tubing of 6 mm o.d. before being connected to a variable-voltage transformer (Matsunaga Mfg Co., Japan) and were kept at a temperature of 120°C throughout the study. Gas flows through the GC were 35 $cm^3 min^{-1}$ nitrogen and 45 $cm^3 min^{-1}$ helium. The optimum flows of hydrogen and air in the atomizer cell were 400 $cm^3 min^{-1}$ and 10 $cm^3 min^{-1}$ respectively. Elution of the butyltin compounds was carried out using a temperature programme. The injection port was kept at 120°C and initially the oven temperature was kept at 70°C for 4 min. It was then increased to 170°C at 30°C min^{-1} with the final temperature maintained for 10 min.

Procedure

Oyster samples (*Crassostrea gigas*) originated from UK estuarine waters. The flesh was freeze-dried, ground and refrigerated prior to analysis. Extraction efficiency studies were carried out using one large sample, to ensure constant background levels. It was split into two equal halves (1.58 g each) and each half was spiked with 900 ng (as Sn) of each of $BuSnCl_3$, Bu_2SnCl_2 and Bu_3SnCl in a total of 20 cm^3 of ethanol. The two samples were equilibrated by continuous reciprocating shaking for 12 h. After evaporation of the ethanol at room temperature the first sample was leached for 12 h (continuous reciprocating shaking) with 45 cm^3 of 2 mol dm^{-3} hydrochloric acid. It was then filtered (Millipore vacuum filtration apparatus, 0.45 μm Millipore cellulose ester filters; Millipore (UK), Harrow, Middlesex, UK) and the solids washed with 45 cm^3 distilled water to give 90 cm^3 of filtrate solution A, which was split into nine subsamples. The following extraction and derivatization procedures were carried out on solution A.

Method 1: Analysis of three samples without further treatment.

Method 2: Analysis of three samples as follows: each was extracted with 5 cm^3 CH_2Cl_2 solution containing 0.5% (w/v) tropolone. The solvent was evaporated at room temperature (overnight) and 10 cm^3 of 1 mol dm^{-3} hydrochloric acid was added prior to analysis.

Method 3: As in method 2, using 0.5% (w/v) NaDDTC (sodium diethyldithiocarbamate) in 5 cm^3 CH_2Cl_2.

The second large sample of spiked oyster tissue was leached with 45 cm^3 2 mol dm^{-3} sodium hydroxide for 12 h (continuous reciprocating shaking). It was then neutralized with 5 mol dm^{-3} of hydrochloric acid and filtered as before. The solids were washed with distilled deionized water and the resultant filtrate was made 1 mol dm^{-3} in hydrochloric acid and 90 cm^3 in total volume (solution B). Nine subsamples of solution B were analysed as follows.

Method 4: Analysis of three samples without further treatment.

Method 5: As in method 2.

Method 6: As in method 3.

For derivatization and analysis, each solution resulting from the above extractions was placed in a 120 cm^3 hypovial and sealed with a Teflon-lined silicon rubber septum (Supelchem, UK, Sawbridgeworth, Herts, UK). After the trap of the GN Concentrator had been cooled to −198°C with liquid nitrogen, the septum was pierced with the two needles of the purge and trap set-up. A 3 cm^3 aliquot of 3% (w/v) $NaBH_4$ was added and the butyltin hydrides were purged to the trap for 20 min. Lowering of the liquid-nitrogen Dewar flask coincided with the desorption of the hydrides by flash heating of the trap from −198°C to 170°C in 5 s. Elution was effected with the initiation of the GC temperature programme.

The oyster samples were subsequently analysed by method 1 since no advantage was obtained from any of the other extraction procedures (Table 1). Each oyster sample was split into two by separating the flesh from the gill. After leaching, each sample was split into three subsamples so that the method of standard additions could be carried out in duplicate (analysis of the sample and two standard additions of 20 ng). Calculation of absolute concentrations was carried out using Eqn [1] below.

$$x = \frac{yA_1}{A_2 - A_1} \qquad [1]$$

where A_1 is the peak area corresponding to amount or concentration x, A_2 is the peak area corresponding to amount or concentration $x+y$, y is the concentration or amount of added standard, and x is the unknown concentration or amount.

RESULTS AND DISCUSSION

Simple dilute acid or base extraction of butyltin compounds may not produce a solution in which the alkyltin is in the form optimal for hydride generation. The tropolone and NaDDTC extractions were therefore tested to investigate whether they produced a solution giving a higher overall extraction/hydride generation efficiency.

Results of the efficiency study have been tabulated in Table 1. Solvent extractions with chelating agents did not improve the efficiencies, nor was there a marked difference between acid and alkali extraction of the samples. Extractions using solvents and chelating agents in general resulted in poorer efficiencies due to an increased number of handling steps and container transfers. Hence simple acid leaching at room temperature with reciprocating shaking over 12 h was adopted for the analysis of all samples. Low-concentration spiking of the test sample was adopted for the calculation of realistic extraction efficiencies. The level of 100 ng spiking per subsample was chosen so that spike/background ratios of 6 to 10 were achieved. The sample chosen contained 92 ng (Sn) g^{-1} as $BuSn^{3+}$ (10 ng per subsample), 103 ng (Sn) g^{-1} as Bu_2Sn^{2+} (11 ng per subsample), and 56 ng (Sn) g^{-1} as Bu_3Sn^+ (6 ng per subsample). High spike-to-background ratios were employed in interpreting the percentage recovery data so that estimates of analytical precision were not biased.[21] The percentage recovery data were calculated from calibration graphs of aqueous standards which were taken through the same analytical procedure as the samples. Results obtained from such calculations, however, may not reveal systematic errors of the procedure due to sample losses during extraction/hydride generation. Thus for the calculation of Bu_3Sn^+ extraction efficiencies, standards of Bu_3SnH were placed in hypovials in 10 cm^3 of water and purged, trapped and analysed in a similar manner to the aqueous standards. Efficiencies calculated from such a calibration graph were lower, revealing losses of Bu_3Sn^+ during the extraction/hydride generation procedures (Table 1). The efficiencies for the other two butyltin compounds were not calculated using butyltin hydride standards because they were not readily available commercially. Because of the low efficiencies of all extraction procedures, the method of standards addition was used for the estimation of concentrations of butyltin compounds in oyster samples. Absolute limits of detection based on 3σ of the baseline noise were 3.5 ng for Bu_3Sn^+, 1 ng for Bu_2Sn^{2+} and 0.5 ng for $BuSn^{3+}$. A typical chromatograph of butyltin standards is presented in Fig. 2, whilst Fig. 3 depicts a chromatogram of sample 1A.

Oyster samples were analysed with the species-specific method of GC AA because determination of the degradation and biotransformation products would establish the persistence and the effects of TBT on non-target organisms. Analyses by solvent extractions and AA[5-8] could inflate the values of TBT present in the matrix analysed

Table 1 Extraction efficiencies of $Bu_nSn^{(4-n)+}$ from oyster tissues

	Recovered Sn (%)			
Method	$BuSn^{3+}$ [a]	Bu_2Sn^{2+} [a]	Bu_3Sn^+ [a]	Bu_3Sn^+ [b]
1	88±7	73±6	69±10	48±9
2	83±5	69±2	66±7	46±7
3	85±10	72±8	69±3	48±3
4	81±3	68±9	56±4	39±4
5	77±3	74±9	71±5	48±5
6	78±6	70±6	66±13	46±13

[a]Efficiencies calculated from calibration graphs of standards in water treated in similar manner to samples. [b]Efficiencies calculated from calibration graph of Bu_3SnH added to aqueous solution and purged, trapped and analysed as usual.

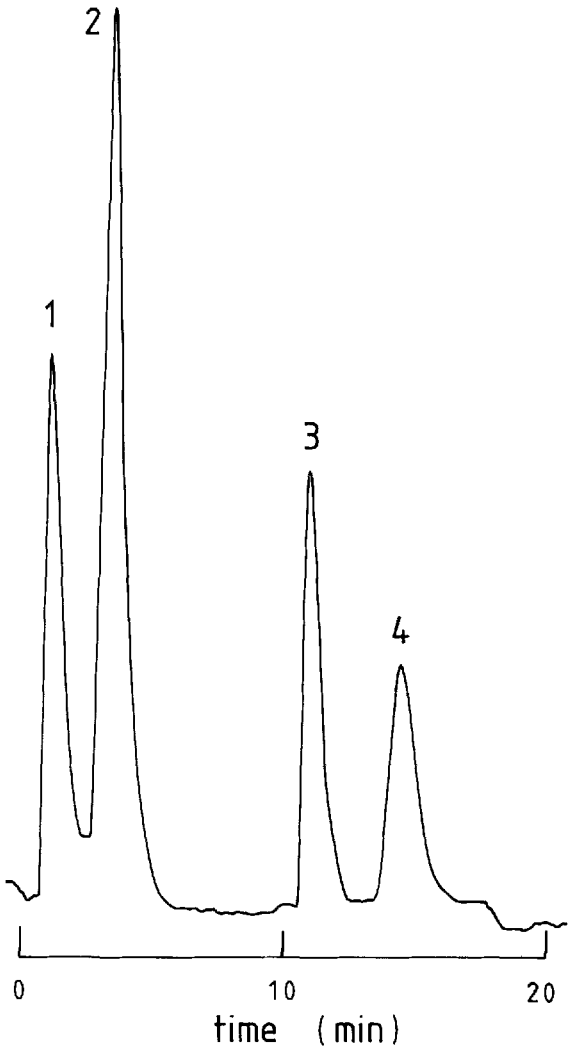

Figure 2 Chromatogram of butyltin standards (50 ng as Sn); 1, blank inorganic Sn; 2, $BuSnH_3$; 3, Bu_2SnH_2; 4, Bu_3SnH.

and would not differentiate between butyltin species present. The toxicity of these compounds towards marine organisms varies according to the number of alkyl chains attached to tin.[2] Furthermore, problems with precision and sensitivity arise from the analysis of butyltins in organic solvents by GF AA (graphite furnace atomic absorption spectrometry) if matrix modifiers are not used.[22]

Speciation of butyltins by solvent extractions, Grignard reagent derivatization and GC FPD (gas chromatography flame photometric detection) increases the handling steps and may introduce errors of contamination[12] and fouling of the chromatographic column. The limitations of the FPD detector are well documented.[19,20]

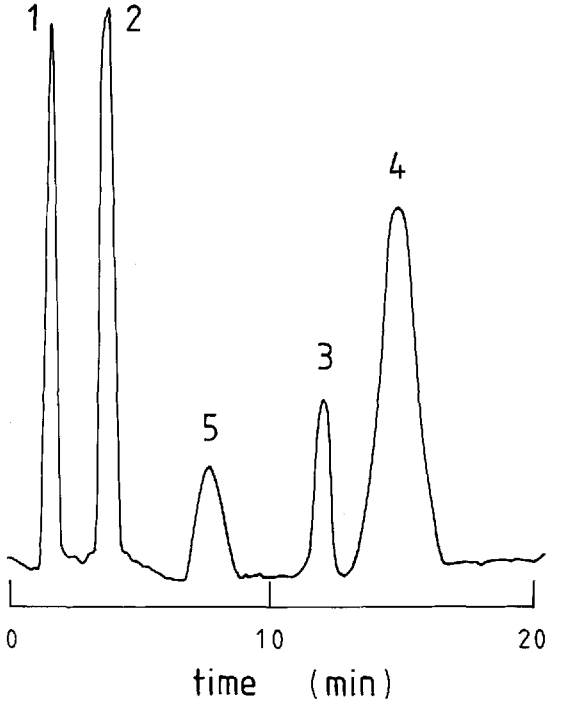

Figure 3 Chromatogram of sample 1A: 1, inorganic Sn; 2, $BuSnH_3$; 3, Bu_2SnH_2; 4, Bu_3SnH; 5, unknown.

Its absolute limits of detection are not much better than those for the quartz cell atomizer[19] and interferences by reduced sulphur and other organic compounds necessitate sample clean-up procedures. Fouling of the detector from organic solvents and by-products of the Grignard reaction also necessitates extensive detector clean-up.[20] *In-situ* derivatization by hydride generation and GC quartz furnace AA used in the present study is a fast, reliable and sensitive method for the speciation of butyltin compounds. The conventional chromatograph employed gave distinct advantages over the purge and trap system described previously.[11] A higher precision in retention times was obtained and analysis time was decreased because a temperature programme was used. However, water vapour condensing in the cryogenic trap and eluting through the column reduced its lifetime to *ca* 30–40 analyses.

Comparative data on butyltin in oysters is sparse. In his recent review, Maguire[23] reports data on Bu_3Sn^+ from two UK coastal studies which give concentrations varying from undetectable to 4500 ng (Sn) g^{-1} (dry weight) and <40–135 ng (Sn) g^{-1} (wet weight) respectively. Rice *et al.*,[24] using hexyl-derivatization and GC FPD analysis, report concentrations of Bu_3Sn^+ of 6–

1570 ng g^{-1} (wet weight) in the oyster, *Crassostrea virginica*. Our data (Table 2) lie within the range of these earlier studies.

Two distinct trends emerge from the results tabulated in Table 2. The gills of the oysters contained very little butyltin and mainly Bu_3Sn^+ whilst the rest of the flesh contained all three butyltin species at higher concentrations. An unidentified tin compound was also detected on three occasions with retention time between that of $BuSnH_3$ and Bu_2SnH_2. In view of the previous identification of mixed methylbutyltin compounds a tentative conclusion would be that it is $BuMeSnH_2$ (Me = methyl). The implications of this finding are either that butyltins are methylated in the environment or that transalkylation reactions between butyl- and methyl-tins take place in chemical or biological matrices. In the first case products of higher toxicity to marine organisms are formed. Once TBT has contaminated the oysters it may be degraded via dismutation or disproportionation reactions, examples of which are shown in Scheme 1. β-Hydroxyl-mediated decay may also occur.[25]

$$2Bu_3Sn^+ \rightleftharpoons Bu_2Sn^{2+} + Bu_4Sn$$

$$2Bu_2Sn^{2+} \rightleftharpoons Bu_3Sn^+ + BuSn^{3+}$$

$$2BuSn^{3+} \rightleftharpoons Bu_2Sn^{2+} + Sn^{4+}$$

$$Bu_2Sn^{2+} \rightleftharpoons 2Bu^+ + Sn^0$$

$$BuSn^{3+} \rightleftharpoons Bu^+ + Sn^{2+}$$

Scheme 1

Table 2 Results of analysis of oyster tissues

Sample	Concentration of Sn per dry weight (ng g^{-1}; as Sn)			
	$BuSn^{3+}$	Bu_2Sn^{2+}	Bu_3Sn^-	Unknown (%)[a]
1A[b]	60	31	154	10
1B	—	53	197	—
2A	581	402	—	—
2B	—	19	370	—
3A	—	—	316	—
3B	—	10	27	—
4A	108	259	75	7
4B	—	29	108	—
5A	—	12	1667	5
5B	15	19	—	—

[a]Unknown was calculated as % Sn of all organotin found.
[b]All A samples were oyster flesh and B samples were oyster gill.

CONCLUSIONS

The apparatus described offers a fast, reliable and precise method for the speciation of butyltins. Evaluation of a number of extraction procedures established that simple acid leaching of oyster flesh is sufficient to extraction butyltins from it. Levels of butyltins in the samples analysed were comparable with other published data. The presence of mixed methylbutyltins is suggested and may arise from environmental methylation or transalkylation processes.

REFERENCES

1. Davies, A G and Smith, P J Tin. In: *Comprehensive Organometallic Chemistry*, Wilkinson, G, Stone, F G A and Abel, E W (eds), Pergamon Press, Oxford, 1982
2. Blunden, S J and Chapman, A Organotin compounds in the environment. In: *Organometallic Compounds in the Environment*, Craig, P J (ed), Longman, Harlow, 1986
3. Alzieu, C, Heral, M, Thibaud, Y, Dardignac, M J and Feuillet, M *Rev. Trav. Inst. Pech. Marit.*, 1982, 45: 101
4. Whelan, E., *Practical Boat Owner*, 1987, 244: 44
5. Waldock, M J, Thain, J E and Waite, M E *Appl. Organomet. Chem.*, 1987, 1: 287
6. Anon, Standard Test Method AA-33, M and T Chemicals Inc.
7. Cleary, J J and Stebbing, A R D *Marine Pollut. Bull.*, 1985, 16: 350; *ibid.*, 1987, 18: 238
8. Waldock, M J and Miller, D ICES Paper CM 1983/E: 12, International Council for the Exploration of the Sea, Copenhagen, 1983
9. Hodge, V F, Seidel, S L and Goldberg, E *Anal. Chem.*, 1979, 51: 1256
10. Braman, R S and Tomkins, M A *Anal. Chem.*, 1979, 51: 1256
11. Donard, O F X, Rapsomanikis, S and Weber, J H *Anal. Chem.*, 1986, 54: 772
12. Maguire, R J, Tkacz, R J, Chau, Y K, Bengert, G A and Wong, P T S *Chemosphere*, 1986, 15: 253
13. Maguire, R J *Environ. Sci. Technol.*, 1984, 18: 291

14. *Proceedings Oceans 86; Proceedings Oceans 87* (Organotin Symposia). Copies of the Conference Records are available from IEEE Service Center, 445 Hoes Lane, Piscatanay, NJ 08854, USA
15. Unger, M A, MacIntyre, W G, Greaves, J and Hugger, R J *Chemosphere*, 1986, 15: 461
16. Matthias, C L, Bellama, J M, Olson, G J and Brinckman, F E *Environ. Sci. Technol.*, 1986, 20: 609
17. Miller, M D *Anal. Chem.*, 1987, 59: 617
18. Donard, O F X, Randall, L, Rapsomanikis, S and Weber, J H *Int. J. Environ. Anal. Chem.*, 1986, 27: 55
19. Andreae, M O and Byrd, J T *Anal. Chim. Acta*, 1984, 156: 147
20. Maguire, R J and Tkacz, R J *J. Chromatogr.*, 1983, 268: 99
21. Provost, P L and Elder, R S *Am. Lab.*, December 1983, 57
22. Benabdallah, M Z PhD Thesis, 1987, University of Pau, France
23. Maguire, R J *Appl. Organomet. Chem.*, 1987, 1: 475
24. Rice, C D, Espourteille, F A and Huggett, R J *Appl. Organomet. Chem.*, 1987, 1: 541
25. Brinckman, F E and Craig, P J In: *Organometallic Compounds in the Environment*, Craig, P J (ed), Longman, Harlow, 1986, p 27