

Division Control No. ECD-109B

This manuscript is submitted as an NWRI Publication

BACTERIAL DEGRADATION OF TRIBUTYLtin

R.J. Maguire, D.L.S. Liu, K. Thomson
and R.J. Tkacz

NWRI Contribution No. 85-82

Environmental Contaminants Division
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario, Canada L7R 4A6

EXECUTIVE SUMMARY

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R.J. Maguire, D.L.S. Liu, K. Thomson and R.J. Tkacz

Environmental Contaminants Division
National Water Research Institute
Department of Environment
Canada Centre for Inland Waters
Burlington, Ontario, Canada L7R 4A6

A study of the degradation of tributyltin by bacteria from Toronto Harbour sediment revealed that at 20 °C the degradation was significantly faster under anaerobic conditions than under aerobic conditions. This finding means that estimates of persistence of tributyltin in aquatic ecosystems should take into account the degree to which the system is aerobic or anaerobic.

Management Implications

This is simply one finding that will be used in making the hazard assessment for tributyltin in aquatic ecosystems.

RÉSUMÉ EXÉCUTIF

DÉGRADATION DES TRIBUTYL-ÉTAINS PAR LES BACTÉRIES

R. J. Maguire, D. L. S. Liu, K. Thomson et R. J. Tkacz

Division des polluants de l'environnement
Institut national de recherche sur les eaux
Ministère de l'Environnement
Centre canadien des eaux intérieures
Burlington (Ontario)
Canada L7R 4A6

Une étude sur la dégradation des tributyl-étains par des bactéries contenues dans les sédiments du havre de Toronto a révélé qu'à 20° C, le processus était beaucoup plus rapide en conditions anaérobies qu'en conditions aérobies. Cette découverte signifie que pour évaluer la persistance des tributyl-étains dans les écosystèmes marins, il faudra tenir compte de leur teneur en oxygène libre.

Applications pour la gestion

Cette découverte sera utilisée simplement pour évaluer les risques que présentent les tributyl-étains pour les écosystèmes marins.

ABSTRACT

A study of the degradation of tributyltin by bacteria from Toronto Harbour sediment revealed that at 20 °C the degradation was significantly faster under anaerobic conditions than under aerobic conditions.

RÉSUMÉ

Une étude sur la dégradation des tributyl-étains par des bactéries contenues dans les sédiments du havre de Toronto a révélé qu'à 20° C, le processus était beaucoup plus rapide en conditions anaérobies qu'en conditions aérobie.

INTRODUCTION

Organotin compounds are used in three main ways, viz., as stabilizers for poly(vinyl chloride), as catalysts and as pesticides (1). They are a class of chemicals about which more information is sought under Canada's Environmental Contaminants Act (2) regarding toxicology and environmental fate.

The main organotin compounds which are likely to be released to the environment in Canada are those of triphenyltin (Ph_3Sn^+), tricyclohexyltin (Cy_3Sn^+), di-n-octyltin ($\text{Oct}_2\text{Sn}^{2+}$), di-n-butyltin ($\text{Bu}_2\text{Sn}^{2+}$), dimethyltin ($\text{Me}_2\text{Sn}^{2+}$) and tri-n-butyltin (Bu_3Sn^+) (1). Triphenyltin and tricyclohexyltin are agricultural pesticides. Di-n-octyltin is used as a stabilizer in some food wrappings. Di-n-butyltin is used as a poly(vinyl chloride) stabilizer, as is dimethyltin, and as a catalyst in a number of industrial processes. Tri-n-butyltin is mainly used as an antifouling agent in some paints for boats, ships and docks, as a general lumber preservative and as a slimicide in cooling water. It is by far the most toxic to aquatic organisms of all organotin compounds used in Canada.

We have been interested in tributyltin because of its high toxicity. Lethal concentrations are in the range 0.04-16 ug Sn/L for short term exposures of copepods (3,4), mussel larvae (5), crab larvae (6), lobster larvae (7), sheepshead minnow (8), bleak (3), guppy (9) and rainbow trout (10,11).

We have recently completed a survey for tributyltin in water and sediment from 265 locations across Canada (12). In 10% of the water samples tributyltin was found at concentrations which could cause growth retardation upon chronic exposure to a sensitive organism, rainbow trout yolk sac fry. Tributyltin was mainly found in areas of heavy boating and shipping traffic, which is consistent with its use as an antifouling agent in some paints for boats, ships and docks.

Our work on the persistence of tributyltin has indicated that abiotic degradation generally occurs, as does biological degradation, through a mechanism of sequential debutylation to inorganic tin (13,14). The main factors limiting the persistence of tributyltin in aquatic ecosystems are sunlight degradation in water and biological degradation in water and sediment, and with the temperatures and sunlight intensities prevalent in Canada, the half-life is likely to be at least a few to several months.

This study was undertaken to determine if tributyltin could be degraded under anaerobic conditions as well as aerobic conditions. We studied the biodegradation of tributyltin by sediment organisms in cyclone fermentors (15) under controlled conditions. Before starting the biodegradation experiments we tested the toxicity of tributyltin to bacteria to ensure that the concentrations of tributyltin used in the degradation experiments would not be toxic to the bacteria. In the course of the toxicity study we noticed strong synergism between tributyltin and pentachlorophenol, another common lumber preservative, at elevated concentrations. These findings are also presented in this report.

EXPERIMENTAL SECTION

For brevity, each of the n-butyltin species is referred to here as though it existed only in cationic form (e.g., Bu_3Sn^+). This formalism is not meant to imply exact identities for these species in water.

Materials

Tri-n-butyltin chloride, bis(tri-n-butyltin) oxide, di-n-butyltin dichloride, n-butyltin trichloride and tin were from Ventron (Danvers, MA, USA). 2-Hydroxy-2,4,6-cycloheptatrien-1-one (tropolone) was from Aldrich (Milwaukee, WI, USA). Pentachlorophenol was from Supelco (Bellefonte, PA, USA). Yeast extract was from Difco (Detroit, MI, USA). All organic solvents were pesticide grade from Caledon (Georgetown, Ont., Canada). Sulfuric and hydrochloric acids were reagent grade, but the HCl was washed with a solution of tropolone in benzene in order to remove traces of inorganic tin. n-Pentylmagnesium bromide was prepared from readily available chemicals. Unless specified otherwise, water was doubly distilled.

Toxicity of Tributyltin to Bacteria

The bacterium Bacillus cereus, originally isolated from activated sludge (16), was employed as the test microorganism. The cells were grown in a liquid medium containing the following ingredients in 1L of distilled water: 2 g each of glucose, sodium acetate and yeast extract, 0.82 g KH_2PO_4 and 2.64 g K_2HPO_4 . The same growth medium was also used in all toxicity tests. Since younger cells of B. cereus were consistently more sensitive to toxic substances, the test bacterium was always cultured for 18 h in 50 mL of growth medium in a 125 mL Erlenmeyer flask on a

rotary shaker at room temperature (21 °C). The culture was used directly in the toxicity tests without adjusting cell concentrations.

The effect of tributyltin on B. cereus was determined using the resazurin reduction method (16) with a minor modification in which resazurin reduction was measured directly in the reaction mixture without solvent extraction. The test mixtures contained 2.75 mL growth medium, (250-X) uL methanol, X uL test solution, 1 mL cell suspension and 1 mL resazurin solution. To provide strong buffering of the test mixture, the resazurin was dissolved in phosphate buffer of twice the ionic strength of the growth medium. The cell control contained 250 uL of methanol without the test toxicant, while the dye control contained no toxicant and no cells. The mixtures were incubated for 15 min in the dark at room temperature, and then the reaction was stopped by adding 50 uL of 10 mg/mL HgCl₂ solution. After 3 s of vortexing vigorously, the samples were centrifuged at 3000 g for 5 min and the absorbance of the supernatant solutions was measured at 610 nm. The % inhibition (IC) was calculated according to the equation

$$IC = \frac{(A-B) - (A-C)}{(A-B)}$$

where A, B and C are the absorbances of the dye control, cell control and test mixture, respectively.

Degradation of Tributyltin

Experiments were done with cyclone fermentors (15) under both aerobic and anaerobic conditions, and with sterile controls. The bacterial inoculum was obtained from Toronto Harbour sediment. Sediment was shaken vigorously with tap water for several minutes and then allowed to settle. The supernatant constituted the inoculum.

Each fermentor contained 1.4 L tap water, 100 mL bacterial inoculum (approx. 5×10^6 /mL), 10 mg/L sodium lignin sulfonate and an initial concentration of tributyltin of 45 ug Sn/L. Controls contained HgCl₂ or KCN at concentrations of 66 or 433 mg/L, respectively. At 0, 2 and 8 d, 100 mL samples were taken from the fermentors and were extracted and analyzed as described below. Although an initial concentration of tributyltin of 45 ug Sn/L is about ten times higher than

concentrations observed in natural waters in Canada (12), we felt that the use of higher concentrations of tributyltin would make it easier to detect products of degradation.

Preliminary experiments with initial tributyltin concentrations at or above its aqueous solubility of about 0.5 mg Sn/L had revealed substantial volatilization losses. The fermentor lids, which were 4-10 cm above the liquid surface smelled of tributyltin, and methanol washes of the lids contained substantial amounts of tributyltin. However, at the initial tributyltin concentration of 45 ug Sn/L used in the experiments reported here, the controls demonstrated negligible volatilization, and lid washings contained only insignificant quantities, if any, of tributyltin and its degradation products.

Analysis of Samples for Tributyltin and Degradation Products

The methods of analysis for water are documented elsewhere (17,18). In essence they involve extraction of Bu_3Sn^+ , $\text{Bu}_2\text{Sn}^{2+}$, BuSn^{3+} and inorganic tin from acidified water with the complexing agent tropolone dissolved in benzene, pentylation of the extract to produce the volatile mixed butylpentyltin derivatives, $\text{Bu}_n\text{Pe}_{4-n}\text{Sn}$, purification by silica gel column chromatography, concentration of the purified solution, and gas chromatographic determination of the derivatives as described below. Extractions of bacterial suspensions containing tributyltin were done in the same way as the water samples.

Determination of the $\text{Bu}_n\text{Pe}_{4-n}\text{Sn}$ derivatives was done by packed column gas chromatography with a quartz tube furnace atomic absorption spectrophotometric detector (19). Considering that a fairly specific detector was used in the analyses, identities of the butylpentyltin derivatives were deemed to be confirmed by co-chromatography with authentic standards on two column packing materials of very different polarity.

In the quantitation of the analytes, use was made of appropriate reagent blanks. The results reported in this article are all above the limit of quantitation, which is defined as the reagent blank value plus ten times its standard deviation (20). In practice, for our work this is equivalent to stating that a chromatographic peak was not accepted as real unless it was at least 2-3 times as large as any corresponding peak in the reagent blank.

Recoveries of Bu_3Sn^+ , $\text{Bu}_2\text{Sn}^{2+}$ and BuSn^{3+} from spiked water samples at

1-10 mg Sn/L varied from 96 ± 4 to $103 \pm 8\%$ (17). Recoveries of Sn(IV) from water at pH 5-8 were poor ($35 \pm 23\%$), probably because of the formation of unextractable SnO_2 (13). Recoveries of tributyltin from the bacterial suspensions used in this study were quantitative. Recoveries of the other butyltin species and inorganic tin from the bacterial suspensions were not determined, but were assumed to be the same as recoveries from water.

The concentrations of butyltin species and inorganic tin in water and bacterial suspensions reported in this article have not been corrected for recovery.

Although Sn(IV) was the only inorganic tin species for which recoveries were determined, the tin present in our water and bacterial suspension samples is reported as total inorganic tin, since any Sn(II) which might have been present would likely have been oxidized to Sn(IV) during pentylation.

RESULTS AND DISCUSSION

Toxicity of Tributyltin-Pentachlorophenol Mixtures to *B. cereus*

The toxicity tests demonstrated that at the concentration of tributyltin to be used in the degradation experiments, i.e., 45 ug Sn/L, there was no inhibition of microbial dehydrogenase activity.

In the course of our evaluation of the toxicity of tributyltin to *B. cereus*, we noticed a large synergistic effect with pentachlorophenol, another common lumber preservative. Table 1 shows the combined effects of tributyltin and pentachlorophenol.

The first observation to be made is that essentially the same results are obtained with tributyltin chloride as with bis(tributyltin) oxide. This agrees with earlier observations that the variation of X within any series of R_3SnX compounds usually has little effect on the biological activity (9,21).

Low concentrations of pentachlorophenol alone or tributyltin alone (either as tributyltin chloride or bis(tributyltin) oxide) produced little or no inhibition of resazurin reduction in *B. cereus*. When pentachlorophenol and tributyltin were both present, however, there was significant inhibition of microbial dehydrogenase activity. For example, at a tributyltin concentration of 2 mg Sn/L, a synergistic effect was noted at 50 ug/L pentachlorophenol. (Although a concentration of 2 mg Sn/L

exceeds the solubility of bis(tributyltin) oxide in pure water, bacterial cells would provide adsorptive sites for tributyltin.) The % inhibition observed for pentachlorophenol plus tributyltin chloride was 28% vs. an expected inhibition of 1%. Conversely when the pentachlorophenol concentration was held constant at 2 mg/L, a synergistic effect was noted at 50 ug Sn/L tributyltin. The data in Table 1 also show that the % inhibition increases faster when the concentration of tributyltin is held constant and that of pentachlorophenol is varied, than vice versa.

The biochemical mechanism of the enhancement of the effect of tributyltin on B. cereus by pentachlorophenol is uncertain. It does not appear to be by uncoupling oxidative phosphorylation since 2,4-dinitrophenol (a classic uncoupler) at 0.01-10 mg/L did not affect the % inhibition produced by 2 mg Sn/L tributyltin. Whatever the mechanism, the synergism between tributyltin and pentachlorophenol will not be significant in natural waters since neither tributyltin nor pentachlorophenol have been observed at concentrations higher than 20 ug/L (12,22).

Degradation of Tributyltin

Figure 1 shows that there is very little, if any, degradation or volatilization of tributyltin in the KCN-poisoned control over an 8 d period. The mass balance for the sum of tributyltin, dibutyltin, monobutyltin and inorganic tin is excellent. These results are taken as proof that there is negligible volatilization of tributyltin from the test fermentors at an initial concentration of 45 ug Sn/L.

Figure 2 shows substantial degradation of tributyltin in the HgCl_2 -poisoned control over 8 d. This strictly chemical debutylation is analogous to the previously observed transfer of a methyl group from methyltin species to mercury (23). In the case of the HgCl_2 -poisoned control, the mass balance for all four species was not constant over 8 d, and indicated that 36% of the initial tributyltin was unaccounted for. One reason for this, as stated above, is that the efficiency of extraction of Sn(IV) from water at neutral pH is poor.

A comparison of Figures 3 and 4 shows that tributyltin can be degraded under anaerobic conditions, and that the rate is significantly faster than under aerobic conditions. The mass balance for all four species was excellent under aerobic conditions. Under anaerobic conditions 31% of the initial tributyltin could not be accounted for after 8 d, again perhaps because of the poor extraction

efficiency of Sn(IV) from water. Bacterial degradation of tributyltin in culture medium and in soil has been demonstrated before under aerobic conditions (24,25). In addition, we have shown previously that the half-life of biological degradation of tributyltin in sediment under aerobic conditions is about 4 months at 20 °C (14). This is the first time that anaerobic degradation has been demonstrated, and means that estimates of the persistence of tributyltin in aquatic ecosystems must take into account the degree to which the system is aerobic or anaerobic.

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Table 1. Reduction of Microbial Dehydrogenase Activity by Tributyltin Chloride, Bis(tributyltin) Oxide and Pentachlorophenol

Pentachlorophenol (mg/L)	% Inhibition											
	Tributyltin Chloride (mg Sn/L)						Bis(tributyltin) Oxide (mg Sn/L)					
	0.05	0.1	1	2			0.05	0.1	1	2		
0.01	E ^a	0 ^a	E	0	E	0	E	0	E	0	E	0
0.05												
0.10												
1												
2	6	40	5	41	-2	58	8	40	6	40	0	69
5											21	95
10											49	98

^aE is expected inhibition (i.e., the sum of the inhibitions of the chemicals applied singly) and 0 is observed inhibition. Negative values for inhibition mean activation.

FIGURE CAPTIONS

- Figure 1. Variation of concentrations of butyltin species and inorganic tin with time in the KCN-poisoned control.
- Figure 2. Variation of concentrations of butyltin species and inorganic tin with time in the HgCl_2 -poisoned control.
- Figure 3. Variation of concentrations of butyltin species and inorganic tin with time in the aerobic test fermentor.
- Figure 4. Variation of concentrations of butyltin species and inorganic tin with time in the anaerobic test fermentor.







