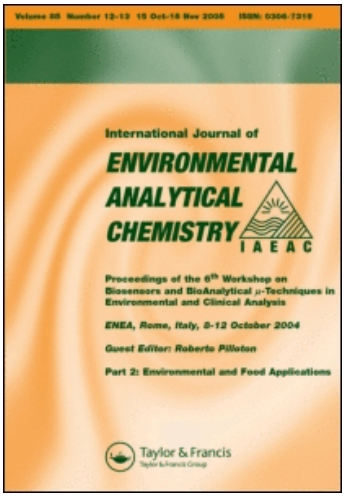

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of DI- and Tributyltin in Sediment and Microbial Biofilms Using Acidified Methanol Extraction, Sodium Borohydride Derivatization and Gas Chromatography with Flame Photometric Detection

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To cite this Article Matthias, Cheryl L. , Bellama, Jon M. , Olson, Gregory J. and Brinckman, Frederick E.(1989) 'Determination of DI- and Tributyltin in Sediment and Microbial Biofilms Using Acidified Methanol Extraction, Sodium Borohydride Derivatization and Gas Chromatography with Flame Photometric Detection', International Journal of Environmental Analytical Chemistry, 35: 2, 61 — 68
To link to this Article: DOI: 10.1080/03067318908028379
URL: http://dx.doi.org/10.1080/03067318908028379

Intern. J. Environ. Anal. Chem., Vol. 35, pp. 61–68
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DETERMINATION OF DI- AND TRIBUTYLTIN IN SEDIMENT AND MICROBIAL BIOFILMS USING ACIDIFIED METHANOL EXTRACTION, SODIUM BOROHYDRIDE DERIVATIZATION AND GAS CHROMATOGRAPHY WITH FLAME PHOTOMETRIC DETECTION

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(*Received 10 February 1988; and in final form 15 August 1988*)

A method for the relatively rapid determination of di- and tributyltin species in sediment and microbial biofilms is presented. Di- and tributyltin species were detected in Chesapeake Bay sediments using this method. Microbial biofilms grown on glass slides exhibited substantial accumulation of tributyltin species from solution. This method should have useful application to sediment analysis of di- and tributyltin species and for studies on the accumulation and fate of tributyltin in microbial biofilms.

KEY WORDS: Antifouling coatings, biofilms, chemical speciation, flame photometric detection, gas chromatography, organotin compounds, sediments, tributyltin species.

INTRODUCTION

The recent concern about the effect of the marine biocide tributylin (TBT) on non-target organisms has spawned the development of a number of analytical methods for the determination of TBT in fresh, estuarine and marine water.[1-6] Unfortunately, relatively few methods have been published for TBT determination in sediment and in microorganisms. Determination of TBT and dibutyltin, a major TBT degradation product, in sediments is necessary in order to understand fully the environmental fate and long-term effect of TBT. Some as yet unanswered questions concerning TBT and its interaction with sediment include: (1) what is the role of TBT sorption on to particulate matter and subsequent sedimentation as a route for removal of TBT from the water column; (2) what is the effect of TBT in sediments on benthic organisms; and (3) is TBT released from sediments to the water column and, if so, at what rate? This last question is important in light of recent bans on the use of TBT paints. Mobilization from sediments could sustain

elevated concentrations of TBT in the water column despite reduced TBT inputs from paints.

It is also important to measure butyltin species in microbial biofilms which coat particles and surfaces in aquatic environments. These ubiquitous films may play an important role in the binding and bioavailability of TBT in the aquatic environment. Blair, *et al.*[7] measured TBT in methanol extracts from laboratory cultures of bacteria which had accumulated TBT.

The first analytical method for TBT determination in sediment was reported by Seidel *et al.*,[8] who leached TBT from sediment using 0.3 N HCl. The leachate was then analyzed by the hydride generation technique described by Hodge and co-workers.[9] Maguire[10] reported TBT determination in sediment by refluxing with tropolone/benzene following by Grignard derivatization. Valkirs *et al.*[5,11] determined TBT in sediment by suspending a small portion (0.1–0.2 mg) of sediment in artificial seawater and then analyzing using their hydride generation method for water. These same investigators have recently reported[12] that some particulate-associated TBT is not available to direct borohydride derivatization, opening to question the utility of this approach to sediment analysis. Rice *et al.*[13] determined TBT in sediment by hexane reflux followed by hexyl Grignard derivatization. Tsuda *et al.*[14] determined TBT by extraction with acidified ethyl acetate-hexane or hexane following by conversion to the hydride derivatives using sodium borohydrides in ethanol. The methods above employ atomic absorption,[8,11] flame photometric,[10,13] and electron capture[14] detectors.

The approach described here involves reflux with acidified methanol, followed by extraction into cyclohexane, and conversion to the respective butyltin hydrides using aqueous sodium borohydride. Speciation and quantitation was accomplished by gas-liquid chromatography using tin-selective flame photometric detection.[1] This approach has the advantage of being more rapid than some other sediment TBT analysis techniques mainly because shorter reflux times were required for TBT extraction. It also utilizes a water miscible solvent (methanol), preventing the need for drying the samples.

EXPERIMENTAL

For the work described herein, a Hewlett-Packard (HP) (Avondale, PA) Model 5730A gas chromatograph equipped with an HP flame photometric detector (FPD) was used. The chromatographic separation was carried out on a 2 mm i.d. × 6 ft (1.8 m) glass column packed with 1.5% OV-101 (liquid methyl silicone) on Chromosorb G HP (Varian, Sunnyvale, CA). Nitrogen gas (zero grade) carrier flowed at the measured rate of 20 mL/min. A hydrogen rich flame was sustained with hydrogen flowing at 150 mL/min, air at 50 mL/min and oxygen at 5 mL/min. The tin molecular emission was monitored with a 600–2,000 nm optical interference filter. The chromatographic column was initially held at a temperature of 30 °C for 2 min following sample injection, then the oven temperature was increased 32 °C/min to a final temperature of 170 °C.

All glassware was cleaned prior to use by washing with laboratory detergent

followed by 12–24 h of leaching with 10% nitric acid. The clean glassware was then rinsed with copious amounts of deionized water.

Spectrograde cyclohexane and methanol was obtained from Fisher Scientific (Silver Spring, MD). Sodium borohydride (sodium tetrahydroborate) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Fresh solutions of sodium borohydride (4% w/v) were prepared daily in deionized water.

Di- and tributyltin chlorides and tripropyltin chloride were obtained from Alfa Chemical (Milwaukee, WI). These compounds were all greater than 95% purity and were used as received without further purification. Stock solutions of butyltin and propyltin chlorides were prepared gravimetrically in spectrograde methanol at about 2,000 ng/μL. These stock solutions were stable for about three months at room temperature in borosilicate glass bottles, if protected from light. Dilutions of these stock solutions to 0.5–1 ng/μL were prepared daily in methanol.

Analytical Procedure

Two to four grams of wet sediment was placed in a 150 mL round bottom flask (water content was determined by drying a separated subsample to constant weight in a 100 °C oven). Fifty microliters of tripropyltin chloride (5.0 ng/μL in methanol) was added as internal standard, followed by 0.5 mL concentrated HCl. The sample was then swirled for about 30 seconds and 25 mL methanol was added. A magnetic stir bar was then placed in the flask and the mixture was refluxed for 30 minutes in an 80 °C water bath using a magnetic stirrer. Initially, reflux times of 30, 60 and 120 min were tested and showed similar recoveries of TBT. All subsequent work used a 30 min reflux. After refluxing, the sample was cooled to room temperature and the slurry transferred to a 50 mL glass centrifuge tube (Corning Glass, Corning, NY). The sample was centrifuged for 5 min at 164 × G. The supernatant was transferred to a 25 mL volumetric flask using a Pasteur pipet, and methanol was added to bring the sample volume to 25 mL. One milliliter subsamples of the resulting solution were placed in 5 mL glass vials (Reactivial, Wheaton Scientific, Millville, NJ) and 1.0 mL of cyclohexane was added. Quantitation was done by method of standard additions with the di- and tributyltin spikes added to three subsamples at this point in the analysis. The methanol solutions were extracted with cyclohexane for 5 min on a mechanical wrist-action shaker (Burrel Corp., Pittsburgh, PA). The cyclohexane layer was removed and an additional 1.0 mL cyclohexane was added to each vial containing the methanol solution and a second extraction was performed. The cyclohexane layers were combined and evaporated to about 0.7 to 1.0 mL using a stream of dry air. Hydride derivatization was achieved by shaking the cyclohexane layer with 1.0 mL of 4.0% (w/v) sodium borohydride in water for 45 min. The aqueous layer was then removed with a Pasteur pipet and a 5–10 μL aliquot of the cyclohexane solution was injected onto the gas chromatographic column.

The analysis of biofilm material was performed in the same manner as above except that the films were refluxed in 10 mL of methanol plus 0.5 mL HCl. Following reflux, the entire solution was extracted with two 4.5 mL portions of

cyclohexane. Microbial films were grown on glass slides immersed in 500 mL of an autoclaved Chesapeake Bay water solution amended with 0.005% peptone and 0.005% yeast extract (Difco, Detroit, MI). The solution was inoculated with a mixed microbial community scraped from a TBT-painted panel in Chesapeake Bay near Annapolis, MD. After four weeks incubation at 22 °C several of the glass slides with biofilms were placed into autoclaved Chesapeake Bay water containing 50 μg/L TBT (as the cation). Control slides consisted of clean glass slides placed into water containing TBT and biofilm slides in water not spiked with TBT. After 4 days incubation at 22 °C, the slides were dipped twice into unspiked Chesapeake Bay water and scraped with a single edge razor blade into glass centrifuge tubes. Six glass slides containing biofilms exposed to TBT were scraped into a single centrifuge tube. An aliquot of the pooled sample was analyzed for TBT content and three other aliquots were spiked with varying amounts of di-, tri-, and tetrabutylin species for method of additions quantitation. An additional aliquot was removed for dry weight determination.

This method was also used to determine TBT in some sediment samples collected in July, 1987 in and around several commercial marinas in the Annapolis, MD area. The samples were collected using a polycarbonate sediment core sampler and the top 2–3 cm of sediment was collected into polycarbonate bottles and frozen at −20 °C until analysis (about 45 days after collection). Previous work has shown TBT is stable in frozen seawater for 2–3 months.[15]

RESULTS AND DISCUSSION

Chromatographic Separation

A chromatogram typical of that seen for sediment samples is shown in Figure 1(a). Di- and tributyltin as well as the internal standard tripropyltin are well resolved. Monobutyltin cannot be determined because of apparent cooling of the flame by the hydrocarbon solvent. Tetrabutyltin cannot be determined because of a very large interfering peak that appears immediately after tributyltin and has a peak width of about 5–7 minutes, overlapping with the tetrabutyltin retention time. Biofilms (Figure 1(b)) do not show the wide interference peaks noted in sediment samples and hence tetrabutyltin is detectable.

Recovery

Recovery of an analyte from a complex matrix such as sediment is often difficult to assess. An NBS standard reference material (SRM) of sediment certified for TBT is under development, but was not available for use at the time this work was done. In the absence of a suitable SRM, the usual approach is to spike a known amount of the analyte into the matrix of interest, allow time for equilibration, then subject the spiked system to the analytical scheme. This approach has the disadvantage that spiking the matrix may not result in the same

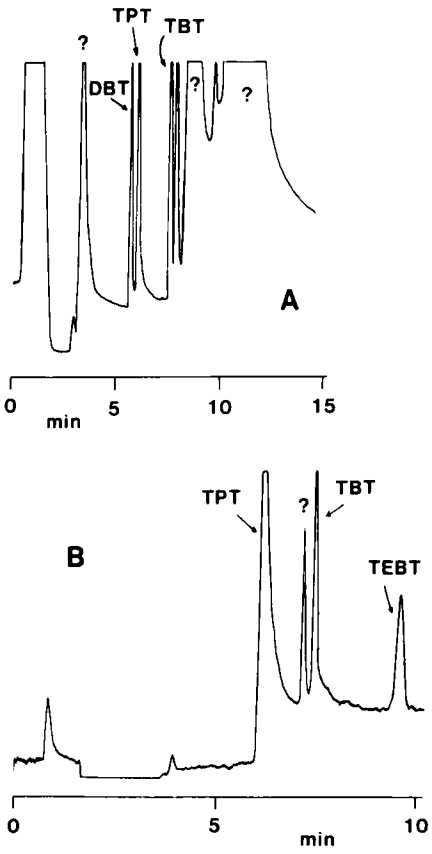

Figure 1 Chromatograms showing the occurrence of (a) di- and tributyltin species in sediment (site 2, Table 1) and (b) tri- and tetrabutyltin in microbial biofilms. TBT denotes tributyltin; DBT, dibutyltin; TEBT, tetrabutyltin; TPT, tripropyltin (internal standard).

type or rate of sample-matrix interactions as are found in the natural sytsem, but in the absence of a natural material with known analyte concentration, it is a reasonable interim approach to determining recovery efficiency.

Spiked sediment samples were prepared by suspending 1.0 g dry Chesapeake Bay surface sediment of low intrinsic TBT concentration (a pristine site in mid-Bay) in 2 mL artificial seawater and spiking the samples to either 0.3 μg TBT/g sediment or 1.2 μg TBT/g sediment dry weight. These spiked samples were swirled and allowed to equilibrate overnight (15 hr), then were refluxed in methanol as described below. Additional amounts of TBT were then spiked into the methanol extracts (method of additions) and the extraction and derivatization completed as above. Recovery of TBT was $79\% \pm 26\%$ for the 0.3 μg/g samples, and $140\% \pm 25\%$ for the 1.2 μg/g samples ($n = 3$). This is in good agreement with Hattori *et al.*[16] who report 93% recovery of tributyltin from sediment spiked to about 0.04 μg/g, and Maguire[10] who found TBT recovery from spiked sediments

Table 1 Concentration (μg/g dry weight) (mean $\pm$ SD of duplicate determinations of duplicate samples at each location)

Site	*Dibutyltin*	*Tributyltin*
1	2.2 $\pm$0.5	1.4 $\pm$0.8
2	0.64 $\pm$ 0.14	0.60 $\pm$ 0.16
3	0.57 $\pm$ 0.24	0.43 $\pm$ 0.20
4	0.40 $\pm$ 0.02	0.62 $\pm$ 0.20
5	0.15 $\pm$ 0.03	0.14 $\pm$ 0.05
6	0.09 $\pm$ 0.04	0.24 $\pm$ 0.20
7	n.d.	0.05 $\pm$ 0.10

ranged from 63 $\pm$ 35 to 108 $\pm$ 11% depending on concentration of TBT spikes (0.01–100 mg/kg).

Quantitation and Interferences

Quantitation of TBT in sediments and biofilms was by method of standard additions rather than a calibration curve because of an apparent interferent in the sediment sample matrix that diminished the flame photometric detector response. This interferent causes no FPD response and may be a hydrocarbon component of the sediment extract. Quenching of the FPD flame by hydrocarbons has been reported.[17] Addition of diesel fuel to a prepared standard of TBT hydride in dichloromethane resulted in a reduction of the FPD response to TBT, similar to that observed with sediment extracts, probably caused by quenching of the excited species produced in the FPD flame. We analyzed sediments in the vicinity of marinas where antifouling paints containing TBT are commonly used. Therefore, detection of TBT was not a problem. Samples with low TBT concentrations would require slight modification of our analytical procedure. Specifically, cyclohexane extraction of a larger subsample of the refluxed methanol, as was performed with biofilm material, would improve sensitivity.

Environmental Samples

Several sediment samples collected from commercial marinas in the Annapolis, MD area were collected and found to contain 0.05 to 1.4 μg TBT per g dry weight of sediment. Dibutyltin levels at these sites ranged from non-detectable (<0.05 μg/g sediment) to 2.2 μg/g dry weight (Table 1).

Biofilms

Substantial quantities of TBT were bound to microbial films on glass slides which

were immersed into Chesapeake Bay water spiked with 50 μg/L TBT. After 4 days incubation, the biofilm material contained 353 μg TBT/g dry weight. Each glass microscope slide contained an average 0.43 mg dry weight biofilm over an area of 25 cm^2. The concentration of TBT in the biofilm was determined by spiking aliquots of the pooled biofilm sample with di- and tributyltin species. There was no evidence of degradation of TBT to DBT species either in the biofilm or in the solution of Bay water in which the biofilm slides were suspended. No TBT was detected in scrapings from control slides. Tetrabutyltin was detected in the biofilm (Figure 1(b)). We do not think that the biofilm produced tetrabutyltin (for example, from a redistribution of TBT to di- and tetrabutyltin) but rather accumulated contaminant tetrabutyltin from solution. We detected tetrabutyltin in the TBT-spiked Bay water before immersion of the biofilm slides. No tetrabutyltin was detected in solution after the four day biofilm incubation period. Other biofilm experiments using Bay water spiked with chromatographically purified TBT did not show tetrabutyltin in solution or in biofilms.

SUMMARY

A relatively rapid method suitable for determination of di- and tributyltin species in sediments and biofilms is described. The method was used to show that marina sediments contained di- and tributyltin species and that microbial biofilms have large capacity for accumulation of TBT species.

Acknowledgements

We thank Lenwood Hall and Stephen Bushong of The Aquatic Ecology Section, Applied Physics Laboratory, The Johns Hopkins University for assistance with collection of the sediment samples. We also thank the Office of Naval Research for support for portions of this work, and thank Paul Schatzberg, David Taylor Naval Ship Research and Development Center, for helpful discussions. Portions of this work will be included in the doctoral dissertation of CLM, in partial fulfillment of the requirement of the Graduate School of the University of Maryland. Certain commercial products are identified in order to adequately describe experimental procedures. Such indentification does not imply endorsement by the National Bureau of Standards.

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