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COMMUNICATION

Analysis of tributyltin in estuarine sediments and oyster tissue, *Crassostrea virginica*

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An analytical method to determine tributyltin (TBT) in oyster tissue, *Crassostrea virginica*, and estuarine sediments is described. Recoveries of TBT from oysters range from 86 to 102% when samples are fortified at concentrations ranging from 22 to 890 $\mu g\,kg^{-1}$ (wet weight); recoveries from sediment range from 92 to 105% for samples fortified from 20 to 500 $\mu g\,kg^{-1}$ (dry weight).

Feral oysters and natural sediments were analysed and shown to be contaminated with TBT. Oysters collected near a marina contained concentrations as high as 1.5 $mg\,kg^{-1}$ (wet weight).

Keywords: Tributyltin, analysis, sediments, tissue, oyster

INTRODUCTION

Tributyltin, a widely used biocide in antifouling paints, has been the focus of considerable scientific scrutiny in both Europe and North America. Laboratory experimental evidence indicates that tributyltin (TBT) can adversely effect marine and estuarine organisms at aqueous concentrations of less than one microgram per liter ($\mu g\,dm^{-3}$) and there is increasing evidence that concentrations of less than one hundred nanograms per liter ($100\,ng\,dm^{-3}$) are harmful to some species.[1] Concentrations at or above these levels have been detected in some waters of North America and Europe.[2–5]

Existing data indicate that organotins can be concentrated by marine and estuarine organisms.[5,6] Oysters have a limited ability to metabolize TBT relative to finfish[7] and therefore have the potential of accumulating the material to levels which may prove harmful to the animal itself or to human consumers.

TBT associates with suspended and bottom sediments. The contaminated sediments can expose benthic infauna epifauna to the substance and can exchange TBT with the overlying water.

At present, the extent to which estuarine biota and sediments are contaminated with TBT is unknown. A major reason for this ignorance is that acceptable analytical methodologies have not been available. This paper reports on development of the needed methods.

EXPERIMENTAL

Oyster tissues, *Crassostrea virginica* (whole body), were removed from the shell and the tissues from either individuals or groups were blended in a Virtis homogenizer to achieve a fluid paste. At this point a subsample could have been dried to constant weight to determine moisture content if dry weight TBT concentrations had been desired. Typically this species of oyster contains 85% water. A 20 g aliquot of the wet blend was desiccated with a mixture of 8 g QUSO G35 (precipitated silica, DeGussa Corporation) and 71 g of anhydrous sodium sulfate in a $1\,dm^{-3}$ glass jar. The desiccants and tissue were thoroughly mixed by hand and the mixture was frozen overnight at −15°C to facilitate the lysing of cells. The sample was then thawed and ground to a fine powder consistency with a blender. The sample was placed in a glass soxhlet thimble with a coarse frit and spiked with a known amount of tripentyltin chloride which acts as an internal standard. The amount of tripentyltin chloride added was chosen depending on the expected range of TBT in the tissue. The concentrations of the internal standard and the TBT should be within a factor of 5 of each other in the sample. The spiked sample was soxhlet-extracted with $400\,cm^3$ n-hexane for 24 h and reduced to $10\,cm^3$ by rotary evaporation at 40°C.

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The extract was then derivatized and analyzed according to the methodology developed by Unger *et al.*[8] It involves converting the tributyltin and tripentyltin to hexyltributyltin and hexyltripentyltin respectively, a clean-up with fluorisil and quantitation by glass-capillary gas chromatography with flame photometric detection.

Sediment samples were treated exactly as tissues with the exception that 48 h was required for near complete extractions.

To determine the efficiency of the methodology to quantitate tributyltin in sediments and oyster tissues, known amounts of tributyltin chloride were added to both substrates. The oysters were from the Rappahannock River, Virginia (37°40′N, 76°34′W) and the sediments were collected from Carter Creek, Virginia (37°20′N, 76°34′W). These locations were chosen because of their isolated nature. The samples were fortified with an *n*-hexane solution of tributyltin chloride at room temperature and allowed to stand at 4°C for up to 24 h before extraction. Analyses were conducted as mentioned above except that the internal standard was added after soxhlet extraction. This was because otherwise relative recoveries of the spike would have been obtained, and absolute recoveries were sought.

To obtain an indication of the variation in 'natural' TBT concentrations between oysters from various locations and among oysters from the same location, an experiment was conducted where oysters were obtained from two areas and individual animals analyzed. Oysters from Sarah Creek, VA (37°16′N, 76°29′W) had been subjected to TBT from the numerous recreational vessels which berth here. Those from King Creek, VA (37°18′N, 76°25′W) which is much more pristine were expected to be less exposed. All oysters collected were intertidal.

An estimate of the precision of the methodology for sediment TBT quantitation was obtained by analyzing replicate subsamples from a single homogenized sample of sediments from Sarah Creek.

To determine the concentrations of TBT in sediments near marinas, ten sediment samples were obtained with a ponar grab sampler from near the mouth of and in the channel of Sarah Creek (Fig. 1). The top 2 cm of each sample was analyzed.

RESULTS AND DISCUSSION

Since tissues and sediments contain water, the samples must be desiccated if a hydrophobic extraction solvent is used. Freeze drying and oven drying (50°C) were tried, but each resulted in a loss of up to 60% of TBT added as a spike. Therefore chemical desiccation was chosen. Additionally, cellulose soxhlet thimbles were tried but abandoned after discovery that some lots contained TBT, presumably as a fungicide.

Results of the oyster fortification experiments are presented in Table 1. At concentrations of $22\,\mu g\,kg^{-1}$ (wet weight) to $890\,\mu g\,kg^{-1}$, the average percentage recovery ranged from 86% to 102%.

Results of the natural TBT concentration in oysters are presented in Table 2. The data clearly show that oysters do concentrate TBT and that organisms living in areas of high boating activity can acquire TBT body burdens which are two orders of magnitude above those from less contaminated areas.

Results of the sediment fortification experiments are presented in Table 3. At spiked concentrations of from $20\,\mu g\,kg^{-1}$ to $500\,\mu g\,kg^{-1}$, the recoveries ranged from 92 to 106%.

Results of the sediment TBT precision experiment are given in Table 4. A relative standard deviation of 5% of the mean was obtained.

Tributyltin concentrations as high as $290\,\mu g\,kg^{-1}$ (dry weight) were found in the bottom sediments from Sarah Creek (Table 5). This concentration was found at station 9, which is near a marina and boatyard. Samples collected at stations 1–5 were relatively low in TBT concentration. Stations 1 and 2 are near the mouth of the creek which experiences more flushing from the York River and stations 3, 4 and 5 are in an arm of Sarah Creek which does not have a marina and only a few vessels.

Table 1 Recovery of tributyltin from replicate spiked samples of oyster tissue

Spike[a]	Mean concentration detected	Percentage recovered
890, $n=5$	910	102
650, $n=5$	560	86
133, $n=5$	122	91
22, $n=5$	19	86

[a]Concentrations of TBT^+ reported as $\mu g\,kg^{-1}$ (wet weight) n = number of replicates.

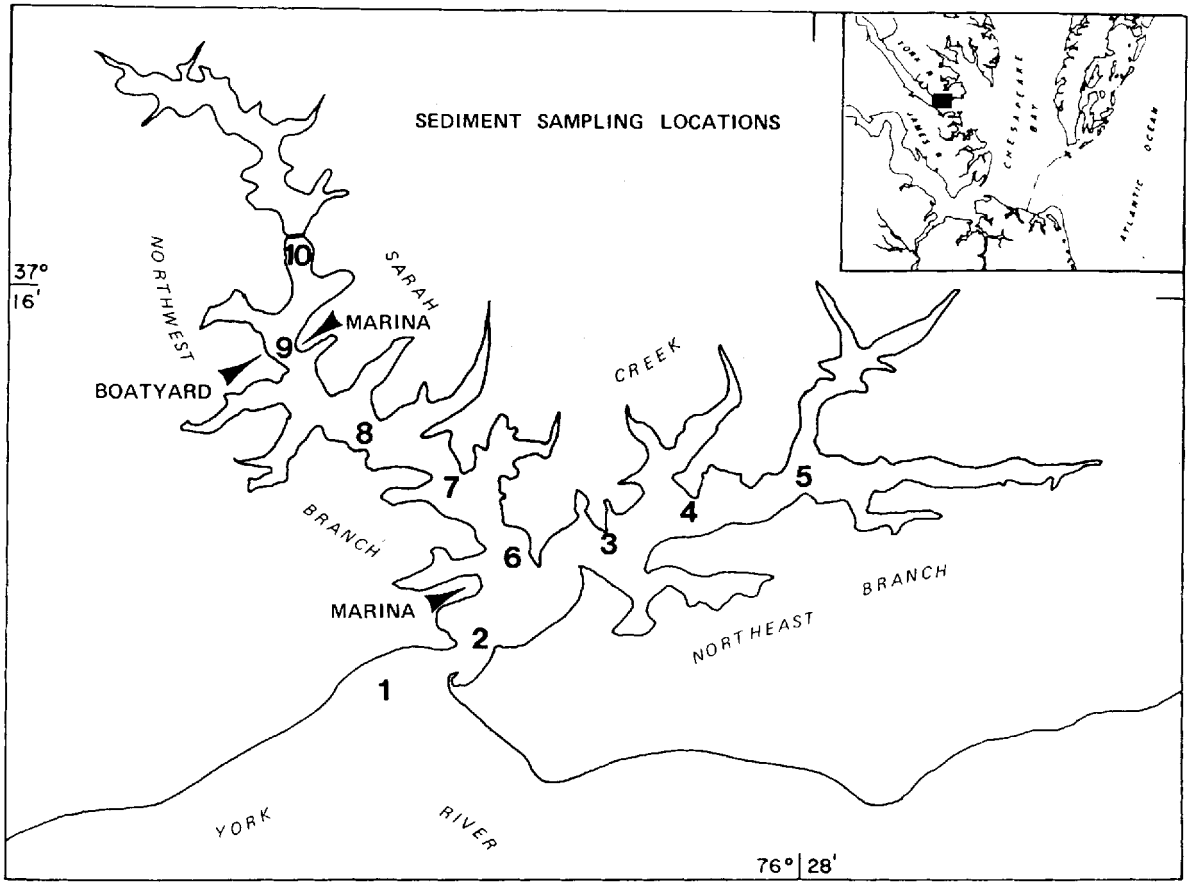

Figure 1 Sediment sampling locations in Sarah Creek, Virginia.

Table 2 Concentrations of tributyltin[a] in feral *Crassostrea virginica* tissue samples

Oyster no.	Sarah Creek	Kings Creek
1	590	6
2	1570	10
3	470	12
4	800	7
5	740	11
Mean ± S.D.	$\bar{x} = 834 \pm 430$	$\bar{x} = 9 \pm 2$

[a]Concentrations of TBT^+ reported as $\mu g\,kg^{-1}$ (wet weight).

Table 3 Recovery of tributyltin[a] from replicate spiked samples of estuarine sediments

	Spike		
Replicate	$20\,\mu g\,kg^{-1}$	$100\,\mu g\,kg^{-1}$	$500\,\mu g\,kg^{-1}$
1	18	92	527
2	22	88	522
3	21	113	537
4	19	88	535
5	19	80	507
Mean ± S.D.	$\bar{x} = 20 \pm 2$	$\bar{x} = 92 \pm 13$	$\bar{x} = 530 \pm 12$

[a]TBT^+ concentrations reported as $\mu g\,kg^{-1}$ (dry weight).

Table 4 Tributyltin subsamples taken from a single, homogenized 'natural' sediment

Subsample	Measured TBT^+ concentration ($\mu g\,kg^{-1}$, dry wt)
1	98
2	110
3	100
4	106
5	108
Mean ± S.D.	$\bar{x} = 104 \pm 5$

Table 5 Concentration of TBT^+ in bottom sediments from Sarah Creek, Virginia (a tributary of the York River with several marinas)

Sample number	TBT^+ concentration ($\mu g\,kg^{-1}$, wet wt)	($\mu g\,kg^{-1}$, dry wt)
1	12	34
2	15	43
3	13	48
4	8.6	32
5	6.1	23
6	44	71
7	40	150
8	53	120
9	74	290
10	33	110

CONCLUSIONS

The analytical methodology described above is capable of determining TBT in oyster tissues and sediments with recoveries of TBT spikes being greater than 85%.

Analysis of samples collected from contaminated and presumed-clean areas confirm that TBT is present in sediment and oysters and that the patterns observed follow what would be expected based on the numbers of boats in the various areas.

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