Fate of ^{14}C-labeled Tributyltin in an Estuarine Microcosm

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A radiotracer experiment was conducted in a controlled experimental ecosystem (microcosm) to determine the persistence and behavior of tributyltin (TBT) under conditions simulating a temperate, shallow estuarine ecosystem. Radiolabeled TBT was introduced to the estuarine microcosm, which contained estuarine water, sediment and fish. TBT and its degradation products were monitored for 40 days. TBT rapidly distributed among the compartments of the microcosm. The TBT half-life in the water column was 2.55 days for the first 11 days and then slowed to 13.4 days. More than 60% of the TBT and its metabolites were found in the sediment, indicating that the sediment was an important sink for butyltins. Higher concentrations of butyltins, relative to the water column concentrations, were found in the surface microlayer. TBT could be bioconcentrated by the fish to levels more than 200 times the exposure concentration, and underwent rapid degradation in the fish body, so that high concentrations of its metabolites were found in the fish. The concentrations of TBT adsorbed on the suspended particles were three orders of magnitude greater than that in dissolved form. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

In recent years, pollution with tributyltin (TBT) compounds used as an antifouling paint has been frequently reported in many aquatic areas over the world.[1] Several countries have banned the use of TBT-containing paints on boats less than 25 m in length, for its extreme toxicity to marine organisms.[2] To assess the risk to the environment from TBT, it is necessary to know both the effects of the chemical and its fate in the environment. Previous studies concerning TBT in the ecosystem focused mainly on the individual processes, such as adsorption onto sediment and suspended particles, degradation in sediment and water, enrichment at the air–water interface and bioconcentration;[3–6] only a few studies investigated the persistence and behavior of TBT at systematic level.[7]

A radiotracer experiment in a controlled experimental ecosystem (microcosm) has been conducted in this study to determine the persistence and behavior of TBT under conditions simulating a temperate, shallow estuarine ecosystem. Radiolabeled TBT was added to the estuarine microcosm, which contained estuarine water, sediment and fish, then the distribution of labeled TBT and its degradation products, dibutyltin (DBT) and monobutyltin (MBT), were followed for 40 days.

EXPERIMENTAL

Reagents

Tributyltin chloride, dibutyltin dichloride, monobutyltin trichloride and tropolone were obtained from Aldrich Corp., Milwaukee, WI, USA. Tri-*n*-[1-^{14}C]butyltin chloride was a gift from Professor Dr Kenneth R. Hinga, University of Rhode Island, NY, USA. The manufacturer (Amersham Corp.) gave the specific activity of the compound to be 21 mCi mmol^{-1}. The TBT as received had contaminants and it was cleaned before use by preparatory thin-layer chromatography (TLC) to reach 99.6% purity as determined by TLC and liquid scintillation counting (LSC) analysis.[8] All solvents were of pesticide grade.

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Estuarine microcosm

The estuarine microcosm was a glass tank, 0.9 m long, 0.3 m wide and 0.5 m high, containing 100 dm^3 of estuarine water and a 2 cm layer of sediment. Sediment and water were taken from the adjacent Haihe river estuary, Tianjin, China, a fairly typical estuarine ecosystem. The sediment was examined to pick out large shells and pieces of gravel, and the water was not modified. The temperature of the water in the tank was controlled at 25 ± 1 °C. Air was gently bubbled up from halfway down the water depth through airstones, to supply oxygen. In the experimental period, a certain amount of distilled water was added every evening to compensate for the evaporated water. Twenty fish (*Tilapia*), purchased from the Institute of Aquaculture, Tianjin City, were put in the microcosm after they had been acclimatized in the laboratory to 25 ± 1 °C, 15‰ salinity for about a week, i.e. in the same conditions as in the microcosm. The fish were 8–10 cm long and the wet tissue weight was about 10–12 g. Cool-white fluorescent lamps were used as the simulating light source, which induced a light intensity of 6000 lx at the water surface with a 12 h:12 h light: dark photoperiod. It should be noted that neither the short-wavelength spectrum nor the full potential intensity of natural sunlight was simulated. The microcosm was installed in a ventilation system in the laboratory, which was licensed for radiotracer work.

Spiking and operation

On 11 April 1996, 8.84 μCi of tri-*n*-[1-^{14}C]butyltin chloride dissolved in ethanol was introduced into the microcosm to give an initial concentration in the water of 500 ng Snl^{-1}.

Samples of every component (water, surface microlayer, sediment and fish) of the microcosm were taken at certain intervals. At each time, the surface microlayer was collected by a glass plate sampler, which could collect a layer 60–100 μm thick.[9] Water samples were siphoned out through a glass pipe at the half-depth of the water column, then an aliquot of each water sample (100–500 ml, unfiltered) was extracted directly, and another aliquot was filtered through a weighed 0.45 μm Micropore filter. The filter was weighed at dryness to calculate the concentration of suspended particles in the water sample. Sediment samples (each about 20 g) were collected by a home-made sampler which could collect the top 2 cm layer of the sediment in the microcosm. Two or three fish were caught at each time. In order to keep the proportion of the fish relatively constant, the second batch of fish were put in the microcosm on the 11th day. The two batches of fish were sampled and analyzed separately.

Analysis

A brief description of the analytical procedures is provided here. Further details of the extraction and separation by thin-layer chromatography (TLC) may be found in Ref. 8. Water samples (unfiltered and filtered) and surface microlayer samples acidified with hydrobromic acid were extracted by 0.1% tropolone in benzene. The benzene phase was transferred to a glass-stoppered graduated tube and was evaporated to 0.3 ml under a gentle stream of air at room temperature. Whole fish was digested in 10% TMAH (tetramethylammonium hydroxide) solution at 60 °C for 1 h. After being cooled, the solution was neutralized with 50% hydrochloric acid (HCl) to pH 8 ± 0.2, and was extracted with 0.3% tropolone in a mixture of ethyl acetate and hexane (3:2). The mixture was centrifuged at 3000 rpm for 10 min. The organic phase was withdrawn, dried with anhydrous sodium sulfate, and evaporated to 0.3 ml. Wet sediment samples were acidified with acetic acid and extracted for 2 h with 0.3% tropolone in a mixture of benzene and hexane (1:2). The mixture was centrifuged to withdraw the organic phase, then the organic phase was dried with anhydrous sodium sulfate and evaporated to 0.3 ml.

A 100–200 μl portion of each concentrated extract was separated by TLC. Silica-gel G60 TLC plates were used and the mobile phase was isopropyl ether–acetic acid (97:1) mixture. This condition gave R_f values of 0.88 for TBT, 0.37 for DBT and 0.004 for MBT. As references, nonlabeled standards of butyltin chlorides (TBT, DBT and MBT) were visualized by spraying with Pyrocatechol Violet in ethanol and dithizone in chloroform. The appropriate sections of TLC plate, containing the extracted samples corresponding to the locations of the standards, were cut out and determined by LSC (Beckman model 5801). All counts were corrected for background and counting efficiency. The concentrations of TBT, DBT and MBT were calculated from the counts, the specific activity of each compound and their recoveries. The limits of detection for TBT, DBT and MBT are 0.059 ng Sn, 0.12 ng Sn and 0.18 ng Sn, respectively.

Table 1 Mass balance of butyltins

	Day 1				Day 4			
	Total butyltins		TBT		Total butyltins		TBT	
	(μg)	(%)	(μg)	(%)	(μg)	(%)	(μg)	(%)
Filtered water	15.9	31.9	9.32	21.7	8.46	17.8	2.99	9.3
Suspended particles	2.3	4.6	3.18	7.4	4.64	9.8	3.58	11.2
Sediment	30.8	61.8	29.8	69.3	33.2	70.0	24.6	76.9
Fish	0.859	1.7	0.700	1.6	1.11	2.3	0.844	2.6
Microcosm	49.9	100	43.0	100	47.4	100	32.0	100
Recoveries (%)	99.7		86.0		94.8		64.0	

RESULTS AND DISCUSSION

Changes in the concentrations of TBT and its metabolites in each phase of the estuarine microcosm are shown in Figs 1–7 (below). The mass balances were calculated for the first and the fourth days to show the fate of butyltins in the microcosm (Table 1). Recoveries are given in terms of the amount of butyltin determined as a percentage of that introduced into the microcosm. After TBT was added to the water of the microcosm, it rapidly distributed to the sediment, the fish and the suspended particles. More than 60% of the substance and its metabolites in the microcosm was found in the sediment, indicating that the sediment was an important sink for butyltins in the aquatic environment. From 25.4 to 54.3% of tributyltin and from 12.6 to 35.4% of total butyltins in the water column were associated with the particulate phase. So particulate deposition and sediment resuspension may be an important transport process for butyltins between the water column and the sediment. In the early stages after TBT had been introduced to the aquatic environment, degradation of TBT was not significant, and the important factors which influenced the changes in TBT concentration in the water were the transport processes of TBT among the compartments of the aquatic environment.

TBT concentrations in the unfiltered water, the filtered water and the surface microlayer decreased rapidly in the first few days, then the rate of decrease became relatively slow (See Figs 1–3). Table 2 provides TBT half-lives in the water phase calculated on the basis of regression analysis of TBT loss according to first-order kinetics. TBT half-lives in the water phase for the first 11 days are shorter than those for days 12 to 39, because TBT could be rapidly removed from the water phase by the distribution processes in the early stages of the experiment. The half-lives of TBT in the unfiltered

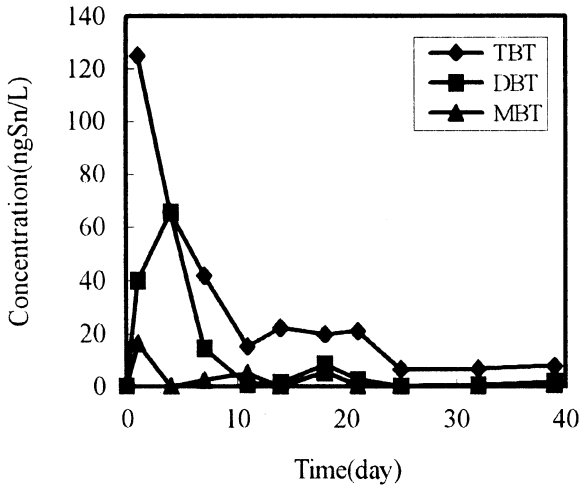

Figure 1 Changes of butyltin concentrations in unfiltered water with time.

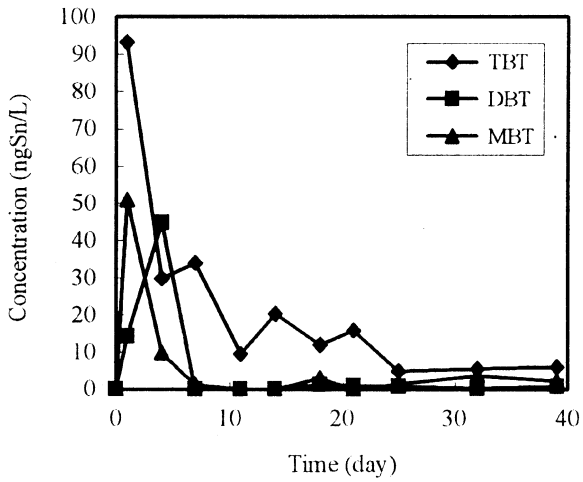

Figure 2 Changes of butyltin concentrations in filtered water with time.

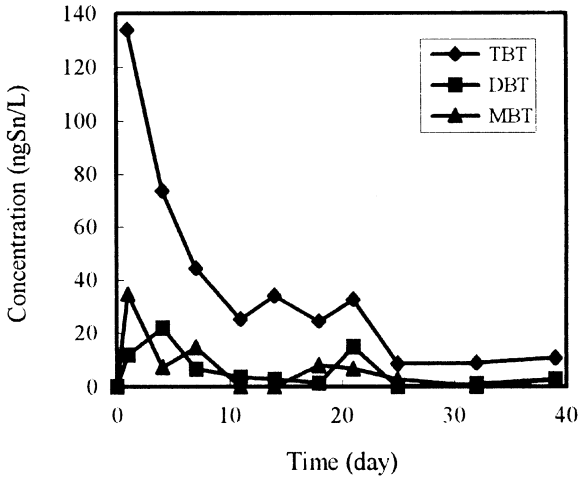

Figure 3 Changes of butyltin concentrations in surface microlayer with time.

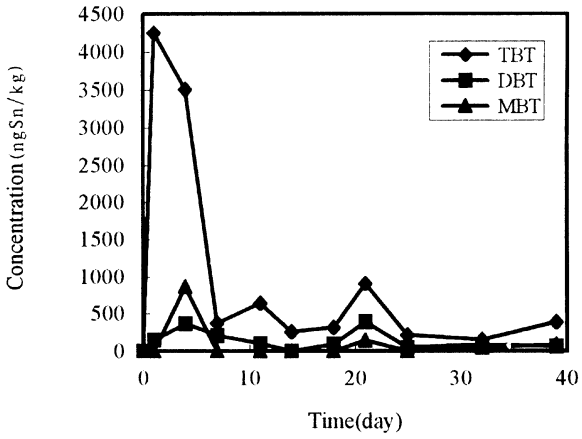

Figure 4 Changes of butyltin concentrations in sediment with time.

Table 2 Half-lives of TBT calculated from linear regression of disappearance of TBT parent compound

	Half-life of TBT (days)		
	Days 0–39	Days 0–11	Days 12–39
Unfiltered Water	7.77	2.55	13.4
Filtered Water	7.92	2.34	13.7
Surface microlayer	8.36	2.96	12.5

Table 3 Ratios of butyltin concentrations in surface microlayer to that in unfiltered water

	Time (days)									
	1	4	7	11	14	18	21	25	32	39
TBT	1.07	1.12	1.06	1.65	1.54	1.24	1.56	1.26	1.27	1.36
DBT	0.297	0.333	0.450	5.00	2.00	0.170	5.52	—	1.54	1.41
MBT	2.12	—	6.01	—	—	1.45	13.5	6.21	—	2.83

water, the filtered water and the surface microlayer are approximately similar, indicating that the transport processes of TBT between the water and the suspended particles, the bulk water and the surface microlayer are extremely fast relative to the TBT degradation process. The half-lives of TBT degradation in natural water ranged from several days to several weeks;[10–12] these experimental results are in agreement with previous work.

The ratios of butyltin concentration in the surface microlayer to that in the unfiltered water were usually higher than one, indicating that butyltins enrichment could occur in the surface microlayer (Table 3).

Tributyltin appeared to concentrate in the sediment. In the early stages of the experiment, TBT concentration in the sediment rapidly increased, then it decreased along with the decrease of TBT concentration in the water column (Figure 4). It can be concluded that the transport process of TBT between the water column and the sediment was fast and reversible. Tributyltin adsorbed on the sediment could return to the water column through simple desorption, sediment resuspension or ingestion by benthic biota. Thus, the biological availability and toxicological significance of residues of tributyltin in the sediment should be paid special attention. The partition coefficient (K_p) between the sediment and water may be calculated by dividing the TBT concentration in the sediment by that in the filtered water. During the experimental period, the partition coefficient for TBT in the sediment ranged from 11.1 to 117 l kg^{-1}. Several researchers have shown that the equilibrium partition coefficients for TBT in the sediment ranged between 10^2 and 10^4 l kg^{-1}.[3,4,13,14] The partition coefficient obtained from this experiment is relatively low, mainly because the partitioning process of TBT between the sediment and the water did not reach equilibrium due to the rapid biological degradation of TBT in the sediment. Concentrations of DBT and MBT in the sediment did not increase significantly, indicating that they might be degraded to inorganic tin under these experimental conditions.

TBT could be removed from the water and accumulated by the fish. At the beginning of the

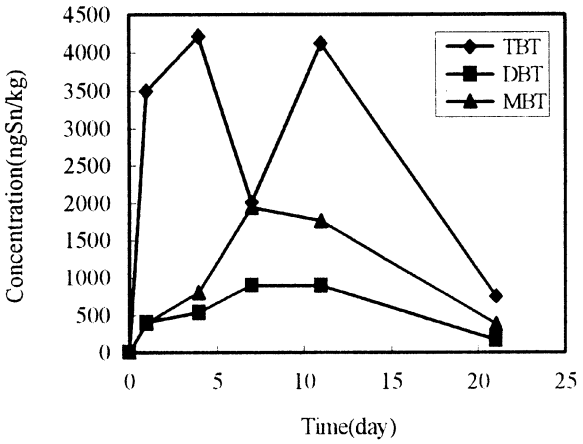

Figure 5 Changes of butyltin concentrations in fish (batch 1 only) with time.

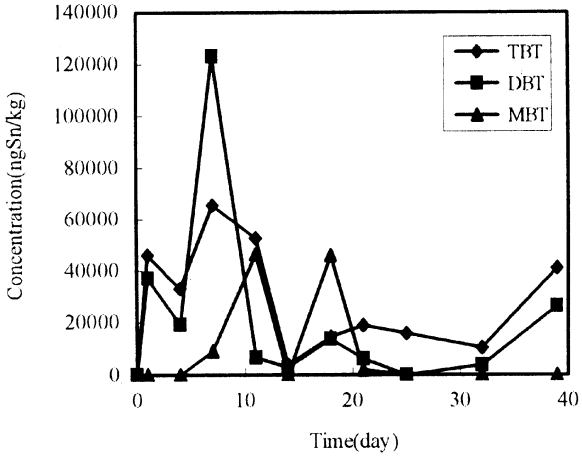

Figure 7 Changes of butyltin concentrations on suspended particles with time.

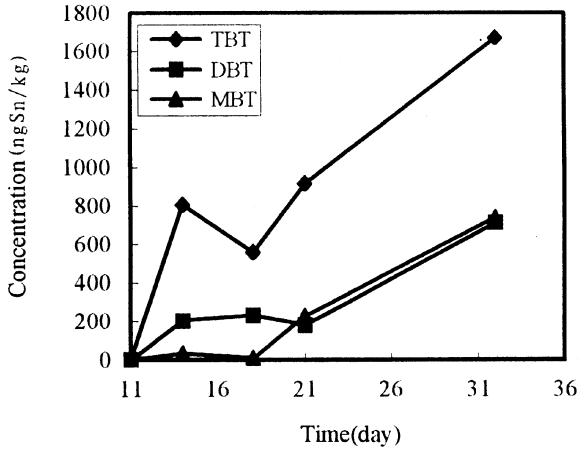

Figure 6 Changes of butyltin concentrations in fish (batch 1, and batch 2 added on day 11) with time.

experiment the TBT concentration in the water was high, so the first batch of fish took up TBT from the water more rapidly than the second batch (See Figs 5 and 6). The maximum accumulation factors (concentration in fish/concentration in filtered water) of the two batches of fish are 434 l kg^{-1} and 295 l kg^{-1}, respectively. Higher DBT and MBT concentrations were found in the fish, indicating that once TBT was taken up by the fish this chemical could undergo rapid degradation in the fish body. Because of the metabolism of the fish and the decrease in the uptake rate due to the decline of the TBT concentration in the water, the TBT concentration in the first batch of fish decreased with time after reaching the maximum accumulation factor (Fig. 5). After the second batch of fish were put into the microcosm on the 11th day, the TBT concentration in the water declined slowly and became relatively steady (Figs 1–3). Thus the TBT concentration in the second batch of fish could increase until it reached the accumulation factor of 295 l kg^{-1} on the 39th day.

Butyltin concentrations adsorbed on the suspended particles were calculated from butyltin concentrations in the water and the filtered water and the concentrations of the suspended particles in the water. Butyltin concentrations on the suspended particles were affected by many factors such as

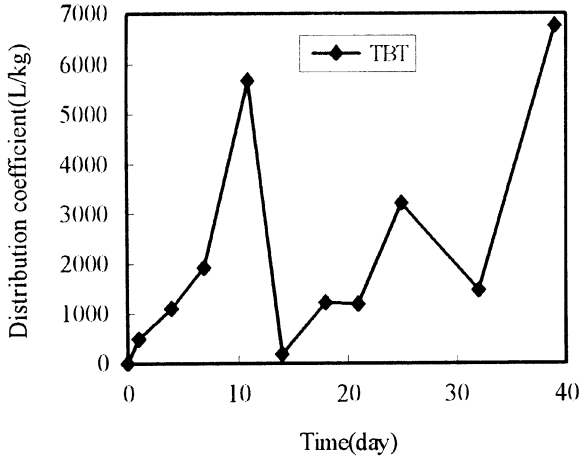

Figure 8 Changes of TBT distribution coefficients on suspended particles with time.

butyltin concentrations in the dissolved state, the concentrations of the suspended particles in the water and the rates of particulate deposition and sediment resuspension. Changes in butyltin concentrations on the suspended particles were therefore complicated (Fig. 7). The distribution coefficients (concentration on the suspended particles/concentration in the filtered water) of TBT tended to increase with time due to the decline of both the TBT concentration and the concentration of the suspended particles in the water (Fig. 8), when the fish underwent a violent perturbation, much sediment became resuspended in the water column and so the distribution coefficients of TBT for the first and the 14th days were relatively low.

CONCLUSIONS

Once TBT was introduced to one compartment (such as water) of the microcosm, it would rapidly distribute among all the compartments of the microcosm. The factors which affect the rates of TBT transport processes among the different media, such as water current velocity, tides and perturbation of the organisms, would have significant effects on the distribution of TBT among the media in the aquatic environment. TBT was mainly removed from the water column by degradation and partitioning to sediment. Sediment was an important sink of TBT in the microcosm. TBT could be bioconcentrated by fish and rapidly degraded in fish bodies. The concentrations of the metabolites of TBT did not increase significantly, indicating that the metabolites DBT and MBT could be rapidly degraded to inorganic tin.

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