APPLIED ORGANOMETALLIC CHEMISTRY
Appl. Organometal. Chem. 2002; **16**: 355–359

Organotin speciation analyses in marine biota using sodium tetraethylborate ethylation and gas chromatography with flame photometric detection

Roberto Cassi, Imma Tolosa, Jean Bartocci and Stephen de Mora*

Marine Environmental Studies Laboratory, International Atomic Energy Agency–Marine Environment Laboratory, 4, Quai Antoine 1 er, BP 800, MC 98012 Monaco

Received 11 February 2002; Accepted 19 March 2002

The analysis of organotin compounds based on the *in situ* simultaneous derivatization and extraction with sodium tetraethylborate has been optimized to overcome the most common matrix effect problems with biological samples. To obtain a complete dissolution of the samples, free of colloidal interfaces problems, mechanical agitation has been coupled with stirring in a warmed ultrasonic bath. A strict quality control system using three internal standards was implemented. Tripropyltin chloride was used as internal standard to assess the derivatization reaction and tetraoctyltin was used to check the overall extraction efficiency. Tetrabutyltin was used to verify the gas chromatography (with flame photometric detector) performance of the analyses. The technique was validated using Certified Reference Materials (NIES-11 and BCR-477) and has been applied successfully to different biological matrices (fish, mussels, oyster and barnacles). Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: organotin compounds; tributyltin; derivatization; sodium tetraethylborate; GC-FPD

INTRODUCTION

Butyl- and phenyl-tin compounds are used worldwide, mainly as antifouling agents and biocides. The large-scale application of these compounds to submerged marine structures, commercial vessels and pleasure crafts has resulted in dispersion to many marine environmental compartments. These compounds, especially the tributyltin (TBT) moiety, exhibit broad spectrum biocidal properties and so have elicited considerable research attention.[1,2] Owing to deleterious effects on non-target organisms, the use of organotin compounds as antifouling agents on boat hulls of small vessels has been widely prohibited. The International Maritime Organization recently resolved that TBT should also be banned from use on larger vessels within the next decade.[3] Despite strict regulations, the contamination of the marine environment from organotin compounds will continue to pose a long-term risk to aquatic wildlife due to their persistence in sediments and the leaching of contaminated sediments. For this reason, interest in these contaminants remains high, and several monitoring programmes are carried out every year.

Many analytical procedures based on chromatographic separation coupled to various detection techniques have been developed.[4] Organotin compounds must be extracted from the matrix and derivatized into suitable forms for gas chromatographic analysis. The most common derivatization procedures include hydride generation in the aqueous phase using sodium borohydride,[5] or the extraction of organotin complexes with tropolone or diethyldithiocarbamate followed by Grignard derivatization.[6] The instability and volatility of butyltin hydrides can lead to losses of compounds and consequently to an underestimation of contamination.[7] The Grignard derivatization requires scrupulously dry conditions and is rather time consuming.[8]

To avoid such difficulties, an organotin derivatization procedure directly applied to the aqueous phase using sodium tetraethylborate ($NaBEt_4$) was developed, whereby derivatization and extraction of organotin compounds can be carried out simultaneously.[7] This technique allows the determination of several butyl- and phenyl-tin compounds.

---

*Correspondence to: S. d. Mora, Marine Environmental Studies Laboratory, International Atomic Energy Agency–Marine Environment Laboratory, 4, Quai Antoine 1 er, BP 800, MC 98012 Monaco.
E-mail: S.de_Mora@iaea.org

Nevertheless, the determination of organotin compounds in complex matrices, such as biological matrices with a high lipid content, has led to several problems, including low recovery and low derivatization efficiency.[9] Other effects that have been observed during the gas chromatography (GC) analysis include distortion of the baseline, the appearance of large bands and the disappearance of some peaks.[10] Such problems are due to matrix effects, and thereby call into question the reliability and the accuracy of the use of $NaBEt_4$ for biological samples.

In this work, an optimization of the derivatization and extraction technique of organotin compounds for marine biota based on $NaBEt_4$ is described. Certified Reference Materials for mussels and fish (BCR 477 and NIES-11) were processed to validate the method, which was then tested using a number of different biological matrices.

MATERIALS AND METHODS

Standards and reagents

Dibutyltin (DBT) dichloride (98%), TBT chloride (96%), tetrabutyltin (TeBT) (>97%), tetraoctyltin (TeOcT) (75%), tripropyltin (TPhT) chloride, diphenyltin (DPhT) dichloride (98%) and triphenyltin (TPhT) chloride (99%) were obtained from Merck (Darmstadt, Germany). Monobutyltin (MBT) trichloride (95%) was purchased from Aldrich (Milwaukee, WI). Individual stock solutions of organotin compounds were prepared by dissolving approximately 60–80 mg of the original standard compound in 100 ml *n*-hexane (for tri- and tetra-substituted organotin species) or acetone (for mono- and di-substituted organotin species). Individual intermediate solutions were prepared by dissolution of 2 ml of the concentrated stock solution in 25 ml *n*-hexane. A mixed working standard was obtained by dilution of 1 ml of each of the intermediate standard solutions in 10 ml of acetone. All solvents and reagents were of pesticide-grade and used without further purification.

$NaBEt_4$ was purchased in 1 g sealed aliquots from Strem Chemicals (Newburyport, MA). A solution of 1% $NaBEt_4$ in 2% KOH was prepared with deionized water and stored in a freezer (−18 °C). A sodium acetate–acetic acid buffer was prepared by dissolving 2 mol of reagent-grade sodium acetate and 2 mol of reagent-grade glacial acetic acid into deionized water to give a final volume of 1 l. Tetramethylammonium hydroxide (25% solution in water) was purchased from Aldrich (Milwaukee, WI). Florisil cartridges (Supelclean™ LC-Florisil® SPE Tubes 6 ml, 1 g) were purchased from Supelco Bellefonte, PA.

Glassware and the Teflon tubes were cleaned for 15 min in an ultrasonic bath containing an aqueous solution of a liquid detergent (2%) (MICRO, Bioblock, France). They were then rinsed in turn with tap water, acetone and hexane. The glassware was then baked overnight in an oven at 240 °C.

Sample preparation

Using approximately 0.5 g of freeze-dried tissue, a series of organotin standards was added to samples in order to quantify the overall recovery of the analytical procedures. TPrT chloride was used to serve as an internal standard and TeOcT was added to quantify the extraction efficiency. Biota samples were dissolved in 10 ml of tetramethylammonium hydroxide. To optimize the dissolution, samples were stirred in an ultrasonic bath for 1.5 h at 50 °C. After complete dissolution of the tissue, buffer and acetic acid were added to stabilize the pH between 4 and 5.

The samples were simultaneously derivatized and extracted using 1 ml of the $NaBEt_4$ solution and 5 ml of *n*-hexane. The samples were mechanically shaken for 20 min, then centrifuged at 5000 rpm for 15 min at a temperature of 0 °C. The organic phase was recovered. A second extraction with 5 ml of *n*-hexane was performed, followed by a second centrifugation. The organic phase was recovered and combined with the first one. The combined extract was then dried with activated sodium sulfate and concentrated to ~0.5 ml by evaporation under a gentle stream of pure nitrogen. The samples were then cleaned up using SPE (solid phase extraction) Florisil cartridges and eluted with 10 ml of *n*-hexane. The purified samples were then again concentrated to about 0.5 ml under a gentle stream of pure nitrogen prior to injection for capillary GC using a flame photometric detector (FPD).

Two standard addition experiments were carried out in order to assess the extent of possible matrix effects. Approximately 0.5 g of freeze-dried oyster and fish tissue samples were spiked with different amounts of a standard solution of MBT, DBT and TBT. The difference between the slopes of the curves obtained for the two matrices was analysed statistically.

Analysis by GC-FPD

An HP 5890 gas chromatograph equipped with an FPD (610 nm filter and with a hydrogen-rich flame) was used for all organotin determinations. A 30 mm × 0.25 mm × 0.25 μm HP-5 (Hewlett-Packard) capillary column (5% phenyl methyl silicone) was used with splitless injection (250 °C) and the FPD was maintained at 270 °C. Helium, at a flow of 1 ml min^{-1}, was used as the carrier gas. The GC temperature program was 60 °C for 2 min, then 60–270 °C at 6 °C min^{-1} and 270 °C for 20 min.

RESULTS AND DISCUSSION

Optimization

The method for organotin compounds presented in this paper is based on ethylation with $NaBEt_4$.[9,11] The simultaneous derivatization and extraction with $NaBEt_4$ converts butyltin and phenyltin moieties into their corresponding volatile derivatives in the organic phase. This derivatization reaction is the most critical step of the protocol. Firstly, it should be appreciated that $NaBEt_4$ is extremely air-sensitive and must be handled with care to keep its chemical integrity. For optimal derivatization efficiency, the solution of $NaBEt_4$ should be either freshly prepared just prior to sample processing, or stored frozen (−20 °C) for no longer than 2 weeks.[7] Optimal conditions for $NaBEt_4$ comprise a 30 min reaction time at a pH between 4 and 5.[8,12]

While processing biological matrices, foams and colloidal interfaces often appear between the organic and the aqueous phases. These interfaces interfere with the recovery of the organic phase and can lead to underestimation and low reproducibility. Many experiments were carried out to prevent the formation of such colloidal interfaces: samples have been shaken vigorously for long time, frozen down to −80 °C, treated with NaCl solutions and exposed to a low non-focused microwave field. However, all these methods were found to be ineffective. It was observed that the complete dissolution of the samples to give a homogeneous and limpid solution reduces the appearance of the colloidal phases. To improve dissolution and homogenization, samples were stirred in an ultrasonic bath at a temperature between 40 and 60 °C. Thereafter, centrifugation at 5000 rpm at 0 °C resulted in excellent phase separation and recovery levels, even in the case of complex matrices such as fish and mussels with high lipid content. Figure 1 reports chromatograms of a spiked blank, oyster tissue and mussel tissue (BCR 477). Both baseline and peaks are very well defined, which allows for an accurate and precise quantification. The detection limits of this method, depending on the compounds, are in the order of 3–4 ng g^{-1} as organotin moiety.

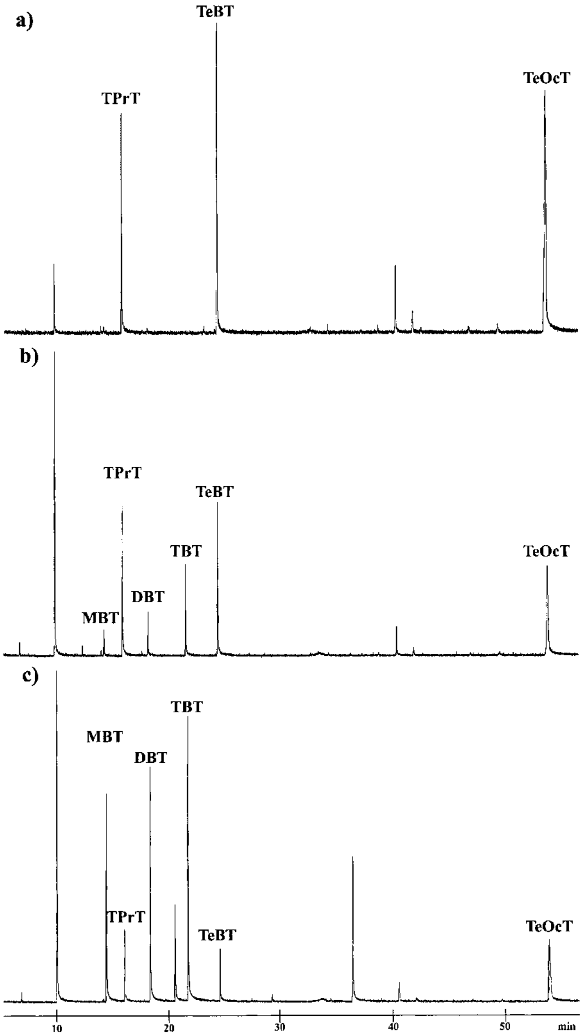

Figure 1. GC-FPD chromatograms of samples spiked with the three internal standards TPrT, TeBT and TeOcT: (a) blank, (b) oyster tissue, (c) mussel tissue BCR 477

Results quantification and quality control

A matrix-matched calibration curve was made, for every working batch of six samples, by spiking known amounts of both butyl- and phenyl-tin compounds into a non-contaminated fish tissue matrix. For each organotin compound, an average response factor was obtained from the calibration curve. The response factors were then used to quantify all butyl- and phenyl-tin concentrations in the samples.

A quality control system based on three internal standards was implemented. Each critical step of the procedure was assessed by the recovery of the corresponding internal standard. Firstly, TPrT chloride was used to indicate the derivatization reaction efficiency. All the chromatographic

Table 1. Examples of recoveries of tripropyltin and tetraoctyltin (internal standards) in different matrices

Sample matrix	Tripropyltin			Tetraoctyltin		
	Quantity spiked (ng)	Quantity measured (ng)	Recovery (%)	Quantity spiked (ng)	Quantity measured (ng)	Recovery (%)
Blank	66.8	65.2	98	317.8	252	79
Fish	267	243	91	1271	1189	93
Mussel	267	312	117	1271	1166	92
Oyster	133.5	136	102	317.8	295	93
Barnacles	133.5	136	102	317.8	253	80

Q2

Table 2. Certified and measured butyltin concentrations (mg kg^{-1} as organotin moiety) in Certified Reference Material BCR 477 (mussel tissue)

Sample no.	MBT	DBT	TBT
1	1.0	1.5	2.3
2	1.4	1.3	2.6
3	1.4	1.4	2.7
4	1.3	1.3	2.4
5	1.3	1.7	2.0
Mean	1.27	1.44	2.39
SD	0.16	0.18	0.25
Certified value	1.50 ± 0.28	1.54 ± 0.12	2.20 ± 0.19

Table 3. Certified and measured TBT and TPhT concentrations (μg g^{-1} as chloride) in Certified Reference Material NIES 11 (fish tissue)

Sample no.	TBT	TPhT
1	1.1	3.6
2	1.2	4.6
3	1.2	5.3
4	1.2	4.3
Mean	1.2	4.5
SD	0.06	0.67
Certified Value	1.3 ± 0.1	6.3[a]

[a] Reference value.

Table 4. Results (ng g^{-1} as chloride) of five independent determinations of organotin compounds in oyster tissue

Sample no.	MBT	DBT	TBT
1	191	332	535
2	195	371	558
3	192	330	543
4	186	329	538
5	191	349	574
Mean	191	342	550
SD	3.2	18	16

peak areas were normalized to that of derivatized TPrT. Low recoveries for TPrT usually indicated that the NaBEt$_4$ solution had not been correctly prepared or that it had been kept too long. Secondly, TeOcT was used to check the overall solvent extraction efficiency. Low TeOcT recoveries were usually associated with incomplete sample dissolution, manifested as opaque solutions. Thirdly, TeBT was used as a GC internal standard to quantify the recoveries of both internal standards.

Table 1 reports an example of the recoveries of tripropyl tin and tetraoctyltin in different matrices. The recovery values are reported as percentages and represent the ratio between the quantity measured and the quantity spiked. The values usually range between about 80% and 115% and are shown to be matrix-dependent.

Application to marine biota samples

Certified Reference Materials were analysed in order to assess the accuracy and the precision of the method proposed. NIES-11 (fish tissue) and BCR 477 (mussel tissue) were analysed several times and the results obtained for organotin compounds were compared with the certified values (Tables 2 and 3). The concentrations reported in Tables 2 and 3 are expressed in the same units as those reported on the certification sheets. Butyltin values obtained for both BCR-477 and NIES-11 were consistently in excellent agreement with the certified values. The available value for TPhT in NIES-11 is not certified but it is given as a reference value, and the results obtained here seem to be slightly underestimated. However, since TPhT degrades rapidly, and considering the age of the material, the low levels are possibly due to photodegradation[13] of the material rather than to an underestimation.

After optimizing and validating the method by analysing Certified Reference Materials, this method was subsequently applied successfully to real marine biota samples: mussels, oysters, fish and barnacles. Table 4 reports the results of five independent analyses on the same oyster tissue, and demonstrates the excellent reproducibility of the technique.

Table 5. Slopes of standard addition experiments for oyster and fish. The 95% confidence limits are given in parentheses; the calculated and critical *t* values for 95% confidence limits are also listed

			t value	
Compound	Oyster	Fish	Calculated	Critical[a]
MBT	$4.15 \times 10^{-3} \pm (0.15 \times 10^{-3})$	$3.91 \times 10^{-3} \pm (0.46 \times 10^{-3})$	0.562	3.18
DBT	$3.09 \times 10^{-3} \pm (0.30 \times 10^{-3})$	$3.10 \times 10^{-3} \pm (1.3 \times 10^{-3})$	0.0017	3.18
TBT	$3.88 \times 10^{-3} \pm (0.65 \times 10^{-3})$	$4.03 \times 10^{-3} \pm (0.20 \times 10^{-3})$	0.252	3.18

[a] Critical *t* values for a significance test at 95% confidence limit with three degrees of freedom.

Standard addition experiments

Two different matrices (oyster and fish) were spiked with varying amounts of butyltin standard solution. The amounts added were approximately the double and the quadruple of the amounts already present in the samples. The slopes of the curves obtained for each compound for both the oyster and fish matrices were compared. To verify if the slopes were statistically identical, and thus that there was no matrix effect, a significance test was carried out. Table 5 reports the slopes of the curves with their associated confidence limits (95%). The last two columns show the calculated *t* values and the critical *t* values for 95% confidence limits. Because the observed *t* values are less than the critical values, there is no evidence that the differences between the slopes are significant. It is thus evident that the method proposed allows the quantification of the organotin concentration in different matrices, overcoming the most important matrix effects.

CONCLUSIONS

In this paper, a simple and effective method for measuring organotin compounds in biological samples is presented. This method is based on the *in situ* simultaneous derivatization and extraction with $NaBEt_4$ and has been successfully applied both to Certified Reference Materials (NIESS-11 and BCR-477) and to different biological samples (oyster, fish, mussels and barnacles). The matrix effect problems and disturbances linked in previous studies to the use of $NaBEt_4$ have been overcome. The complete dissolution of the samples before the derivatization reaction was found to be necessary to obtain accurate and precise results. The use of three organotin internal standards (TPrT chloride for the derivatization step, TeOcT for the solvent extraction and TeBT as a GC internal standard) allowed assessment of the efficiency of each step of the procedure. Organotin compound concentrations found in Certified Reference Materials were consistently in excellent agreement with the certified values. Repeated analyses and standard addition experiments demonstrated high reproducibility and negligible matrix effects. The accurate results and the speed of the method make it ideal for routine analysis of organotin compounds in biological samples.

REFERENCES

1. de Mora SJ (ed.). *Tributyltin: Case Study of an Environmental Contaminant*. Cambridge University Press: Cambridge, 1996.
2. Champ MA and Seligman PF. *Organotin, Environmental Fate and Effects*. Chapman & Hall, 1996.
3. Smedes F, de Jong A and Davies IM. *J. Environ. Monit.* 2000; **2**: 541.
4. Quevauviller Ph. The analysis of butylated tin compounds in the environment and in biological materials. In *Tributyltin: Case Study of an Environmental Contaminant*, de Mora SJ (ed.). Cambridge University Press: Cambridge, 1996; 62–93.
5. Donard OFX, Randall L, Rapsomanikis S and Weber JH. *Int. J. Environ. Anal. Chem.* 1986; **27**: 55.
6. Meinema HA, Burger-Wiersma T, Versluis-De Haan G and Gevers EC. *Environ. Sci. Technol.* 1978; **12**: 288.
7. Michel P and Averty B. *Appl. Organomet. Chem.* 1991; **5**: 393.
8. Carlier-Pinasseau C, Lespes G and Astruc M. *Appl. Organomet. Chem.* 1996; **10**: 505.
9. Chau YK, Yang F and Brown M. *Anal. Chim. Acta* 1997; **338**: 51.
10. Montigny C, Lespes G and Potin-Gautier M. *J. Chromatogr. A* 1998; **819**: 221.
11. Schmitt VO, Szpunar J, Donard OFX and Lobinski R. *Can. J. Anal. Sci. Spectrose.* 1997; **42**: 2.
12. Ceulemans M, Szpunar-Lobinka J, Dirkx WMR, Lobinski R and Adams FC. *Int. J. Environ. Anal. Chem.* 1992; **52**: 113.
13. Caricchia AM, Chiavarini S, Cremisini C, Morabito R and Scerbo R. *Anal. Chim. Acta* 1994; **286**: 329.