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Butyltin Compounds in Severn Sound, Lake Huron, Canada

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Severn Sound is a heavily used recreational and boating area in the southeast corner of Georgian Bay, Lake Huron, Canada. Because of the concern over the possible release of tributyltin species (TBT) from antifouling paints on boat hulls and marinas, surveys were carried out in 1989 and 1992 to determine the presence of this species and its degradation products dibutyltin (DBT) and monobutyltin (MBT) in this area. Many fish (pike and young-of-the-year spottail shiners) and sediment samples collected in 1989 contained detectable levels of TBT. A maximum concentration of TBT was recorded in northern pike in the spring to be 240 ng Sn g^{-1}. Maximum levels occurred in marinas during the beginning of the boating season and significantly reduced during the summer and early autumn, although the maximum value of TBT in sediment (392 ng Sn g^{-1}) was observed in the summer of 1989. The seasonal variation of TBT levels was further substantiated in the subsequent 1992 study, in which sediments from three areas in a marina were sampled at monthly intervals from May to October. TBT levels were much higher in May and then generally decreased with time. Mussels (*Elliptio complanta*) caged in the marina for three months also contained TBT. DBT was frequently detected in the sediments but less frequently in fish and mussels. MBT was generally below detection limits. Plants (macrophytes and cladophora) contained very small amounts of butyltin compounds.

Keywords: Butyltin, marinas, Severn Sound, Lake Huron, Canada

INTRODUCTION

In the past 30 years organotin compounds have been used in many important industrial, agricultural and environmental applications. Organotin compounds are molecules that contain one or more tin-to-carbon covalent bonds. There are four classes, referring to the number of alkyl, aryl or other ring organic groups associated with the tin (Sn) atom, namely mono, di-, tri- and tetra-substituted organotin compounds. Mono-organotin and diorganotin compounds are mainly used as poly(vinyl chloride) stabilizers, triorganotins as biocides and tetraorganotins as industrial catalysts. The most significant environmental usage is that of tributyltin in some antifouling paint formulations against algae, snails and barnacles.

Once in the environment, these tin compounds are subject to photodegradation, chemical reactions and biological methylation and degradation.[1] Although the various butyltin species in water are in cationic or in complexed forms with other ligands, they are referred to in this study as tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) for brevity. Various tin compounds, including their degradation and methylation products, have been detected in significant levels in environmental samples around the world.[2,3] Tributyltin appears to concentrate in sediments of harbours, marinas and shipping channels. The highest concentrations in sediment (7–11 mg Sn kg^{-1} dry weight) have been found in marine harbours in Canada.[4] Over the past several years we have developed methods of analysis for butyltin and methyltin species in water, sediment and biological materials, studied their toxicity to aquatic organisms, determined their persistence in fresh water and sediments, and followed the uptake of butyltin compounds by mussels.[4–8]

Severn Sound, a group of bays including Penetang Bay, Midland Bay, Hog Bay and Sturgeon Bay, is a heavily used recreational and boating area in the southeast corner of Georgian Bay. There are 24 private marinas, five federal Small Craft Harbour facilities and two ports located in the area. Boating (pleasure cruising and for sport fishing) is a major recreational

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attraction.[9] In response to concerns about the possible release of tributyltin from antifouling paints on boat hulls and its contamination of the aquatic environment, in 1989 our laboratories investigated the occurrence of butyltin compounds in 100 samples of sediment, fish, mussels and aquatic plants taken from three heavily used marinas (Tiffin Harbour marina, and Penetanguishene Harbour west and south marinas) in the Severn Sound area. Since sediments in Tiffin Harbour were found to contain significant levels of butyltin compounds, in 1992 we again investigated the seasonal variations of these compounds in sediments in this area. This paper presents results from the 1989 and 1992 studies.

METHODS

Sediments, fish, mussels and plants were collected from several locations in the Severn Sound area (Fig. 1). The locations were primarily in the three large marinas where there were high densities of pleasure and fishing craft, so that the source for butyltin compounds entering the waters would be high. Station 207, upstream in Wye River, served as a control station. Station 208 was right at the mouth of Wye River. Stations 209 to 219 were locations in the Tiffin Harbour marina; 221 to 229 and 231 to 237 were locations respectively in the south and west marinas of Penetanguishene Harbour. Sediments were obtained by means of an Ekman grab sampler or scoop. Fish (northern pike and young-of-the-year spottail shiners) were caught by gill net. Indigenous freshwater mussels (*Elliptio complanata*), 6.0–7.0 cm long, from Balsam Lake, Ontario, were caged in study locations for approximately three months in summer before analysis. These mussels were analysed and found not to contain any detectable amounts of butyltin compounds. Mussels and plants (macrophytes and cladophora) were collected by divers, and were frozen immediately after collection.

Monobutyltin trichloride, dibutyltin dichloride and tributyltin chloride were obtained from Alfa Products, Ward Hill, MA, USA. Tropolone and ethylmagnesium bromide (0.2 M in tetrahydrofuran) were from Aldrich, Milwaukee, WI, USA. All solvents were of pesticide grade; water was distilled and deionized by the Millipore system. Standard butyltin solutions (1000 μg Sn cm^{-3}) were prepared by dissolving appropriate amounts of butyltin compounds in water. The internal standard addition technique was used for the determination of the concentration of butyltin.

Sample extraction

Tissue (5 g) from whole fish including organs or plant or whole mussel was digested in a 50 cm^3 beaker in 10 cm^3 of a 20% TMAH (tetramethylammonium hydroxide) solution on a hotplate at 60 °C for 1–2 h until the solution turned pale yellow. After cooling and removal of the undissolved debris, the solution was neutralized with 50% hydrochloric acid (HCl) to pH 8 ± 0.2, and extracted with 3 cm^3 of 0.5% tropolone in hexane solution for 1 h in a mechanical shaker. The mixture was centrifuged and 1 cm^3 of the hexane phase was transferred to a glass-stoppered graduated centrifuge tube for ethylation with 0.2 cm^3 of ethylmagnesium bromide. The excess ethylmagnesium bromide was destroyed by shaking with 2 cm^3 of 0.5 M sulphuric acid (H_2SO_4). The hexane layer was transferred to a small vial containing *ca* 0.5 g of anhydrous sodium sulphate for further clean-up in a silica-gel column.

Freeze-dried sediment samples (2 g) were extracted for 1 h with 5 cm^3 of 0.5% tropolone–hexane solution after addition of 20 cm^3 of water, 6 g NaCl, 2 g sodium benzoate and 1 g potassium iodide. Then 1 cm^3 of the tropolone extract was removed for ethylation with 0.2 cm^3 of ethylmagnesium bromide in a small graduated centrifuge tube. Excess ethylmagnesium bromide was destroyed by shaking with 2 cm^3 of 0.5 M H_2SO_4. The hexane phase was stored in a small vial containing anhydrous sodium sulphate for analysis in the gas chromatography–atomic absorption spectrometry (GC AA) system. There was no need for sample clean-up for sediment samples.

Clean-up of tissue samples

Sample clean-up was carried out in a glass column (15 cm × 1.5 cm i.d.) packed with 8 cm of height of kieselgel 60 (3% water) (E. Merck) suspended in hexane. The column was covered with a 1 cm layer of anhydrous sodium sulphate with a layer of glass wool on top to prevent disturbance of the column bed during elution and to remove any water from the sample. Exactly 0.5 cm^3 of the ethylated sample was loaded onto the column. When the sample had almost passed through the sodium sulphate layer, and the interior walls of the column were rinsed with a few drops of

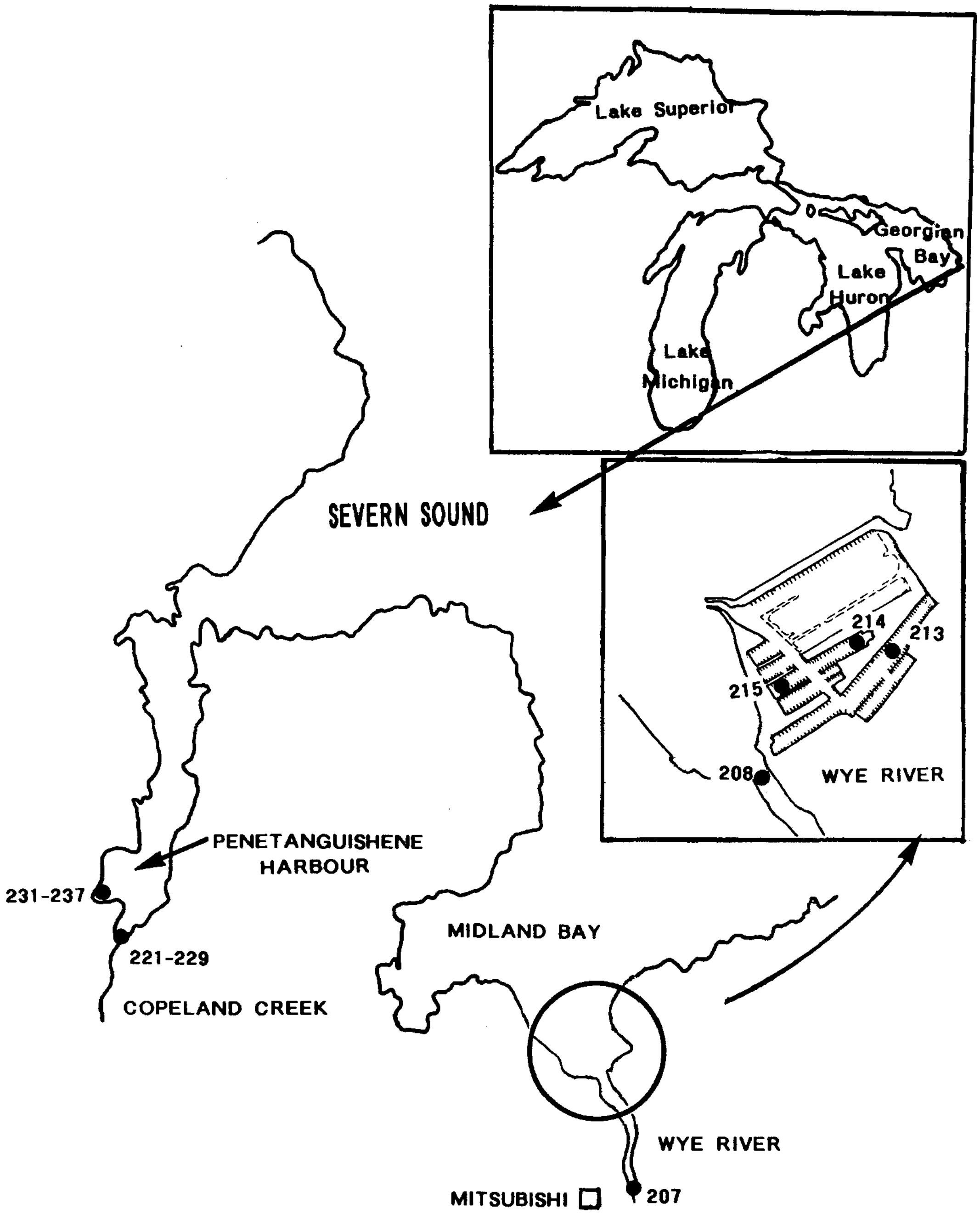

Figure 1 Sampling locations in Severn Sound, Lake Huron, Canada.

hexane, elution was initited with 30 cm^3 of hexane at a rate of 1 cm^3 min^{-1}. The eluate was collected in a 100 cm^3 round-bottomed flask. After 0.5 cm^3 of iso-octane had been added to the hexane eluate as a keeper, the eluate was evaporated first in a rotary evaporator at 30°C, and then in a vortex evaporator at room temperature, to a final volume of 0.5 cm^3. The finished sample was transfered to a small vial and tightly sealed. A 5 μl aliquot of sample or standard or a 20 μl aliquot of blank was injected into the GC AA system for analysis.

The GC AA system

The GC AA system has been described in a previous publication,[5] except that a J&W fused silica megabore column (DB-1, 30 m, 1.5 μm film thickness) was used instead of the 2 m glass column with 3% OV-1 on Chromosorb previously used. Sensitivity, resolution and analytical time were all improved. Temperatures of the injection port and transfer line were both 150 °C. The nitrogen carrier gas flow rate was at 10.5 $\mathrm{cm^3\,min^{-1}}$; the temperature program was 90–200 °C at 20 °C $\mathrm{min^{-1}}$. The AA furnace gases were hydrogen, 84 $\mathrm{cm^3\,min^{-1}}$ (137.89 kPa or 20 lb $\mathrm{in^{-2}}$); air, 21 $\mathrm{cm^3\,min^{-1}}$ (206.84 kPal or 30 lb $\mathrm{in^{-2}}$). The 224.6 nm tin line was generated by an electrodeless discharge lamp operated at 8 W. Deuterium background correction was used. Peak areas were measured with an HP3392A integrator. Tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) were determined against a mixed standard solution of the three butyltin species carried through the same analytical process. Detection limits for sediment (dry weight) and tissue (wet weight) were respectively 15 ng Sn $\mathrm{g^{-1}}$, and 4 ng Sn $\mathrm{g^{-1}}$.

For quality control, the recovery of the three organotin species was evaluated by analysing sediment and tissue samples, spiked with mixed butyltin standards (1 μg of each compound as tin) and comparing with standards carried through the same analytical procedure without the samples. The recoveries for TBT and DBT in sediments were 76–106% and 53–109%, respectively. However, the recoveries for MBT were variable, ranging from 33 to 82%, which indicated some sample matrix interferences for this compound. For biological samples, over 80% recoveries for the three butyltin compounds were found. Results of analyses were not corrected for incomplete recovery.

Table 1 Concentrations of tinbutyltin compounds in northern pike from Severn Sound, Lake Huron (spring survey, 1989)

Sex	Wet weight (kg)	TBT ($\mathrm{ng\,g^{-1}}$)[b]
M	2.35	21
M	1.48	69
M	1.37	92
M	1.20	74
F	2.23	—[c]
F	1.83	150
F	1.67	—[c]
F	1.62	57
F	1.54	240
F	1.52	239

[a] No MBT was detectged. DBT was deleted only in the first sample (66 ng Sn $\mathrm{g^{-1}}$). [b] ng butyltin (as Sn) per g wet weight fish. [c] Below detection limit of 4 ng $\mathrm{g^{-1}}$.

RESULTS AND DISCUSSION

In the 1989 survey, tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) were determined in 100 samples of fish, mussels, plants and sediments collected from Severn Sound, Ontario. Since northern pike is the important species for sport fishing in this area, the occurrence of the butyltin compounds in northern pike was investigated. The results are shown in Tables 1 and 2. Only fish caught in the spring contained detectable levels of TBT (higher than 4 ng Sn $\mathrm{g^{-1}}$). Three out of ten pike samples contained TBT levels higher than 100 ng Sn $\mathrm{g^{-1}}$. The higher concentrations of TBT in the spring probably related to the release of TBT from the sediments and from the leaching of TBT from freshly painted boats placed in the water. Both male and female pike contained TBT, with higher TBT levels in female pike (Table 1). There was no direct rela-

Table 2 Concentrations of butyltin compounds in young-of-the-year spottail shiners from Severn Sound, Lake Huron (autumn survey, 1989)[a]

Site	Wet weight (kg)	Mean ± S.D.[b] ($\mathrm{ng\,g^{-1}}$)	
		TBT	DBT
Penetanguishene Harbour	0.028	9 ± 5	—[c]
Midland Bay	0.025	40 ± 6	5 ± 1

[a] MBT was not detected. [b] Mean ± standard deviation of three samples: ng butyltin (as Sn) per g wet weight fish. [c] Below detection limit of 4 ng $\mathrm{g^{-1}}$.

Table 3 Concentrations of butyltin compounds in sediments from Severn Sound, Lake Huron (spring survey, 1989)[a]

	Mean ± s.d. (ng g^{-1})[b]	
Station no.	TBT	DBT
209	92	—
211	214	97
213	317 ± 83	247 ± 12
214	220 ± 50	100 ± 56
215	118	—
219	106 ± 19	68 ± 1
221	17	—
223	26	—
224	44 ± 10	—
226	—	—
228	—	—
231	208	60
235	107	91
236	32	46
237	142	—

[a] MBT was found only at Stations 219 (92 ng g^{-1}, s.d. = 37) and 236 (35 ng g^{-1}). [b] Mean (± standard deviation) of two samples: ng butyltin (as Sn) per g dry weight sediment. [c] Below detection limit of 15 ng g^{-1} dry weight.

Table 5 Concentrations of butyltin compounds in sediments from Severn Sound, Lake Huron (autumn survey, 1989)[a]

	Mean ± s.d. (ng g^{-1})[b]	
Station no.	TBT	DBT
209	46 ± 8	26 ± 16
211	163 ± 42	70 ± 17
213	108 ± 23	70 ± 8
214	66 ± 16	52 ± 37
215	51 ± 6	46 ± 6
219	24 ± 2	—
221	20 ± 9	17 ± 5
223	37 ± 6	—
224	—	—
226	—	—
228	—	—
231	—	—
233	—	—
235	—	—
236	18 ± 5	—
237	20 ± 1	—

[a] MBT was found only at station 214 (20 ng g^{-1}; s.d. = 9). [b] Mean (standard deviation) of three samples: ng butyltin (as Sn) per g dry weight sediment. [c] Below detection limit of 15 ng g^{-1} dry weight.

tionship between fish size and TBT level, suggesting that fish did not accumulate TBT from the food chain. Instead, the TBT was probably taken up directly from water. With the exception of one sample, no DBT or MBT was found in pike from the spring and autumn samples. The smaller young-of-the-year spottail shiners were found to contain low but still detectable levels of TBT (Table 2). In another study on the seasonal variations of TBT in pike and carp from Whitby and Oshawa Harbours, higher concentrations of TBT were found in fish taken in spring than from the same fish species from the autumn.[7] In addition, pike contained twice as much TBT as carp. The causes of differences in butyltin contents among fish species are not clear but could be caused by differences in the rate of uptake, depuration or metabolism of the compound.

Table 4 Concentrations of butyltin compounds in sediments from Severn Sound, Lake Huron (summer survey, 1989)

	Mean ± s.d. (ng g^{-1})		
Station no.	TBT	DBT	MBT
209	75 ± 36	50 (15)	—[b]
211	392 ± 64	338 (79)	—
213	250 ± 95	205 (38)	60 ± 20
214	266 ± 33	256 (42)	62 ± 9
215	166 ± 25	155 (52)	33 ± 12
219	81 ± 30	76 (40)	62 ± 13
221	96 ± 3	—	—
223	55 ± 10	—	—
224	—	—	—
226	—	—	—
228	—	—	—
231	—	—	—
233	90 ± 8	—	—
235	28 ± 17	—	—
236	47 ± 23	—	—
237	93 ± 16	—	—

[a] Mean (standard deviation) of two samples: ng butyltin (as Sn) per g dry weight sediment. [b] Below detection limit of 15 ng g^{-1} dry weight.

Mussels have been used extensively as an indicator of chemical contamination in the aquatic environment.[8, 10] Chau *et al.*[8] caged mussels, *Elliptio complanata*, in harbour sediments (Severn Sound, Lake Huron) for five months. They found the levels of TBT in mussels slowly increased and reached the maximum level after three months of incubation. Concentration factors ranging from 4800 to 18 500 were obtained in their experiments. Since the sink for TBT leached out from biocides in harbour areas would probably be in the sediment, the concentration of TBT in water is therefore transient in nature. In

Table 6 Seasonal variation of concentrations of butyltin compounds in sediment in Wye River Marina, Severn Sound, Lake Huron (May–October 1992)[a]

Month	Station no.	Mean ± s.d. (ng g^{-1})[b] TBT	DBT
May	207	14 ± 1	—
	208	—	—
	213	717 ± 17	228 ± 7
	214	256 ± 42	99 ± 21
	215	166 ± 29	57 ± 6
June	207	24 ± 9	8 ± 1
	208	—	—
	213	857 ± 115	209 ± 26
	214	266 ± 1	94 ± 15
	215	179 ± 10	54 ± 3
July	207	—	—
	208	—	—
	213	762 ± 18	318 ± 69
	214	188 ± 10	59 ± 2
	215	138 ± 10	51 ± 4
August	207	43 ± 3	—
	208	—	—
	213	516 ± 31	208 ± 4
	214	190 ± 18	76 ± 6
	215	195 ± 3	69 ± 1
September	207	—	—
	208	—	—
	213	578 ± 24	196 ± 7
	214	169 ± 10	70 ± 2
	215	108 ± 7	32 = ± 3
October	207	20 ± 2	—
	208	—	—
	214	31 ± 1	13 ± 1
	215	151 ± 14	43 ± 3

[a] MBT was not detected in any of the samples. [b] Mean (standard deviation) of 3 samples: ng butyltin (as Sn) per g dry weight sediment. [c] Below detection limit of 15 ng/g dry weight.

these studies, mussels were also caged in sediments in several locations in Severn Sound for three months to allow for maximum accumulation before they were analysed for the presence of all butyltin species. Only mussels from Station 208 were found to contain TBT, at around 66 ng Sn g^{-1} (s.d. = 14, mean of three samples; results are in ng butyltin (as Sn) per g of soft tissue wet weight of whole mussel). DBT and MBT were below the detection limit of 4 ng g^{-1}. Because no DBT or MBT was found in mussels in this study, the *in vivo* degradation of TBT in mussels was unlikely. Since butyltin compounds were not detected in the water samples, no bioconcentration factor can be calculated.

In addition to fish and mussels, three samples of plants (macrophytes and cladophora) from Severn Sound were analysed for butyltin species. All samples contained non-detectable levels (less than 4 ng g^{-1}) of TBT and MBT. Only one sample of cladophora (at Wye River mouth) had 142 ng Sn g^{-1} of DBT (s.d. = 52, mean of three samples; results are in ng butyltin (as Sn) per g dry weight of plant).

As expected, the concentrations of butyltin compounds were much higher in the sediments (Tables 3–5). TBT and DBT were more frequently detected than MBT. Similarly to our fish data, concentrations of TBT and DBT were generally higher in the spring samples than in the summer and autumn samples. It was anticipated, and also found, that there would be much higher concentrations of TBT and other butyltin species in sediments inside the marine (Stations 211–219). The presence of DBT and MBT in some sediment samples suggested that degradation of TBT occurred in the sediments. The degradation could be biological, chemical or both.

To substantiate the seasonal variation of butyltin compounds, a marina with high leverls of these compounds was selected for further study. Three stations inside (213, 214 and 215) and two stations outside the marine were chosen (Fig. 1). Sediments were taken at monthly intervals from May to October, 1992. The analyses of butyltin compounds indicated the presence of TBT and DBT in the three stations inside the marina but not in the stations outside it (Table 6). No MBT was detected. Sediments taken in the spring (May) had higher concentrations of TBT and DBT than those taken at other times of the year.

There are few other data with which to compare our sediment results. The levels of TBT in the sediments in Severn Sound were below the high concentrations in Vancouver Harbour but higher than the levels found earlier in Ontario harbours.[4] The toxicological significance of sediment-associated TBT is present difficult to assess. Cardwell and Meador[11] calculated that sediments containing less than 1593 ng Sn g^{-1} TBT should not be acutely toxic to invertebrate species, while sediments possessing less than 141 ng Sn g^{-1} TBT should not be acutely toxic to invertebrate species, while sediments possessing less than 141 ng Sn g should not be chronically toxic to 95% of the invertebrates. Using these calculations, many sediments in Severn Sound containing TBT at levels higher than 141 ng g^{-1} (Tables 4–6) would be chronically toxic to the invertebrates. The biological availability of sediment-associated TBT has been established. Maguire and Tkacz[12] found that oligochete worms could accumulate TBT from sediments. Mussels were also shown to accumulate sediment-associated TBT.[8]

In summary, the presence of detectable butyltin compounds in fish, mussel and sediment samples strongly suggests the use of these compounds in this area. The high TBT concentrations found in sediments at marinas may be a potential threat to the organisms.

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