of an adsorption model showed that the observed effects could be qualitatively accounted for by postulating simple competitive adsorption reactions. The exception was calcium, which appears to form a surface precipitate at medium to high pH.

Acknowledgments

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Registry No. Ca, 7440-70-2; Mg, 7439-95-4; goethite, 1310-14-1.

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Accumulation and Persistence of Tributyltin in Eelgrass (*Zostera marina* L.) Tissue

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■ Decomposition of tributyltin in filtered seawater is compatible with a consecutive debutylation pathway and can be described by consecutive first-order kinetics. Tributyltin is also rapidly taken up from seawater and concentrated by eelgrass (*Zostera marina* L.), suggesting that estuarine plants could provide a sensitive means to monitor tributyltin pollution in coastal areas. In the plant tissue, the decomposition rate of tributyltin is slower than in filtered seawater, while that of dibutyltin is faster. Monobutyltin then gradually moves from the plant material into the surrounding water. Eelgrass could therefore play a role in detoxifying tributyltin. However, since eelgrass is at the base of estuarine food chains, it could also be an important step in the transfer of tributyltin to higher trophic levels.

Introduction

Tributyltin (TBT) is a biocide used extensively in antifouling paints to prevent growth of organisms on marine structures. It is extremely toxic to aquatic life, and evidence indicates that it can harm a wide range of nontarget species, some of which are economically important (*1*, *2*). Since the early 1970s, when TBTs widespread use in paints began, it has caused various cases of serious pollution problems, especially in coastal areas with restricted water circulation and intensive recreational boating (*3*-*6*). This situation prompted several countries to introduce regulations aimed at reducing the impact of this toxic compound in the environment. Such regulations are based on the expected persistence of TBT in the environment. Once released in seawater, it can be either decomposed (chemically or biologically) by a stepwise debutylation process (*7*) or absorbed by lipophilic phases (*8*) such as the lipid fractions of organisms and sediments. Although, at nanomolar concentration levels, the half-life of tributyltin in seawater appears to be a relatively short 3-19 days (*9*, *10*), concentration in lipophilic phases could result in increasing environmental hazard.

Our recent development of a method to measure tri-

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Table I. Concentrations of Butyltin Compounds in Seawater and Eelgrass Tissue[a]

	time, days								
	0	2	3	4	7	8	10	11	14
				Seawater, ng/L					
before spiking	0	<20	41	100	73	191	147	202	67
after spiking	783	540	902	1349	687	1938	820	1810	
				Eelgrass, ng/g					
Bu_3Sn^+	0	36	64	351	459		746		809
Bu_2Sn^{2+}	0	7	3	14			23		61
$BuSn^{3+}$	0	0	19	82			229		319

[a] Seawater was spiked with Bu_3SnCl at the time indicated, and the butyltin concentrations in the eelgrass were determined.

butyltin in eelgrass (*Zostera marina* L.) tissue (*11*) makes possible studies of this important material. Eelgrass meadows form the basis of many estuarine and coastal ecosystems (*12, 13*). Seagrass communities are best known for their role as nurseries and breeding grounds for coastal fish and invertebrate populations, as supporters of complex trophic food webs, as filters of estuarine water, and as suppliers of quantities of detrital organic material to estuarine and offshore environments (*14, 15*). Coastal pollution has been shown to have a major impact on eelgrass populations, primarily resulting from the detrimental effects of eutrophication and sedimentation on water quality (*16*). Effects of metal pollution on eelgrass health have not been reported, but the uptake of numerous heavy metals has been well documented (*17, 18*). This led to the development of a method for using eelgrass as a monitor of heavymetal pollution in coastal areas (*19*). These and other studies have shown that metals are readily taken up by eelgrass, but are not translocated to any great extent inside eelgrass tissues (*20, 21*). Analysis of eelgrass specimens retrieved from a mesocosm suggested that eelgrass could accumulate butyltin species from seawater and could possibly be used as a sensitive pollution indicator (*11*). This observation prompted us to further investigate the ability of eelgrass to concentrate tributyltin from seawater. The objective of this study is to evaluate the impact of this uptake on the residence time of tributyltin in the environment and on its accumulation in estuarine food chains.

We confirmed that eelgrass could rapidly concentrate TBT in its tissue, and that the half-life of TBT in plant tissue was longer than in filtered seawater and depended on light conditions. The monobutyltin resulting from decomposition of TBT was gradually released into the surrounding water.

Experimental Section

Experiment I. In this experiment, we collected 20 healthy eelgrass shoots from an experimental mesocosm (*22*) at the Jackson Estuarine Laboratory. Four groups of five shoots each (E1–E4) were immersed in four glass flasks containing 1 L of filtered (with glass fiber filters GF-C) seawater (salinity, 14 g/kg) at a concentration of 550 ± 90 ng/L TBT. (All concentrations in this paper are as Sn ± 1 standard deviation.) The 550 ng/g level, which is lower than maximum levels found in freshwater and estuarine water (*2*), allows measurement of greater than 95% loss from water.

The four flasks, and three others containing the same spiked seawater but without the eelgrass (S1–S3), were placed in a south-facing window behind a shade (light reduced to 30% of the ambient level) and continuously stirred by a small stream of air bubbles. Evolution of concentrations of mono-, di-, and tributyltin with time, in the seawater and in the eelgrass, was monitored over a period of 2 months. After 2 days, one eelgrass shoot from E1 and one from E2 were retrieved and analyzed. On the seventh day, samples from E3 and E4 were analyzed. Such alternate sampling was then carried on until all plants were analyzed.

Experiment II. In this experiment, 16 eelgrass shoots were collected from the mesocosm and placed in 1 L of filtered seawater spiked with 780 ng/L Bu_3Sn^+. Tributyltin concentration was measured in seawater over a 2-week period and respiked after each analysis to obtain tributyltin concentrations ranging from 540 to 1940 ng/L (Table I). Mono-, di-, and tributyltin concentrations in eelgrass tissue were also measured during this period. Subsequently, the 10 remaining shoots were retrieved and separated into two groups of five plants each. Those looking the healthiest were immersed in filtered, unspiked seawater and placed, as before, on the windowsill. They were marked at the top of the sheath in order to measure separately the tributyltin concentration in the tissue that grew in the uncontaminated seawater. The dead leaves and the remaining shoots were also immersed in unspiked, filtered seawater, but were stored in the dark. The evolution of mono-, di-, and tributyltin concentrations in the eelgrass tissue and seawater was monitored with time.

Extraction of Eelgrass Samples. Extraction of butyltin compounds from eelgrass tissue was performed following the method proposed by Francois and Weber (*11*). Individual shoots were retrieved and ground with a mortar and pestle in liquid nitrogen. The ground material was then split into two approximately equal portions (ca. 0.15–0.30 g, wet weight) and weighed accurately. Mono- and dibutyltin were extracted from one portion with 2 mL of 6 M HCl for 2 h at 50 °C in a sonicator and eventually centrifuged. Tributyltin was extracted from the other portion with 2 mL of dichloromethane/methanol (2:1 v/v) under the same conditions.

Analytical Method. Butyltin compounds were measured by a hydride generation atomic absorption technique previously described by our group (*23, 24*). The apparatus was slightly modified as reported in Francois and Weber (*11*). LOD (3σ) are 1 ng or less for eelgrass tissue (*11*) and seawater.

Aliquots of 50–400 μL of the acid extracts were added to the reaction flask, with 40 mL of distilled, deionized water and 0.5 mL of glacial acetic acid. Two milliliters of 6% $NaBH_4$ was then gradually added, under a helium stream (200 mL/min) and continuous stirring. The hydrides produced were purged on a cryogenic trap, which consisted of 2 g of silanized Chromosorb G AWDMCS coated with 3% SP-2100 packed in Teflon tubing ($^1/_4$ in. o.d.) and immersed in liquid nitrogen. Monobutyltin was then eluted from the trap into the quartz furnace at room temperature and dibutyltin at ca. 150 °C. Quantitation was done by regularly analyzing standard solutions. Precision was ca. 18% for monobutyltin and ca. 12% for dibutyltin (1σ) for sample sizes ranging from 1 to 8 ng.

Similarly, 50–400-μL aliquots of the dichloromethane/methanol extracts were added to the reaction flask with 40 mL of distilled, deionized water and 0.5 mL of glacial acetic acid. Purging the dichloromethane from the sample prior to sodium borohydride addition allowed hydridization of tributyltin without the use of tetrabutylammonium borohydride as suggested previously (*11*). The trap was then left at room temperature, and the reaction flask was purged with helium to remove dichloromethane. When burning in the quartz furnace, dichloromethane absorbs strongly at the tin wavelength. Monitoring of this adsorption ensures its complete removal before addition of $NaBH_4$ (6%; 2 mL). Tributyltin hydride was trapped at room temperature on 0.5 g of packing material (instead of 2 g for mono- and dibutyltin determinations) and eluted at ca. 180 °C. Quantitation was performed by analyzing standard solutions. The precision was ca. 10% (1σ) with sample sizes ranging from 1 to 8 ng.

Model Calculations

Data points from experiments in seawater were fitted to kinetics equations describing consecutive first-order processes. This model assumes successive debutylation processes, i.e.,

$$Bu_3Sn^+ \xrightarrow{k_1} Bu_2Sn^{2+} \xrightarrow{k_2} BuSn^{3+} \xrightarrow{k_3} Sn^{4+}$$

The curve fitting was done by an iterative method using RS/1 software (BBN Research Systems).

The rate constants for first-order decomposition were calculated from the following equations:

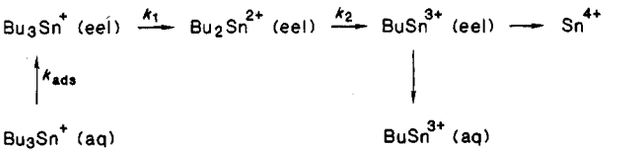
$$d[Bu_3Sn^+]/dt = k_1[Bu_3Sn^+] \quad (1)$$

$$d[Bu_2Sn^{2+}]/dt = k_1[Bu_3Sn^+] - k_2[Bu_2Sn^{2+}] \quad (2)$$

$$d[BuSn^{3+}]/dt = k_2[Bu_2Sn^{2+}] - k_3[BuSn^{3+}] \quad (3)$$

The rate constant of decomposition of TBT (k_1) was first calculated by fitting the function resulting from the integration of eq 1 to the TBT data points. We then evaluated successively k_2 and k_3 according to eq 2 and 3, respectively.

In the presence of eelgrass, the following sequence was partially modeled; "eel" represents association with eelgrass.



The rate of TBT uptake by the plant (k_{ads}) and the rates of TBT (k_1) and DBT (k_2) decomposition in the tissue could also be described in terms of a first-order kinetics model. However, the rate of decomposition and release from eelgrass tissue of monobutyltin could not be fitted by this model.

Results

Two of the filtered seawater samples without eelgrass (S1 and S2) yielded similar results (Figure 1a). They suggested a stepwise debutylation process that could be adequately described by successive first-order decomposition processes. We calculated the rate constants (Table II) by fitting data points to the appropriate first-order equations. There was a rapid exponential decrease in the tributyltin concentrations with a half-life of ca. 2.5 days. The main product of decomposition was dibutyltin, whose concentration reached a maximum at ca. 270 ng/L after 7 days and decreased gradually afterwards (half-life, ca. 6.8 days). Monobutyltin concentrations were lower, but

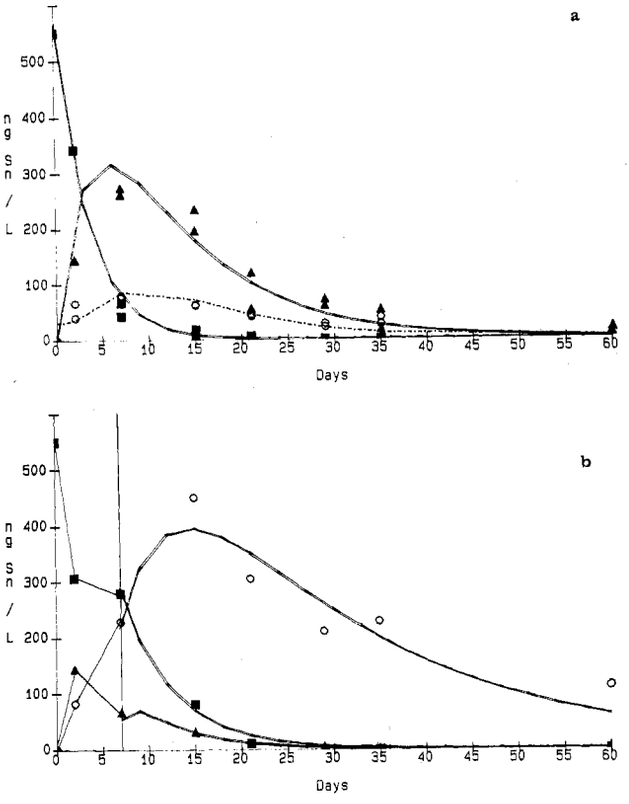


Figure 1. Decomposition of tributyltin in filtered seawater. Key: (a) S1 and S2; (b) S3; (O) monobutyltin; (▲) dibutyltin; (■) tributyltin. Data points in S3 were fitted to the model beginning with day 7.

Table II. Decomposition Rate Constants and Half-Lives of Butyltin Compounds in Seawater and Eelgrass Tissue (Experiment 1)

	rate constants, day^{-1}	half-lives, days	r^2
	Seawater (S1 and S2)		
Bu_3Sn^+	0.28 ± 0.02	2.5 ± 0.2	0.995
Bu_2Sn^{2+}	0.10 ± 0.01	6.8 ± 0.9	0.927
$BuSn^{3+}$	0.33 ± 0.05	2.1 ± 0.4	0.938
	Seawater (S3 after Day 7)		
Bu_3Sn^+	0.17 ± 0.015	4.0 ± 0.4	0.996
Bu_2Sn^{2+}	0.57 ± 0.03	1.2 ± 0.1	0.996
$BuSn^{3+}$	0.048 ± 0.008	14 ± 3	0.976
	Eelgrass Tissue		
Bu_3Sn^+	0.072 ± 0.006	9.6 ± 0.8	0.988
Bu_2Sn^{2+}	0.38 ± 0.06	1.8 ± 0.4	0.820
$BuSn^{3+}$			

followed a similar pattern with a half-life of ca. 2.1 days. The third sample (S3) behaved in a totally different manner (Figure 1b; Table II). Between the second and seventh day, tributyltin decomposiiton seemed to stop, monobutyltin concentration increased, and dibutyltin concentration decreased. After the seventh day, the system followed a consecutive first-order process, as the decomposition of tributyltin resumed with a half-life of ca. 4.0 days. In comparison to S1 and S2, however, dibutyltin decomposed much more rapidly (half-life, ca. 1.2 days) and relatively high concentrations of monobutyltin remained for several weeks (half-life, ca. 14 days). The reasons for these differences are not known, but because of the similarities between S3 and the eelgrass samples (Figure 2), we suspect phytoplankton growth in S3.

In eelgrass tissue (Figure 2), tributyltin concentrations increased rapidly within the first week of the first experiment, to a maximum of 130 ± 10 ng/g (wet weight). The

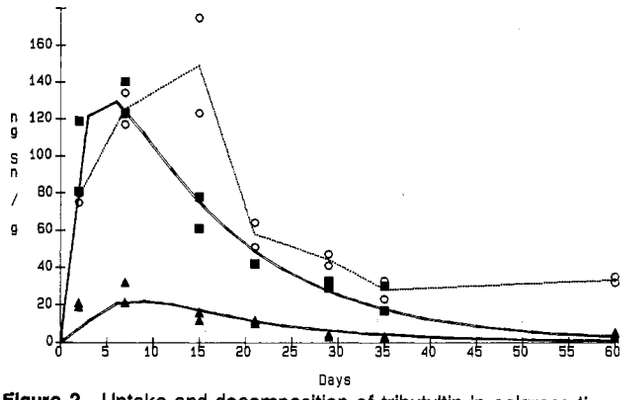


Figure 2. Uptake and decomposition of tributyltin in eelgrass tissue (first experiment). Key: (O) monobutyltin (not fitted); (▲) dibutyltin; (■) tributyltin.

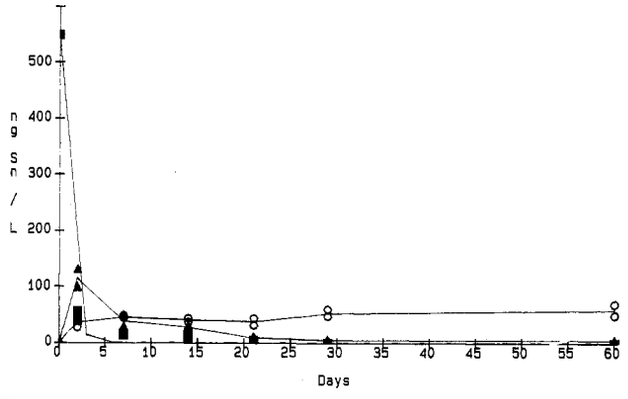


Figure 3. Butyltin compounds concentrations in seawater in the presence of eelgrass. Key: (O) monobutyltin (not fitted); (▲) dibutyltin (not fitted); (■) tributyltin.

rate of adsorption could also be adequately described by a first-order adsorption rate constant (0.45 ± 0.06 day^{-1}). Subsequently, tributyltin concentrations decreased exponentially with a half-life of ca. 9.6 days (Table II). In contrast to seawater samples S1 and S2, dibutyltin concentration remained relatively low (maximum, ca. 25 ng/g) while monobutyltin attained more substantial values with a maximum at 150 ± 20 ng/g reached 2 weeks after the start of the experiment. The evolution of the dibutyltin concentration was consistent with a first-order decomposition rate (half-life, ca. 1.8 days). On the other hand, the changes in monobutyltin concentrations were not compatible with this model. The accumulation of monobutyltin in eelgrass tissue during the first 15 days of the experiment suggests a low rate of decomposition, which is not compatible with the sharp decrease observed in the following weeks. This decrease probably resulted from desorption of monobutyltin from the eelgrass into the seawater, as will be shown in the second experiment.

Concentrations of butyltin compounds in seawater containing eelgrass followed a pattern similar to S1 and S2 (Figure 3). However, tributyltin concentration decreased much more rapidly, reflecting uptake by the plant. We calculated a disappearance rate constant of 1.22 ± 0.06 day^{-1} by fitting the data points for tributyltin to an exponential decrease. Resulting mono- and dibutyltin concentrations were lower than in S1 and S2 (Figure 1a). Dibutyltin was produced by decomposition of tributyltin still present in seawater. Monobutyltin concentration persisted at ca. 50 ng/L for at least 2 months, presumably reflecting the gradual release of this compound from the eelgrass.

In the second experiment, in which the filtered seawater containing the eelgrass was regularly spiked with tri-

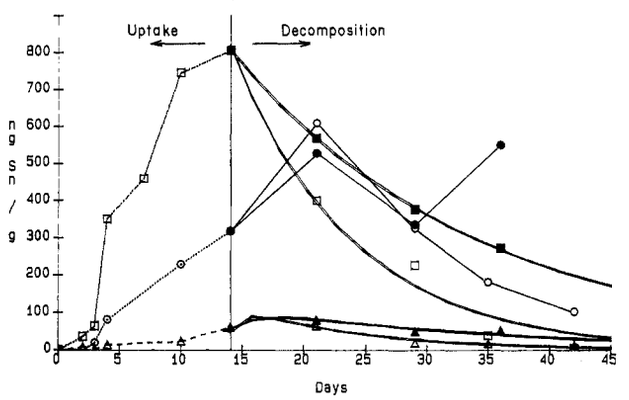


Figure 4. Uptake and decomposition of tributyltin in eelgrass tissue (second experiment). The decomposition of tri- and dibutyltin compounds was fitted to the first-order kinetic model. Monobutyltin in light (O), in dark (●); dibutyltin in light (△), in dark (▲); tributyltin in light (□), in dark (■).

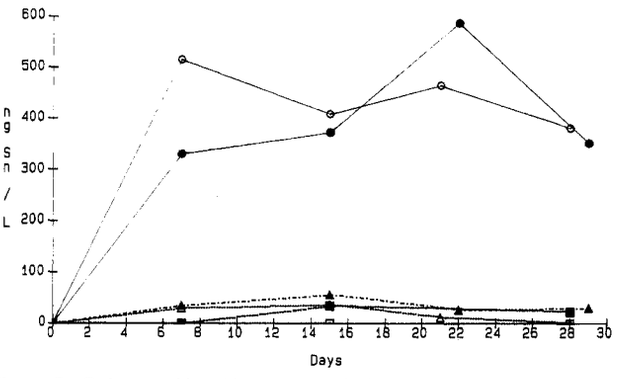


Figure 5. Release of butyltin compounds from contaminated eelgrass to seawater. Monobutyltin in light (O), in dark (●); dibutyltin in light (△), in dark (▲); tributyltin in light (□), in dark (■).

Table III. Decomposition Rates of Tri- and Dibutyltin in Eelgrass Tissue under Light and Dark Conditions (Experiment 2)

	rate constants, day^{-1}	half-lives, days	r^2
	Light		
Bu_3Sn^+	0.10 ± 0.01	6.7 ± 0.9	0.991
Bu_2Sn^{2+}	0.76 ± 0.06	0.91 ± 0.08	0.986
	Dark		
Bu_3Sn^+	0.050 ± 0.001	13.8 ± 0.3	1.000
Bu_2Sn^{2+}	0.39 ± 0.04	1.8 ± 0.2	0.985

butyltin over a period of 2 weeks (Table I), tributyltin concentrations in the eelgrass tissue increased with time (Figure 4; Table I). After 2 weeks and the addition of eight spikes ranging from 540 to 1900 ng/L, the tributyltin concentration in the eelgrass reached ca. 800 ng/g (wet weight). Monobutyltin concentration also increased gradually to ~320 ng/g, while dibutyltin concentration stayed relatively low (up to ca. 60 ng/g), in accordance with the previous experiment.

After contaminated plants were placed in butyltin-free filtered seawater, the tributyltin concentration in eelgrass tissue decreased exponentially, while that of di- and monobutyltin increased to a maximum of 600 and 80 ng/g, respectively, after ca. 7 days (i.e., day 22; Figure 4). There is a significant difference in the half-life of TBT in eelgrass under light (half-life, ca. 6.7 days) and dark (half-life, ca. 13.8 days) conditions (Table III). Similarly, dibutyltin decomposed twice as fast under light conditions. A relatively large amount of monobutyltin was released from the eelgrass into the seawater (Figure 5). Only small quan-

tities of dibutyltin were released, and tributyltin concentration in seawater was below 30 ng/L.

Discussion

Eelgrass quickly takes up and concentrates tributyltin (Figure 2; Table I), thus confirming the potential of eelgrass analysis as a tool for monitoring butyltin pollution in estuaries. Once in the eelgrass tissue, TBT decomposes with a longer 9.6-day half-life (Table II; Figure 2) compared to a 2.5-day half-life in filtered seawater (Table II; Figure 1a). Our observed ca. 2.5-day half-life of tributyltin in seawater is shorter than the 6–19 days reported for seawater samples from San Diego Bay (*10*), but is similar to the removal rate of radiolabeled tributyltin from the water column of the MERL enclosed ecosystem in Narragansett Bay (*9*). It is also of the same order as the half-life obtained in a culture of the wood-decay fungi *Coniophora puteana* (*25*). Some half-life variations observed probably reflect differences in incubation conditions and in species within the biological population. Tributyltin half-lives seem to be significantly longer in the presence of phytoplankton (*26*) and in sediment suspensions (*27*). The seawater used here was filtered through a glass fiber filter (GF-C); the shorter half-life of tributyltin may therefore be due, in part, to the removal of plankton and other suspended particulates.

The decomposition products are also different. Dibutyltin concentrations reached comparatively higher values in filtered seawater samples (Figure 1a). In the eelgrass tissue (and sample S3), monobutyltin is the main decomposition product of tributyltin (Figure 2). This was also observed in the second experiment, there monobutyltin concentrations in eelgrass reached much higher values than dibutyltin during the 2-week spiking period (Table I; Figure 4). If TBT decomposition proceeds via a stepwise debutylation mechanism, then eelgrass tissue seems able to accelerate the rate of decomposition of dibutyltin into monobutyltin (Table II). Barug (*25*) made a similar observation during a study of aerobic degradation of TBT by a strain of *Pseudomonas aeruginosa*. Because dibutyltin is decomposed by reducing sugars (*25*), eelgrass tissue or phytoplankton could accelerate the decomposition of this compound. Similarly, the rate of removal of tributyltin from seawater in the presence of eelgrass (rate constant, 1.22 ± 0.06 day^{-1}) is significantly higher than the sum of the decay rate constant in seawater and adsorption rate constant on eelgrass [0.28 ± 0.02 day^{-1} (Table II) and 0.45 ± 0.06 day^{-1}, respectively]. This suggests that the rate of TBT decomposition in seawater in the presence of eelgrass is significantly higher than in the filtered seawater samples S1 and S2.

The second experiment confirmed the ability of eelgrass to concentrate TBT from seawater (Figure 4; Table I). At the end of the 2-week spiking experiment, 809 ng/g (wet weight) tributyltin was found in the eelgrass tissue in the presence of 67 ng/L in seawater. This corresponds to a concentrating factor of ca. 12000. Since there was no clear indication that TBT accumulation in the eelgrass of this experiment had reached a maximum or that the seawater concentration was at its lowest possible value, this concentration factor could most likely be higher.

After incorporation in eelgrass tissue, TBT decomposition can be described by an exponential curve (Figure 4). The difference in decay rate constants between the light and dark experiments (Table III) suggests that photosynthesis and/or photolysis are significant components of the decomposition process. Factors such as water turbidity and depth of eelgrass habitat could therefore have an influence on the residence time of TBT in eelgrass meadows. No TBT was found in parts of eelgrass leaves that grew after immersion into uncontaminated seawater, suggesting that TBT is not circulating within the plant biomass after uptake. Similar findings have been made for a variety of metals (*20*, *21*).

The substantial release of monobutyltin from the eelgrass into the surrounding water, observed when immersing contaminated plants into alkyltin-free seawater (Figure 5), probably reflects the high hydrophilicity of this compound. The more hydrophobic di- and tributyltin compounds remained essentially in the plant tissue. This could also explain the fast decrease of monobutyltin in eelgrass tissue, observed in the first experiment (Figure 2), and the persistence of monobutyltin in the surrounding water (Figure 3).

The implications of these findings are twofold. In a sense, eelgrass could be contributing to detoxification of tributyltin since eelgrass plants take up this chemical from seawater very rapidly and later release monobutyltin which is much less toxic. On the other hand, since eelgrass is a base of the food chain in temperate estuaries, accumulation of this toxic chemical in its tissue or that of other primary producers may be responsible for its accumulation at higher trophic levels. The fact that the half-life of TBT in eelgrass tissue is significantly longer than in filtered seawater could also aggravate this potential problem.

Conclusions

The short residence time of tributyltin in seawater alone is not an adequate criterion to evaluate its potential environmental hazards. Considering its propensity for uptake by plant materials, researchers must also establish the pathway for tributyltin transfer within the food chain.

Registry No. TBT, 36643-28-4; Bu_2Sn^{2+}, 14488-53-0; $BuSn^{3+}$, 78763-54-9.

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Analysis of Inorganic and Organic Chloramines: Derivatization with 2-Mercaptobenzothiazole

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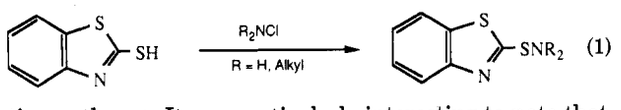
■ A technique has been developed for the analysis of chloramine and organic chloramines present in water using 2-mercaptobenzothiazole as the derivatizing agent. The resulting sulfenamides (-S-N<) are stable and can be conveniently analyzed by HPLC using ultraviolet or electrochemical detection.

Introduction

Knowledge of the amine content present during water renovation processes involving chlorination is important to define more adequately the content of a matrix now described only as "combined residual chlorine" or "chlorine produced oxidants" (*1*, *2*) and because of the variation in disinfectant capability and other chemical and biological activities of the chloramine species that are generated. However, the presence of "organic nitrogen" and the instability of many organic chloramines continue to challenge the analyst (*1*, *2*).

The use of derivatization offers one possible approach to determining the content of the chloramine "pool." However, the design of any process must not only consider generating stable derivatives that can be readily analyzed, but also must address the need to have a derivatization that is rapid relative to chloramine exchange (*3*-*8*).

Reports from the Firestone Research Laboratory on the synthesis of stable sulfenamides as polymerization modifiers by the reaction of 2-mercaptobenzothiazole with chloramines (eq 1) (*9*, *10*) suggested a possible derivatization pathway. It was particularly interesting to note that the sulfenamide products were described as having been generated in high preparative yields from inorganic chloramine as well as primary and secondary chloramines.



(1)

Experimental Section

Analytical high-pressure liquid gradient chromatography (HPLC) was employed using a Perkin-Elmer Series 3 pumping system, a Rheodyne Model 7126 injector, a C-18 column (Perkin-Elmer Sil-X or Rainin) or coupled C-8 columns (Hewlett-Packard and Altex), and a Waters Associates 440 UV detector. Data were taken with a Hewlett-Packard 3390A integrator or a Waters Associates Data Module. A Graphic Controls PHM 7900 pH meter with a Corning combination electrode was used to measure pH. The gas chromatograph-mass spectrometer (GC-MS) was a Hewlett-Packard 5995C fitted with a 12-m SE-54 fused-silica column.

The fluorescence spectra were obtained with a 650-10S Perkin-Elmer spectrophotometer equipped with excitation and emission monochrometers and standard 1-cm quartz cells.

Cyclic voltammetry experiments were carried out with a Bioanalytical Systems DCV-4 potentiostat and a three-electrode system: reference (Ag/AgCl; 3 M chloride), auxilliary electrode (Pt wire), and working electrode (glassy carbon). Cyclic voltammograms were recorded on a Houston Instruments Model 100 XY recorder. Chromatographic detection was effected with a Bioanalytical Systems LC-4B amperometric controller and TL-5 glassy carbon flow cell with Ag/AgCl reference electrode and a Hewlett-Packard 3390A recording integrator.

The chemicals were obtained as follows: 2-mercaptobenzothiazole (2-MBTZ), Aldrich Chemical Co.; cyclohexylamine, Eastman Chemical Co.; piperidine, City Chemical Corp.; morpholine, Jefferson Chemicals; NaOH, CH_3NH_2-HCl, J. T. Baker Chemical Co.; Na_2CO_3, Mallinckrodt; CH_3CN, NaOCl, Fisher Scientific. Water used in this work was purified by a Type 1 "Milli Q" system.

All experiments took place in a 15 °C water bath and were protected from light.

Preparation of Standards. Benzothiazole-2-sulfenamide (*10*). 2-Mercaptobenzothiazole (0.50 g, 2.99 × 10^{-3} mol) dissolved in a solution of aqueous NaOH (1.33 M, 2.25 mL) and NaOCl (5%, 4.5 mL, 3.29 × 10^{-3} mol) was added to a cooled and stirred solution of concentrated aqueous ammonia (9.0 mL, 0.135 mol). The reaction was conducted in an atmosphere of nitrogen over a period of 45 min. The resulting white precipitate was washed with water, recrystallized from $CHCl_3$, and dried in vacuo: 0.23 g, 42%; mp 122 °C (lit. (*10*) mp 122-124 °C); MS, M^+ = 182.

Benzothiazole-2-cyclohexylsulfenamide (*9*). 2-Mercaptobenzothiazole (1.00 g, 5.98 × 10^{-3} mol), NaOH (0.48 g, 1.2 × 10^{-2} mol), and cyclohexylamine (1.39 g, 1.40 × 10^{-2} mol) were dissolved in 20 mL of H_2O. To this solution was added dropwise 50 mL of an aqueous solution of I_2 (1.53 g, 6 × 10^{-3} mol) and KI (1.65 g, 10^{-3} mol) over a period of 1 h. The white product was washed with water

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