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BUTYLTINS IN *CRASSOSTREA VIRGINICA* FROM NINE REEFS IN MISSISSIPPI SOUND

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ABSTRACT Tributyltin (TBT), a very effective anti-foulant, has been banned from most marine paint uses since 1988 because of its reputed toxicity to nontarget organisms. *Crassostrea virginica* were collected from nine reefs in the Mississippi Sound in 1993 and analyzed for concentrations of butyltins: TBT, dibutyltin (DBT) and monobutyltin (MBT). TBT concentrations ranged from <2 to >20 ng(Sn)/g wet wt. Highest concentrations of butyltins were found at eastern Sound reefs near intense shipping and ship building activities with lowest concentrations found in the western Sound.

Introduction

Tributyltin (TBT) became widely recognized as an extremely effective anti-foulant in marine paints in the early 1980's. This application followed the use of other organotins as pesticides, disinfectants, and cotton, wood, and stone preservatives. The effectiveness of TBT in preventing attachment of fouling organisms to marine craft has been offset by its toxicity to nontarget organisms. Several excellent reviews highlight possible adverse effects from the butyltins (Heard et al 1989; Tas 1993; and Fent 1996). Publicity arising from various scientific studies led to a ban on use of TBT for most marine applications by the U.S. Organotin Anti-Fouling Paint Act of 1988. The National Status and Trends Program (NS&T) of the National Oceanic and Atmospheric Administration was created to assess spatial distributions and temporal trends in chemical contaminants in all major U.S. coastal estuaries. In 1989 NS&T added butyltins [including dibutyltin (DBT) and monobutyltin (MBT), successive degradation products of TBT] as a parameter to be measured in oysters and mussels to determine whether the ban would effectively reduce levels of these compounds in sessile organisms exposed to butyltins near regions of intense boating activity.

Three oyster reefs in Mississippi Sound were chosen as sites for NS&T studies and results are available for 1989-1993 collections in a report by O'Connor and Beliaeff (1995). The purpose of this study was to thoroughly investigate TBT residue distributions in the oyster, *Crassostrea virginica*, from nine oyster reefs in the Mississippi Sound. Results of this study indicated that TBT levels were similar to those found at the three sites reported by NS&T for 1989-1993.

Methods

Oysters were collected at nine reefs in the Mississippi Sound, with locations shown in Figure 1, placed on ice and taken to the analytical chemistry laboratory at the Gulf Coast Research Laboratory on the day of collection. Oysters were collected from Gorenflo, White House, Pass Marina and Front Pass Harbor Reefs on November 4, 1993; from Graveline, East River, West River, Bang's Lake and Middle River Reefs on November 9, 1993.

Analysis of fish tissue followed that of Durell and Uhler (1989) with the following brief details. Three samples from each reef were prepared by homogenizing three oyster tissues per sample. Samples were spiked with tri-*n*-propyltin as internal standard, dried over anyhydrous Na_2SO_4 and after acidification were extracted with a tissue homogenizer three times with tropolone in hexane. Butyltins were converted to pentyl derivatives and cleaned up with florisil chromatography. Samples were analyzed on a Perkin Elmer model 8500 gas chromatograph equipped with splitless injector and fitted with a 30m x 0.24mm (id) fused silica capillary column, 0.25µ film thickness DB-5 (J&W Scientific) and flame photometric detector modified for tin analysis by substitution with a photocell optimized for the 610nm wavelength which was selected by a photometric filter (Ditric Optics). The instrument was operated with He carrier gas set at 30cm/sec linear flow rate, helium make-up gas at 28.6ml/min, and hydrogen and air each set at 100ml/min. Programming temperatures were 75°, hold for 1min., programming to 150° @ 30°/min then to 210° @ 10°/min with 3 min. final hold. Detector and injector were each held at 250°C.

Identification of butyltins was made through retention time comparisons with authentic standards of all four butyltins pentylated in the laboratory. Likewise,

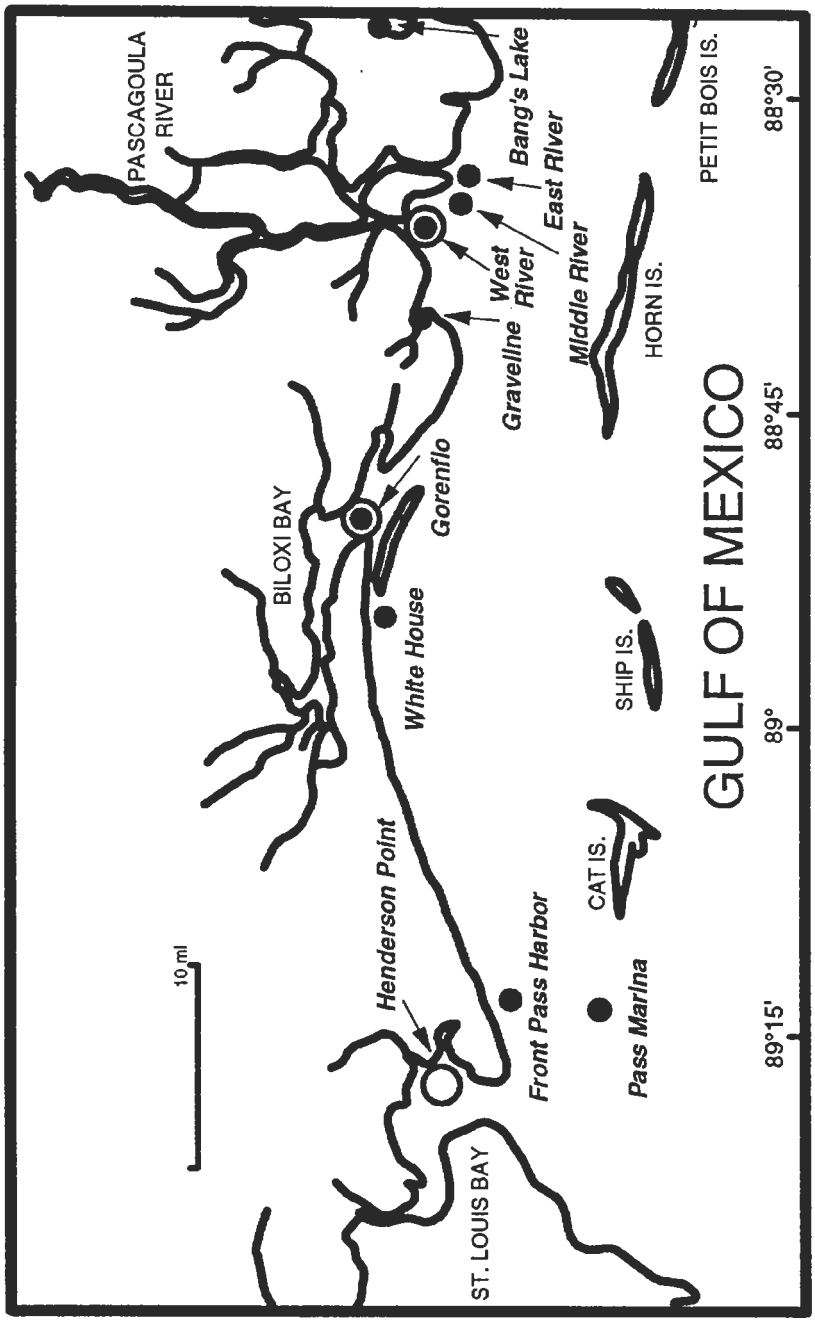

Figure 1. Oyster reefs in Mississippi Sound. Reefs sampled in present study are shown in solid circles; those sampled in NS&T study are in open circles with two sites sampled in both studies.

quantitative measurements were achieved through the use of the internal standard tri-*n*-propyltin and response factors generated through the use of the four butyltin standards. In a separate analytical validation procedure four-12 g aliquots of an oyster homogenate were analyzed before and after spiking with 50 ng(Sn)/g of the four butyltins and tri-*n*-propyltin. Quantitation in this analysis was effected by comparison to a second internal standard, dipentyldipropyltin, added just prior to gas chromatography. Absolute % recoveries of the five organotins were as follows: tri-*n*-propyltin, 64.5±8.3 (standard deviation); tetrabutyltin, 32.3±3.0; TBT, 77.2±5.1; DBT, 101±6.6; MBT, 90.6±4.6. No corrections are made in any reported values for % recoveries. All analyses were conducted under Good Laboratory Practices as described by U.S. Environmental Protection Agency (1983).

RESULTS

Butyltin concentrations for each of three oyster samples from each reef are given in Table 1. Though some values, notably for DBT, are above instrument detection limits, they are slightly below method detection limits and are included for purposes of reef intercomparisons and comparisons to literature from NS&T studies. Only TBT and DBT were detected in oysters from this study, whereas the NS&T study reported measurable levels of TBT, DBT and MBT. O'Connor (1992) proposed that a value of 53 ng(Sn)/g wet wt. be considered a "high" environmental level for total butyltins. This value was based upon results of oyster samples from 214 sites around the United States coastline in 1990 and reported by O'Connor and Beliaeff (1995)[1,2]. Only one sample from Middle River Reef had a value exceeding 53 ng(Sn)/g, and none of the samples from the other eight reefs had a value this high.

O'Connor and Beliaeff (1995) report an observed decline of butyltins at most sites in the U.S. from 1989 to 1993. This decline is presumably due to the ban on TBT use in marine paints. The mean value for all samples collected in the NS&T 1993 study was 4.8 ng(Sn)/g wet wt.[2], so the "high" value for total butyltins designated in 1990 is probably too high for a benchmark comparison for 1993 data. The mean values for total butyltins in oysters from five Mississippi Sound reefs were higher than 4.8 ng(Sn)/g and lower for four reefs.

TABLE 1

Butyltins in *C. virginica* from nine reefs in Mississippi Sound. *= mean of wet weights of 3 individual oysters ± one standard deviation. TBT=tributyltin, DBT=dibutyltin. nd = not detectable; for comparison purposes values are reported for butyltins that yielded a detectable chromatographic peak (Note that for some samples these values are below the method detection limits of: tetrabutyltin (TTBT)<1.2, TBT<2.2, DBT<1.6, monobutyltin (MBT)< 2.3 ng(Sn)/g wet wt). † = values for individual samples are followed by mean ± one standard deviation (TTBT and MBT were not detected in any sample).

Reef Sample Site	Wts, g*	Butyltins, ng(Sn)/g	
		TBT	DBT
Graveline	15.3±4.7	2.9	nd
	14.3±4.8	4.5	nd
	13.8±4.5	3.8	nd
	mean:	3.7±0.78†	nd
East River	10.3±1.9	6.9	1.7
	10.8±2.1	5.9	1.9
	10.1±9.2	3.9	0.73
	mean:	5.5±1.5	1.5±0.64
West River	11.6±5.7	22	3.6
	12.5±1.2	23	3.4
	11.8±1.2	27	3.7
	mean:	24±2.5	3.6±0.14
Bang's Lake	10.8±6.3	29	9.2
	17.2±3.9	10	4.3
	14.4±1.1	25	4.5
	mean:	22±10	6.0 ±2.8
Middle River	13.3±6.0	2.9	1.1
	9.6±3.6	1.3	0.57
	8.6±3.6	62	16
	mean:	22±34	5.9±8.9
Gorenflo	12.9±1.6	9.3	1.6
	12.6±4.3	11	2.5
	13.6±1.7	8.8	1.5
	mean:	9.6±1.0	1.9±0.51
White House	21.7±7.5	2.1	0.32
	21.9±2.6	2.0	0.48
	18.6±5.1	2.6	0.39
	mean:	2.2±0.31	0.40±0.08
Pass Marina	21.0±6.5	1.6	nd
	18.2±4.6	1.3	nd
	15.5±2.5	1.7	nd
	mean:	1.5±0.19	nd
Front Pass Harbor	16.2±2.6	2.8	0.62
	11.2±2.9	4.0	0.67
	14.4±6.3	3.0	0.48
	mean:	3.2±0.66	0.59±0.10

[1] This value was the mean plus one standard deviation of the lognormal distribution of concentrations among sites.

[2] All NS&T butyltin concentrations were reported originally on dry wt. basis and have been converted to wet wt. basis assuming 85% moisture in oysters.

Butyltin concentrations in oysters collected in Mississippi Sound by NS&T are given in Table 2. Butyltin concentrations in oysters from West River (eastern Sound) and Gorenflo Reef (central Sound) can be compared directly to oyster concentrations at the same sites in the present study. NS&T data at Henderson Point can be used indirectly to compare values of butyltins in oysters from western Sound sites reported in this study.

DISCUSSION

In this study all samples collected at West River and Bang's Lake Reefs contained levels of TBT that were four to five times that of East River and Graveline Reefs. The latter two reefs had values very near to the 1993 NS&T mean value of 4.8 ng(Sn)/g. Only one sample at Middle River had a value in excess of the 1993 NS&T mean, and it was also the highest butyltin measured in this study. We expected relatively high levels of TBT at reefs at East River and Middle River considering the proximity to long-term shipbuilding interests in the region of the Pascagoula River. The prevailing westerly river flow across the Mississippi Sound apparently has resulted in greater impact on the West River Reef than on the two reefs further east at the mouth of this river (see Figure 1). This pattern of pollutant transport to the west of river sources in the Sound has also been demonstrated for petroleum hydrocarbons (Lytle and Lytle 1985). The high TBT values at Bang's Lake were not surprising considering connection of this estuarine lake to a number of industrial drainage sites, whereas Graveline Reef oysters, exposed only to residential development, contained the lowest levels of butyltin in the eastern Sound.

The largest sample to sample variation was observed in oysters at Middle River Reef with both TBT and DBT varying over an order of magnitude among samples. The extent of this pollutant range at a single reef, though entirely unlike that observed at other reefs, is not without precedent and was observed in heavy metal content in oysters at a single reef collected by Lytle & Lytle (1990) in Mississippi Sound. T. Lytle has also noted a marked individual variability in ability of oysters to uptake TBT in chronic exposures, with a negative correlation of size to TBT level (unpublished information).

In general, oysters from reefs in the central Sound contained less butyltin residues than in the eastern Sound. Oysters from Gorenflo Reef contained approximately four times higher butyltin concentrations than at White House Reef suggesting that its location in Biloxi Bay, directly in the path of boating activity, may have exposed these oysters to higher levels of butyltins.

TABLE 2

Butyltins in *C. virginica* from National Status & Trends Program (NS&T) study. Data derived from a single analysis of a composite of 20 oysters. Data originally collected on dry weight basis converted to wet weight basis assuming 85% moisture. All data obtained from http://www-orca.nos.noaa.gov/projects/nsandt/nsandt.html on the Internet. nd=not detected; detection limits not specified but presumed similar to those reported in Table 1.

	Year	Butyltins, ng(Sn)/g		
		TBT	DBT	MBT
West River	1989	40	3.7	0.75
	1990	21	nd	nd
	1991	29	3.2	nd
	1993	13	1.1	0.21
Gorenflo	1989	69	4.0	nd
	1990	19	nd	nd
	1991	39	4.3	1.3
	1992	16	1.6	0.76
	1993	32	2.7	0.35
Henderson Point	1989	7.8	nd	0.15
	1990	2.2	nd	nd
	1991	2.8	0.66	1.5
	1993	1.5	0.61	0.19

Front Pass Harbor Reef and Pass Marina Reefs, located in the western Sound, had very low levels of butyltin residues. However, Front Pass Harbor Reef oysters had twice the level of butyltin residues as oysters from further offshore at Pass Marina Reef.

As shown in Tables 1 and 2, both this study as well as the NS&T study have shown that butyltins are highest in the Eastern Sound and lowest in the western Sound. This trend mirrors the decline in commercial boating activities in the western Sound. Though our study found more butyltins in oysters at West River Reef and less at Gorenflo Reef than did the 1993 NS&T study, the year to year fluctuations at both sites are so great that we believe that both sets of data are consistent. The present study has shown that there can be large variations in butyltin levels within and among adjacent reefs.

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