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Extraction Procedure for the Measurement of Butyltin Compounds in Biological Tissues Using Toluene, HBr, and Tropolone

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Tributyltin (TBT), an organometallic compound with impressive biocidal activity, has been used extensively in marine paint formulations to prevent the accumulation of barnacles and slime on boat hulls. In recent years, concern for the environmental impact of TBT and its degradation products dibutyltin (DBT) and monobutyltin (MBT) has prompted diverse studies in the fate and effect of TBT in the marine environment (Champ and Pugh 1987, Maguire 1982, Waldock and Miller 1982, Blunde *et al.* 1984).

One of the key elements in understanding the environmental impact of butyltin compounds rests on the results of laboratory studies conducted to assess the biological effects of TBT on aquatic organisms (Beaumont and Budd 1984, Hall and Pinkney 1984, Walsh 1985, Thain *et al.* 1987, Smith 1981, Beaumont *et al.* 1987). To fully comprehend the fate and effects of TBT in biological systems, TBT and its degradation products must be measured accurately in the tissues of test organisms. Additionally, certain environmental monitoring programs may require a complete speciation of butyltin compounds in the tissues of marine animals.

Recently, butyltin species-specific analytical techniques with sufficient sensitivity have become available for the measurement of these compounds in biological tissues (e.g. Rice *et al.* 1987). The methods used to measure butyltins in tissues yield excellent recovery of TBT and its principal degradation product DBT. This is sufficient for most environmental monitoring studies, since TBT and DBT are significantly more toxic than the very water soluble MBT (Dooley and Vata 1986). However, if a complete mass balance of butyltin compounds in biological tissue is required, current methods do not provide accurate and reproducible measurements for all four principal butyltin compounds, especially MBT. This paper presents modifications of the method of Rice *et al.* (1987) that yields excellent and reproducible recoveries of TBT and DBT, as well as the polar MBT and the TBT manufacturing impurity tetrabutyltin (TTBT).

MATERIALS AND METHODS

The objective of this work was to evaluate the recovery of butyltin species from selected marine animal tissues at environmentally significant concentrations. Tissues from eastern

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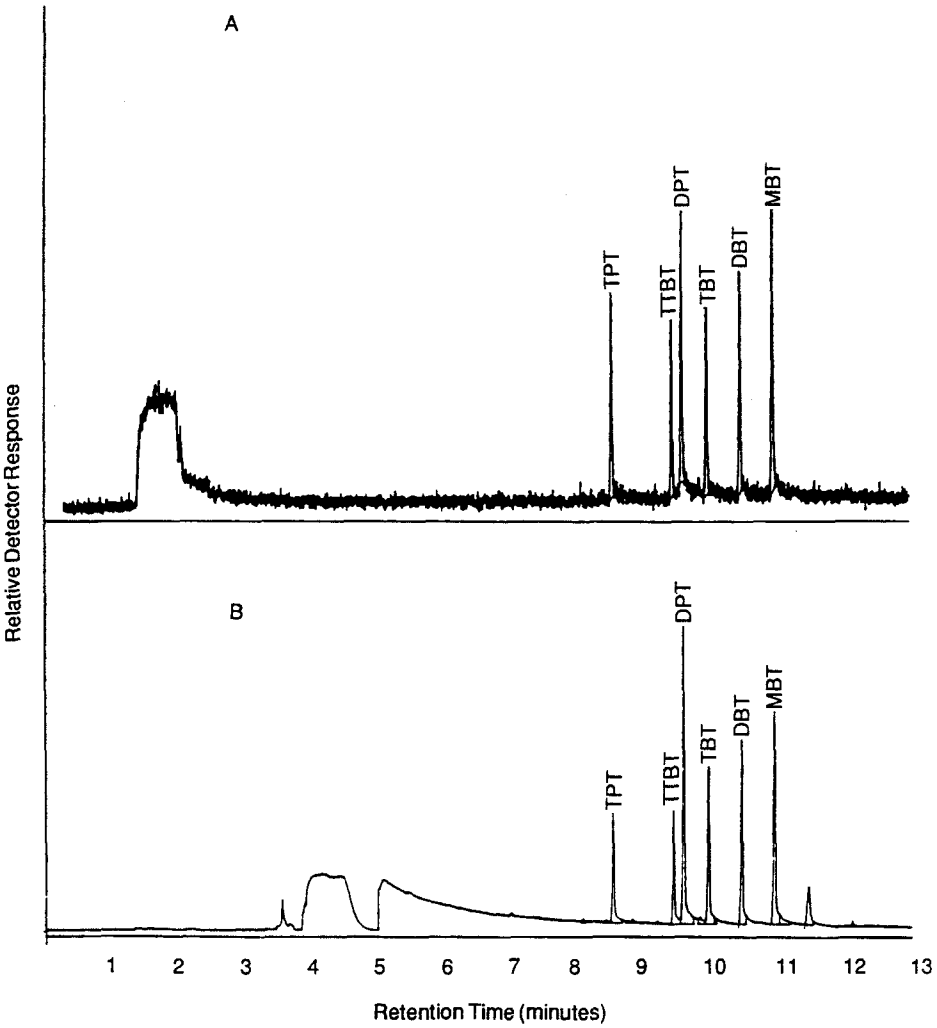

Figure 1. GC/FPD chromatograms of (A) a 2-μL of a mixed butyltin standard at the 0.2 ng/μL level, and (B) an extract of a fortified oyster sample at the 100 ng/g concentration level.

oyster (*Crassostrea virginica*), penaeid shrimp (*Penaeus setiferus*), and sheepshead minnow (*Cyprinodon variegatus*) were selected for study. Recoveries of butyltins at three fortification levels were examined. Fortification levels of 10, 100 and 1000 ng/g (wet weight) were used. These levels encompass a range from near the detection limit (10 ng/g) to a concentration near the highest encountered in biological tissue with ambient butyltin residues (Wade *et al.* 1988, Uhler *et al.* 1989).

In order to assess variability in the recovery measurements, four replicate samples were prepared and analyzed for each tissue type at each fortification level.

Butyltins were extracted from 20 g of wet tissue which had previously been homogenized in a Waring blender. Tissue homogenates were contained in a Teflon jar and fortified with 50 ng of the quantification internal standard (QIS) tripropyltin chloride (TPT) in 100 μL of acetone and with mixed butyltin standards in 100 μL of acetone at the appropriate concentration level. Next, 75 g of anhydrous sodium sulfate (pre-fired at 450°C) and 5 mL of 48 percent HBr (ACS Reagent grade) were added. The mixture was extracted by blending with a Tekmar Tissumizer for 5 min with 60 mL of 0.05 percent tropolone in toluene. Centrifugation facilitated removal of the toluene supernatant, which was dried over 50 g of sodium sulfate. Extraction was repeated two more times and the toluene extracts were combined and rotary evaporated to approximately 10 mL. Hexane (50 ml) was added, and the volume was again reduced to 10 mL. This procedure was repeated two more times until the toluene had been completely exchanged for hexane. The compounds MBT, DBT, TBT, and TPT were derivatized to the corresponding *n*-pentyl derivatives by adding 5 mL of 1.9 M *n*-pentylmagnesium bromide (Alfa, Inc.). The reaction was allowed to proceed for 15 min at room temperature, then quenched by the addition of 25 mL of water followed by 5-10 mL of 10 N sulfuric acid. The hexane fraction was reduced in volume to approximately 4 mL and subjected to liquid chromatographic cleanup. The cleanup column consisted of 16 g of Florisil (EM Science, PR grade, 60-100 mesh, activated at 130°C for a minimum of 5 hr), topped with 7 g of 1-percent deactivated silica gel (Aldrich, grade 923, 100-200 mesh), followed by 2 g of anhydrous sodium sulfate. The column, pre-wet with hexane, was charged with the sample and eluted with 100 mL of hexane. The hexane was collected in a 250-mL Kuderna-Danish apparatus and reduced in volume to ca. 4 mL. The final extract was further reduced in volume to 500 μL by evaporation under a stream of nitrogen gas. An appropriate amount of the recovery internal standard dipropyldipentyltin (DPT) was added. The samples were analyzed by capillary gas chromatography with flame photometric detection (GC/FPD). Gas chromatographic standards were prepared in hexane from the chloride adducts of MBT, DBT, TBT, DPT, TPT, and from pure TTBT (Alfa, Inc.). The standards were derivatized according to the scheme presented above, and diluted to the working range of the samples.

Separation and quantification of the butyltins in tissue extracts were performed by high resolution GC/FPD. A Hewlett-Packard model 5890A was used in this work. The GC was fitted with a 30-m x 0.25-mm i.d. (0.25-μm film thickness) DB-5 capillary column (J&W Scientific, Inc.) using helium at 32 cm/sec as the carrier gas. The FPD was operated using a hydrogen/air flame, and fitted with a 610-nm bandpass filter that is selective for compounds containing tin (Kaplia and Voight 1980). The GC temperature program was 60°C for 1 min, then ramp to 250°C at 20°C/min.

Typical GC performance is presented in Figure 1. Figure 1A is a chromatogram of a 2-μL injection of a mixed butyltin standard containing approximately 0.2 ng/μL of each species. Figure 1B is a chromatogram of an oyster test tissue fortified at the 100 ng/g level. GC/FPD chromatograms of tissue extracts are typified by baseline resolution of the butyltin components and lack of interference in the region of the chromatogram where the target analytes elute.

Butyltins detected in samples were quantified using the method of internal standards. A three-point calibration was run prior to every batch of samples, using TPT as the quantification internal standard. The average response factor (RF) for each analyte from

the three-point calibration typically were less than 15 percent relative standard deviation (RSD) versus the mean RF.

RESULTS AND DISCUSSION

Tissue used in this study were pooled from 100-200 individual animals, homogenized and characterized. Results for percent moisture and percent lipid content were: eastern oyster (85% moisture; 13% lipid); penaeid shrimp (84% moisture; 7% lipid); and sheepshead minnow (74% moisture; 21% lipid).

Table 1 presents the recovery of butyltin species from fortified samples of eastern oyster, penaeid shrimp and sheepshead minnow at the 10, 100 and 1000 ng/g level. These recoveries were measured relative to the QIS TPT. As might be expected, the greatest degree of variability in recovery measurements occurred for all analytes at the lowest (10 ng/g) fortification level.

Table 1. Recovery of butyltin species from biological tissue at the 10-, 100- and 1000 ng/g fortification levels

Tissue[b]	Fortification Level (ng/g)	Percent Recovery[a] MBT	DBT	TBT	TTBT
Oyster	10	77 (4)	87 (2)	97 (9)	72 (3)
Shrimp	10	109 (14)	95 (14)	91 (17)	66 (14)
Minnow	10	69 (12)	90 (16)	89 (18)	69 (13)
Oyster	100	110 (2)	104 (2)	100 (4)	75 (6)
Shrimp	100	109 (14)	95 (14)	91 (17)	66 (14)
Minnow	100	69 (12)	90 (16)	89 (18)	69 (13)
Oyster	1000	103 (9)	97 (8)	95 (7)	75 (4)
Shrimp	1000	66 (7)	65 (6)	66 (6)	61 (6)
Minnow	1000	87 (3)	86 (4)	80 (2)	62 (2)

[a]Results reported as mean recovery ± 1 standard deviation.

[b]Test tissues are eastern oyster (*Crassostrea virginica*), penaeid shrimp (*Penaeus setiferus*), and sheepshead minnow (*Cyprinodon variegatus*).

Recovery of TBT and DBT at all fortification levels were comparable with those reported elsewhere (Rice *et al.* 1987, Uhler *et al.* 1989). MBT recoveries were a factor of 2-3 times better than can be expected using current extraction methods. The combination of sample acidification with HBr and extraction with toluene in place of hexane or methylene chloride resulted in this significant increase in MBT recovery. Tetrabutyltin recovery was somewhat lower than the other butyltin species, likely it is a reflection of loss of the analyte during sample preparation, due to the relatively high volatility of the compound.

Absolute recovery of added butyltin species from the tissue matrices was excellent. The recovery of the QIS TPT was measured versus the RIS DPT (added to the samples just before instrumental analysis). Average recovery of TPT over all three spike levels was: oyster (82%), shrimp (109%) and minnow (96%).

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REFERENCES

Champ MA, Pugh WL (1987) Tributyltin antifouling paints: Introduction and overview. Proc Oceans 87, pp 1296-1308

Maguire RJ (1982) Occurrence of organotin compounds in lakes and rivers. Environ Sci Technol 16:698-702

Waldock MJ, Miller D (1982) The determination of total and tributyltin in seawater and oysters in areas of high pleasure craft activity. International Council for Exploration of the Sea, 1982, E:12

Blunde SJ, Hobbs CA, Smith LJ (1984) In "Environmental Chemistry", ed. H.J.M. Bowen, pp 51-77 London, Royal Society of Chemistry

Beaumont AR, Budd, MD (1984) High Mortality of the larvae of the common mussel at low concentrations of tributyltin. Mar Poll Bull 15:402-405

Hall LW, Pinkney, AE (1984) Acute and sublethal effects of organotin compounds on aquatic biota: An interpretative literature evaluation. CRC Critical Reviews of Toxicology 14:159-209

Walsh GE (1985) Effects of organotins on growth and survival of two marine diatoms, *Skeletonema costatum* and *Thalassiosira pseudonana*. Chemosphere 14:383-392

Thain JE, Waldock MJ, Waite, ME (1987) Changes in concentration of organotins in UK rivers and estuaries following legislation in 1986. Proceedings Oceans 87 pp 1352-1356

Smith BS (1981) Male characteristics of female and male mud snails caused by antifouling bottom paints. J App Toxicol 1:22-25

Beaumont AR, Mills DK, Newman, PB (1987) Some effects of tributyltin (TBT) on marine algae. Proceedings Oceans 87 pp 1488-1493

Rice, CF, Espourteille DJ, Huggett RJ (1987) A method for analysis of tributyltin in estuarine sediments and oyster tissue, *Crassostrea virginica*. App Organomet Chem 1:541-544

Uhler AD, Coogan TH, Davis KS, Durell GS, Steinhauer WG, Freitas SY, Boehm PD (1989) Findings of tributyltin, dibutyltin and monobutyltin in bivalves from selected U.S. coastal waters. J Env Tox Chem 8:971-979

Wade, TL, Garcia-Romero B, Brooks, JM (1988) Tributyltin in bivalves from selected U.S. Coastal estuaries. Environ Sci Technol 22:1488-1492

Kaplia S, Voight, CR (1980) Some aspects of organic tin analysis by gas chromatography with flame photometry. J Chromat Sci 18:144-147

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