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UPTAKE AND FATE OF TRI-N-BUTYLTIN CATION IN ESTUARINE BACTERIA.(U)
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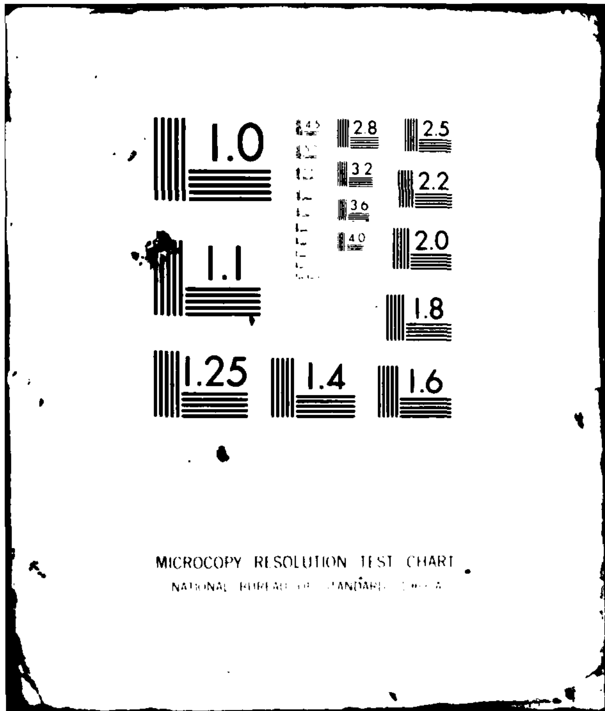

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UPTAKE AND FATE OF TRI-N-BUTYLTIN CATION IN ESTUARINE BACTERIA

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Abstract. The uptake and possible metabolic transformation of tri-*n*-butyltin cation by tin-resistant estuarine bacteria was studied. The bacterial isolates accumulated tributyltin to 3.7 to 7.7 mg tin per g dry weight of cells by a non-energy requiring process, probably by adsorption to the cell envelope. Chemical speciation of cell extracts and culture media by combined liquid chromatography-atomic absorption spectrophotometry and tin-selective purge and trap flame photometric gas chromatography for possible tributyltin degradation products revealed no significant biotransformations of tributyltin cation by the tributyltin-resistant isolates. Apparently the isolates accumulate, but do not metabolize, tributyltin.

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Introduction

Although many studies have documented the uptake of heavy metals by microorganisms, relatively little is known regarding uptake and possible microbial transformations of organometallic compounds[1]. Silverberg et al. [27] reported that Aeromonas apparently was accommodated to organoleads by binding lead in the cell envelope, however, the chemical form of lead bound in the envelope was not ascertained. Aeromonas also produces volatile tetramethyllead from trimethyllead salts [27], perhaps as a detoxification process. Others have studied organomercury biodegradation by mercury-resistant microorganisms. Methylmercury can be degraded to volatile elemental mercury and methane [19,29] and phenylmercuric acetate is degraded to volatile elemental mercury and benzene [23]. Fang [8] found that guppy, snail, elodea, and coontail took up radioactivity when incubated with ^{203}Hg-labeled phenylmercuric acetate (PMA), but did he not speciate the bound radiolabeled mercury. Most of the PMA was converted to inorganic mercury. Guard et al. recently demonstrated microbial volatilization of tetramethyltin from trimethyltin, presumably by a biologically-catalyzed methyl redistribution [11]. Hallas and Cooney [12] as well as Yamada and co-workers [31] found trialkyltins, especially tripropyltin (TPT) and tributyltin (TBT), had the highest antim[illegible]ial activity. Yamada et al. [31] found TPT inhibited membrane bound A[illegible] and DNA and RNA synthesis, but not respiration. TPT caused leakage of cell

[1]Use of terms for organometallic derivatives such as tributyltin, methylmercury, or trimethyllead find increased use in biological literature as this field develops. Readers are cautioned that these represent commonly used but incomplete descriptions of full chemical names. We employ such terms as a space-saving convenience, but with the note that such terms modify descriptions of "species," "salts," "derivatives," or "compounds," and the like in all circumstances, and do not imply free radicals.

constitutents, and in some cases lysis with *Escherichia coli* and *Bacillus subtilis*, yet was rapidly bound (probably to membrane phospholipids) by *E. coli* by a temperature independent process. Barug and Vonk [3] reported that ^{14}C-labeled tributyltin oxide was degraded to dibutyltin derivatives and CO_2 in soil incubated in the laboratory, though no direct tin-cell interactions were investigated. Recently, Barug showed [2] that pure cultures of certain bacteria and fungi convert added bis(tri-butyltin)oxide into mono- and dibutyltin derivatives after several days incubation.

Organotins are being used in increasing amounts as plastics stabilizers, catalysts, and biocides [33]. The U.S. Navy is investigating the use of organotins (especially tributyltin-containing polymers) as biocidal controlled-release agents in antifoulant paints for its fleet. The potential environmental fate of these toxic organotin compounds, which are slowly leached into the aquatic environment, requires attention. This work was undertaken to gain a better understanding of the interaction of tin-resistant estuarine microorganisms with such toxic organotin compounds. Specifically, estuarine bacteria resistant to tributyltin were studied to examine the mode of tributyltin uptake and possible degradation and/or detoxification processes.

Materials and Methods

Chemical and Speciation Procedures

All chemicals used were reagent or purified grade; organotins were obtained from commercial sources and used without further purification since their chromatographic and NMR purities were greater than 98 percent [18]. Stock solutions and freshly prepared dilutions for

experiments and calibrations employed spectrograde methanol and sterile deionized water (18 MΩ-cm resistivity, Millipore Corp.). Speciation of organotins in solutions or from cell extracts was accomplished with appropriate ion exchange columns in an automated dual-pump high performance liquid chromatograph (HPLC) coupled with a tin-specific graphite furnace atomic absorption spectrophotometer (GFAA). Full details of the HPLC-GFAA system and its operation are reported elsewhere [18]; generally, detection limits for tributyltin and dibutyltin (speciated as the cations) were 0.16 and 0.20 mg L^{-1}, respectively. In typical solution growth media (Nelson medium, [23]) less than 3 percent of dibutyltin was detectable relative to tributyltin present at the 10 mg L^{-1} concentration used for uptake experiments. In the ion exchange speciation method, only ionizable organotins are detected as discrete cations at characteristic retention times independent of the labile geganion (e.g., Cl^-); those involatile butyltins, for example, not reversibly ionized from dissolved or particulate materials in the growth medium required a different method for speciation using hydridization.

Both headspace gases above solution growth media and aliquots of these media were examined by column gas chromatography (GC) using a tin-specific flame photometric detector (FPD). Respirant atmospheres were injected directly (1 mL) into the GC-FPD system to detect possible volatile tin species. For the detection of possible non-volatile tributyltin degradation products samples (10 μL) of growth solutions were pretreated with excess $NaBH_4$ in an automated purge-and-trap system (P/T) directly coupled with the GC-FPD and the sparged organotin hydrides formed were speciated. Details of these methods and their sensitivities towards a variety of potential organotin metabolites are reported elsewhere [17].

Total tin (i.e., not speciated) was analyzed by GFAA using 20 μL sample aliquots which were dried (100 °C, 20 s) then atomized (2700 °C, 7 s). Generally a char cycle, sometimes required when analyzing highly carbonaceous samples [18], was not needed since samples were diluted (20 percent HNO_3) prior to analysis. Calibration mixtures of tri-n-butyltin chloride or $SnCl_4 \cdot 5H_2O$ in 20 percent HNO_3 were used in constructing standard curves for cell uptake experiments. Detection limits [25] of 2.4 ng for Sn(IV) and 17 ng for tributyltin chloride were readily achieved.

Organisms and Cultural Conditions

Eight tributyltin-resistant bacterial isolates were obtained by surface plating of sediment from three different sites in Baltimore Harbor, Chesapeake Bay on Nelson medium [23] contining 20 mg L^{-1} tin (as tributyltin chloride). After incubation at 29 °C for one week, morphologically distinct colonies were restreaked on Nelson agar, and after incubation and a third streaking, were considered axenic. All isolates were gram negative rods. Three other tributyltin-resistant strains (*Pseudomonas fluorescens* strains B1 and B69, *Pseudomonas* strain 244) were obtained from Prof. R. R. Colwell of the University of Maryland. Cultures were grown in Nelson broth in 250 mL Erlenmeyer flasks on a rotary shaker operated at 200 rpm and held at 29 °C.

Preparation of Cell Suspensions

Cells were harvested (4600 xg, 15 min) during the exponential growth phase, washed twice in distilled water, and resuspended in 5.0 mM piperazine-N, N'-bis(2-ethanesulfonic acid) buffer (PIPES, [10], at pH 7.6) to a cell density of 2-4 mg (dry weight) mL^{-1}. The cell suspensions were further incubated for 6 to 10 h to deplete cellular energy reserves [24].

Tin Accumulation Studies

Starved cell suspensions were added (2.0 mL aliquots) to a series of 250 mL Erlenmeyer flasks containing 17.6 mL of 5.0 mM PIPES buffer. Distilled water or glucose (10 mM final concentration) was added 15 min before tributyltin chloride (10.0 mg L^{-1} final concentration). In some cases, sodium azide (to give 1 mM final concentration) or formaldehyde (to give 1 percent final concentration) was added 5 min before tributyltin chloride to flasks containing glucose and cells. The final volume in each flask was 20 mL. One flask contained glucose and boiled (10 min, 100 °C) cells. Samples were withdrawn periodically after tributyltin chloride addition and the cells were collected by centrifugation (12,000 xg, 3 min), followed by two washes in 5 mM PIPES buffer or filtration (25 mm diameter, 0.45 µm membrane Millipore filters), followed by a rinse with 5 mL of 5 mM PIPES buffer. Cells and filters were digested by 0.2 mL concentrated HNO_3 in 2 mL polypropylene centrifuge tubes for 30 min at 80 °C, the volume was brought to 1.0 mL with deionized water, and samples were analyzed for total tin using a Perkin Elmer Model 460 graphite furnace atomic absorption spectrophotometer (GFAA). In one experiment, cells which had been incubated 2 h in the presence of tributyltin (10 mg L^{-1}) were washed twice in deionized water, and broken by ultrasonic disruption (five 1.0-min bursts, microtip setting 6.5, Heat Systems Ultrasonics, Inc.). The cell wall-membrane component was separated from soluble material by centrifugation of the mixture at 20,000 xg for 30 min. Total tin in the resulting pellet (digested in concentrated HNO_3), and the supernatant, was also determined by GFAA.

Chemical Speciation of Cell Bound Tin

Cells were grown in Nelson broth containing 10 mg L^{-1} tin (as tributyltin chloride) for 2.5 hours. The cells were then centrifuged (12,000 xg,3 min), washed three times in deionized water, and extracted with 10 mL methanol. After an additional centrifugation, the methanol extract was analyzed directly for organotin species using the HPLC-GFAA system.

Tributyltin Chloride Metabolism Studies

Cultures of bacteria growing on Nelson agar plus 5 mg L^{-1} tributyltin chloride were inoculated into 250 mL Erlenmeyer flasks or mininert (Supelco) capped bottles containing 100 mL Nelson broth plus 10 mg L^{-1} tributyltin chloride and incubated overnight at 29 °C without shaking. The next day headspace gas from the capped bottles was analyzed for volatile tin species using GC-FPD equipped with tin-selective interference filters [17]. Aliquots (10 µL) of the culture medium from Erlenmeyer flasks were analyzed by purge and trap GC-FPD [17], following $NaBH_4$ reduction to detect possible non-volatile tributyltin degradation products.

Results and Discussion

Previous studies [12,13,20,31] have shown that many microorganisms, including estuarine bacteria [11,12] are sensitive to low concentrations of organotin compounds. Hallas and Cooney found that only a small percentage of bacteria in sediment samples from Chesapeake Bay were resistant to 15 mg L^{-1} dimethyltin dichloride in culture medium [13]. Tributyltin is one of the most toxic forms of tin; a few mg L^{-1} is sufficient to completely inhibit the growth of many microorganisms [12,13,20,30,31].

Other triorganotins are also toxic in low concentrations to molluscs, insects, higher plants, and mammals depending on the number of carbon atoms in the organic substituents [20,28,30].

Our tin-resistant isolates (Table I) were obtained from Baltimore Harbor, an industrially polluted portion of the Chesapeake Bay, which contains organotin compounds in the water column [17], and appreciable quantities of inorganic tin in sediments [12]. The bacteria which grew on Nelson agar containing 20 mg L^{-1} tin as tributyltin chloride were considered as tributyltin-resistant. All tributyltin-resistant isolates were also resistant to 100 mg L^{-1} inorganic tin ($SnCl_4 \cdot 5H_2O$), a concentration that Hallas and Cooney found to inhibit the growth of a majority of bacteria in Chesapeake Bay sediments [13]. *Pseudomonas* strain 244 and *Pseudomonas fluorescens* strains B1 and B69, also isolated from the Chesapeake Bay, were resistant to tributyltin chloride (20 mg L^{-1}) and inorganic Sn(IV) (100 mg L^{-1}). Since *Pseudomonas* 244 produces a number of volatile tin compounds [17] from inorganic tin(II) and (IV) it was the most intensively examined organism in this study.

Pseudomonas 244 rapidly bound tin when placed in PIPES buffer containing 10 mg L^{-1} tin as tributyltin chloride (Fig. 1). The cell bound tin was chemically speciated by HPLC-GFAA and showed an identical retention time with that of authentic tributyltin (Fig. 2). The binding appeared to be a chemical adsorption process rather than active transport since starved cells, starved cells plus glucose, and starved cells plus glucose and sodium azide (a metabolic inhibitor) accumulated similar amounts of tributyltin (Fig. 1). Yamada et al. [32] found *Escherichia coli* rapidly bound tripropyltin also by a non-metabolic process. Cells killed by boiling or formaldehyde treatment

bound nearly twice as much tributyltin as live cells, a phenomenon observed by other investigators (using other organisms and metals) and attributed to increased cell envelope binding sites made available by these treatments [5,9,15,22]. Pseudomonas 244 and isolate Ac were also tested for the ability to bind inorganic tin (as $SnCl_4 \cdot 5H_2O$). Inorganic tin(IV) was bound much more rapidly than tributyltin, within 2 to 3 minutes binding was complete and tin had accumulated to 1.5 to 2.0 percent of the cell dry weight (Fig. 3). These data also suggest a rapid surface binding of inorganic tin, rather than progressive metabolic uptake into the cell. Thus, methylation of Sn(IV) by Pseudomonas 244, previously demonstrated in this laboratory [16,17], may occur in the cell envelope rather than intracellularly.

Tributyltin was also bound by the other isolates (Table 2). As with Pseudomonas 244, binding was nearly complete after 1.5 hours. Again, no appreciable differences in the amount of tributyltin bound by starved cells and starved cells plus glucose was noted, indicating uptake was not a metabolically dependent process.

Dry weight concentration factors for tributyltin (Table 2) indicate that these organisms have the capacity to adsorb and concentrate substantial amounts of tributyltin from solution. In the natural aquatic environment tributyltin may occur largely associated with microorganisms and could, therefore, be accumulated in a food web as has been observed with mercury [4].

The majority of tributyltin bound by the tin-resistant isolates occurred in the cell envelope (Table 3). Cells of seven of the isolates from Baltimore Harbor and Pseudomonas 244 were incubated with tributyltin (10 mg L^{-1}) for 2 h, washed, and then broken by ultrasonic disruption

and centrifuged to pellet the cell wall-membrane complex and unbroken cells. Only a small amount of tin was detected in the supernatant fraction, 95 to > 99 percent was detected in the pellet. Since > 92 percent of the cells were broken (except Pseudomonas 244, 70 percent) by ultrasonic treatment (as determined by Petroff-Hausser direct count) the pellet consisted mainly of cell wall-membrane fragments. Thus, virtually all the tributyltin bound by these isolates was associated with the cell envelope. Yamada et al. [32] observed that the cell wall-membrane complex and protoplast membranes of Escherichia coli bound tripropyltin.

Cells that bound tributyltin were collected on membrane filters, and when rinsed with 5 mL EDTA (1 mM) instead of PIPES buffer, 50 to 70 percent of the bound tributyltin was removed, suggesting a weak mode of chelation of tributyltin [7,24]. Yamada et al. have suggested "EDTA does not react directly with trialkyltins" [31]. Perhaps, as Yamada et al. suggested for E. coli [31], EDTA treatment results in defects in membrane structure or stability. Such defects may have resulted in the release of membrane bound or intracellular tributyltin from cells on filters.

The identity of the tin species bound by Pseudomonas 244 and other isolates was confirmed by HPLC-GFAA analysis of methanol extracts of cells which had been incubated with tributyltin chloride. To our knowledge this is the first report in which chemical speciation of a cell-bound organometallic compound has been reported. These analyses also indicated no significant biotransformations of bound tributyltin to dibutyltin. After 2.5 hours of incubation only tributyltin cation was detected in methanol extracts from cells (Fig. 2); similar experiments performed after 8 days incubation showed no tributyltin transformations. Compared

to a methanol wash, very little tributyltin was released by the cells in a deionized water wash. No dibutyltin species, which has been a reported tributyltin breakdown product in rat liver microsomes and mammals [20] in unsterilized soils [3] and in some microorganisms [2], could be detected. Based on system sensitivity, biotransformation of 1 percent of tributyltin in solution or 3 percent of cell-bound tributyltin to dibutyltin would have been detected. The HPLC-GFAA method would not, however, detect volatile tin compounds nor the production of inorganic tin (by microbial C-Sn bond breaking). Therefore additional experiments were performed to determine if volatile degradation products or inorganic tin were produced by the action of the tin-resistant isolates on tributyltin, especially since Pseudomonas 244 produces volatile organotins from $SnCl_4 \cdot 5H_2O$ [16,17] and estuarine microorganisms have been reported to produce volatile tetramethyltin from trimethyltin [11], or Sn(IV)[14].

Possible tributyltin degradation to volatile and non-volatile products was studied with a purge and trap flame photometric gas chromatograph (P/T-GC-FPD) using interference filters selective for tin emission [17]. Culture medium containing 10 mg/L tin as tributyltin chloride and inoculated with tin-resistant isolates was analyzed either by direct P/T-GC-FPD or by borohydride reduction P/T-GC-FPD. Non-volatile inorganic and organic tin compounds are converted to volatile tin hydrides by sodium borohydride [17] and were collected on a Tenax GC trap by purging the sample with nitrogen. The trapped sample was desorbed onto the GC column for separation and detection of tin species. Detection limits for organotins using this system are 13 to 52 ng L^{-1} depending on the tin species examined [17]. In addition to these experiments, direct injection of the headspace gas in closed container experiments was also performed. All

bacterial isolates were examined, and in no case were volatile tin containing degradation products detected. Thus, Pseudomonas 244, which methylates Sn(IV) to volatile tetramethyltin [17] and the other tributyltin-resistant bacteria we isolated, are apparently unable to metabolize or transform tin in the tributyl form, unlike certain microorganisms which apparently transform organometals as a detoxification process [23,26,29]. The tributyltin-resistant estuarine bacteria that we examined do not metabolize tributyltin in laboratory culture, although they accumulated tributyltin in the cell envelopes. This raises the question of both environmental persistence and bioaccumulation of tributyltin in food chains in the estuarine environment.

The exact mechanism of tributyltin resistance in the bacterial isolates is unclear but apparently does not involve metabolic degradation of the organometal. Results with Pseudomonas 244 indicated that accelerated efflux (as in tetracycline resistance in E. coli [1,21]) or metabolic exclusion probably does not account for tributyltin resistance since metabolically inhibited cells do not accumulate more tributyltin than metabolizing cells (Fig. 1). Tributyltin may be sequestered in some manner in the cell envelope, as suggested by Yamada, et al. [32] for E. coli and tripropyltin and Silverberg, et al. [27] for Aeromonas and organic lead, affording some degree of protection to the bacterial cells. Gram-negative bacteria in general are more resistant to many bacteriocidal compounds than are gram positive bacteria by virtue of their complex outer membrane structure [6,31]. Yamada et al. [31] reported gram-negative bacteria were more resistant to trialkyltins than gram-positive bacteria. Barug [2] recently reported that certain fungi and bacteria slowly degraded tributyltin to

mono- and dibutyltin, but suggested that the capacity to degrade tributyltin is not widespread among bacteria. Our results support that suggestion. Thus, a system of organotin detoxification analogous to the well-known organomercurial enzymatic detoxification mechanisms [23,26,29] apparently is not common among organotin-resistant bacteria.

Barug and Vonk [3] detected biodegradation of tributyltin to dibutyltin and CO_2 in soils. It is possible that under actual environmental conditions a similar process occurs in estuaries. From our results we know that tributyltin resistant bacteria do not seem to readily biodegrade tributyltin in laboratory culture, however, under actual environmental conditions other processes may occur. In an attempt to address this question we have undertaken some preliminary analyses of tributyltin spiked sediments from Baltimore Harbor. Initial short-term results have indicated no volatile tins are produced from tributyltin spiked sediments or waters however, in some cases, the analyses are difficult due to interferences from volatile organosulfur compounds in the sediments. New studies employing gas capillary GC-FPD tin speciation techniques should help in peak resolution and allow an assessment of the biological stability of tributyltin species in estuarine waters and sediments.

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Table 1

The organotin resistant isolates obtained from sediments of Chesapeake Bay in (Jones Falls, Colgate Creek) and near the mouth of (Sparrows Point) Baltimore Harbor. The sediments were plated on Nelson agar containing 20 mg L^{-1} tin as tributyltin chloride and resistant colonies were picked and purified. Some mercury-resistant isolates from Chesapeake Bay were obtained from Prof. R. R. Colwell. These were also resistant to tributyltin.

Tin Resistant Isolate	Origin
Ce	Jones Falls[a]
Cf	Jones Falls
Cg	Jones Falls
Ab	Colgate Creek[a]
Ac	Colgate Creek
Bd	Colgate Creek
Dh	Sparrows Point[a]
Dj	Sparrows Point
Pseudomonas fluorescens-B1	Chesapeake Bay
Pseudomonas fluorescens-B69	Chesapeake Bay
Pseudomonas-244	Chesapeake Bay

[a]Baltimore Harbor sites

Table 2

Tributyltin cation uptake by tin-resistant bacteria from Chesapeake Bay. Washed cells were incubated 6 h at 22 °C in 5 mM PIPES buffer to deplete cellular energy reserves then were exposed to tributyltin cation for 1.5 h, with and without glucose (10 mM), trapped on membrane filters, rinsed with 5 mL PIPES buffer, digested in HNO_3, and total tin was determined by GFAA.

Organism	Tin Uptake[a]		Concentration Factor[b]	
	No Glucose	Glucose	No Glucose	Glucose
Ps-244	3.7	4.1	438	487
Ac	7.7	9.2	855	1039
Bd	3.0	3.2	356	381
Dh	7.0	7.2	807	834
Ab	3.9	4.0	464	471
Dj	7.2	7.2	834	834
Cf	4.9	5.0	579	588
Ce	4.9	5.3	543	593
Cg	4.4	4.6	524	544

[a] μg per mg dry weight of cells

[b] $\frac{\text{\% TBT bound to cells/dry wt. of cells}}{\text{\% TBT remaining in medium/wt. medium}}$

Table 3

Adsorption of tributyltin to cell envelope fragments. Cells accumulated tributyltin for 2 h and then were washed and broken by ultrasonic disruption. After centrifugation (20,000 xg, 30 min) tin concentrations in the pellet and the supernatant were determined by GFAA.

Isolate	Pellet	Supernatant	% TBT in Pellet
Ac	204[a]	8.2[a]	96
Cf	180	7.9	96
Ce	302	4.9	98
Dh	365	9.6	97
Dj	166	5.3	97
Cg	155	7.0	95
Ab	156	3.5	98
244	280	0.4	99

[a] µg/mL

Figure Legends

Figure 1. Tributyltin uptake by Pseudomonas 244. Cells were harvested in exponential growth, washed, held in PIPES buffer (5 mM) for 6 h to deplete cellular energy reserves, then exposed to tributyltin chloride (B). Other cells received glucose (C, 10 mM, final concentration) or sodium azide (D, 1 mM final concentration). Some cells were boiled (A) or treated with formaldehyde (E, 1 percent final concentration) prior to tributyltin exposure. Cells were collected by centrifugation (12,000 xG, 3 min), washed twice, digested in concentrated HNO_3 and total tin was determined by GFAA.

Figure 2. Speciation of cell-bound organotin from microbial isolates Ps 244, Ac, Ce, Cg, Dj. Cells were grown in Nelson broth containing 10 mg L^{-1} tin as tributyltin chloride. After 2.5 h cells were harvested by centrifugation, washed three times in deionized water, then extracted with 10 mL methanol. The methanol extract after centrifugation was injected directly onto a liquid chromatograph-graphite furnace atomic absorption spectrophotometer system for speciation of cell-bound tin.

Figure 3. Inorganic Sn(IV) uptake by Pseudomonas 244 (•) and isolate Ac (o). Cells were harvested in exponential growth, washed twice in deionized water and resuspended in 5 mM PIPES buffer plus 10 mg L^{-1} Sn (as $SnCl_4 \cdot 5H_2O$). Cells were collected on membrane filters, rinsed, and digested in concentrated HNO_3 prior to analysis for total tin by GFAA.

FIG 1

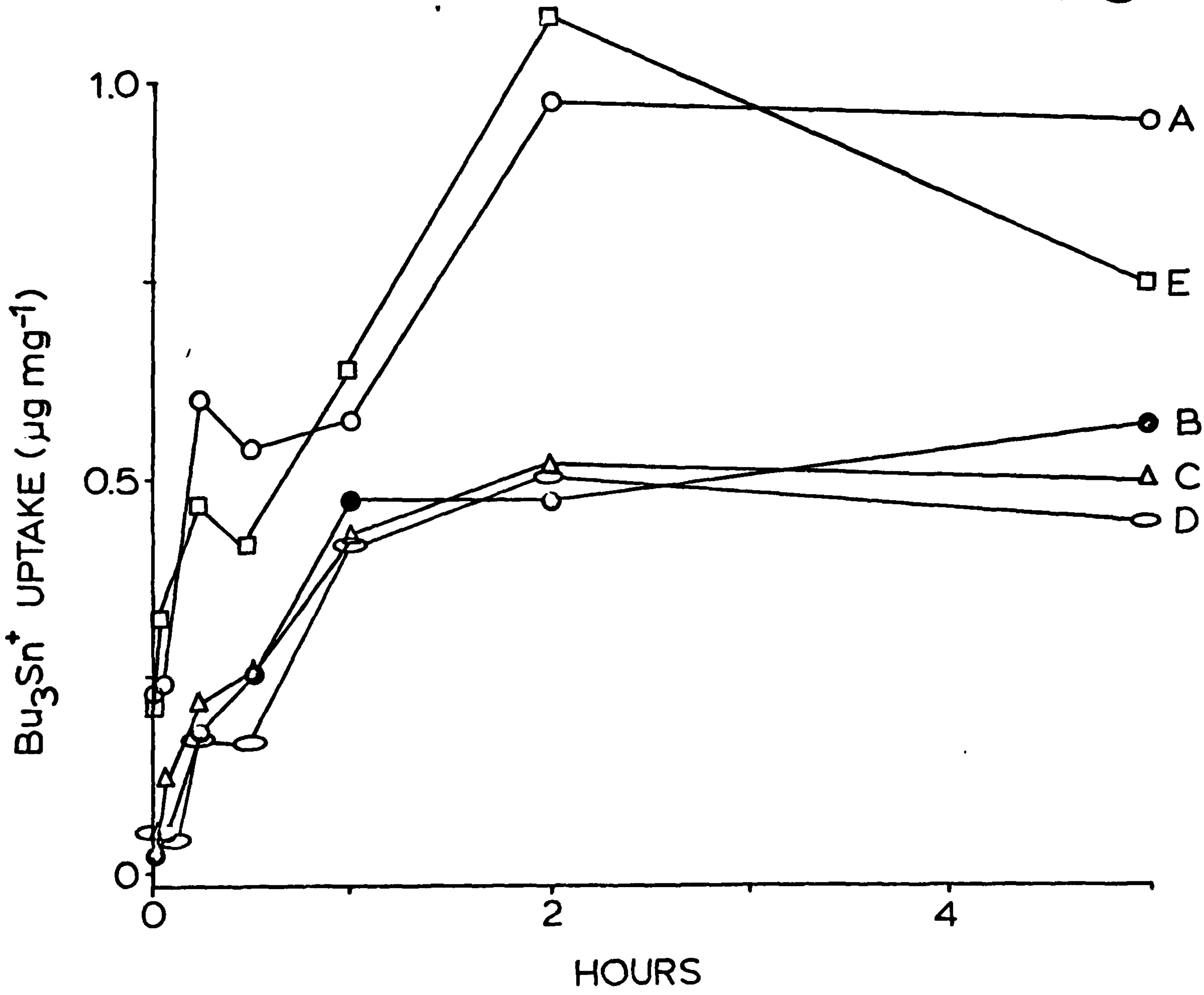
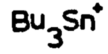
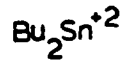
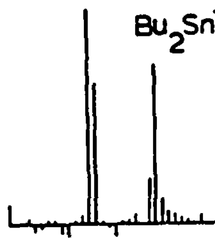


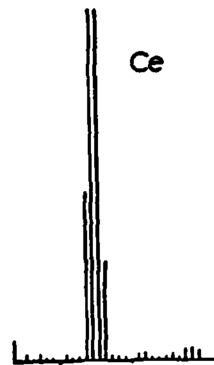
FIG 2

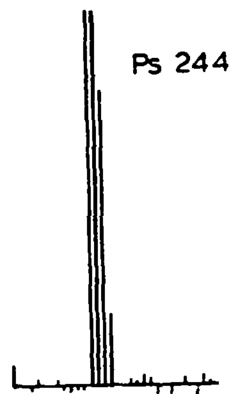
CALIBRATION
250 ng ea

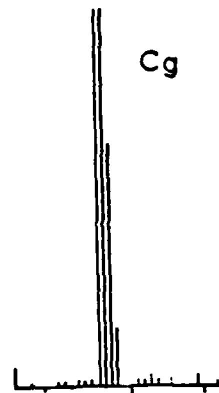


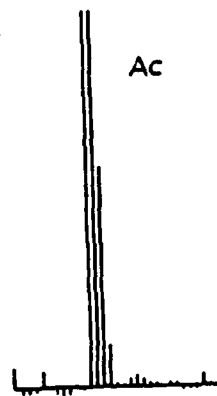


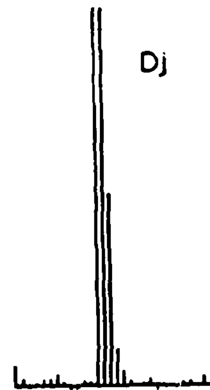












0 10 20 30
min

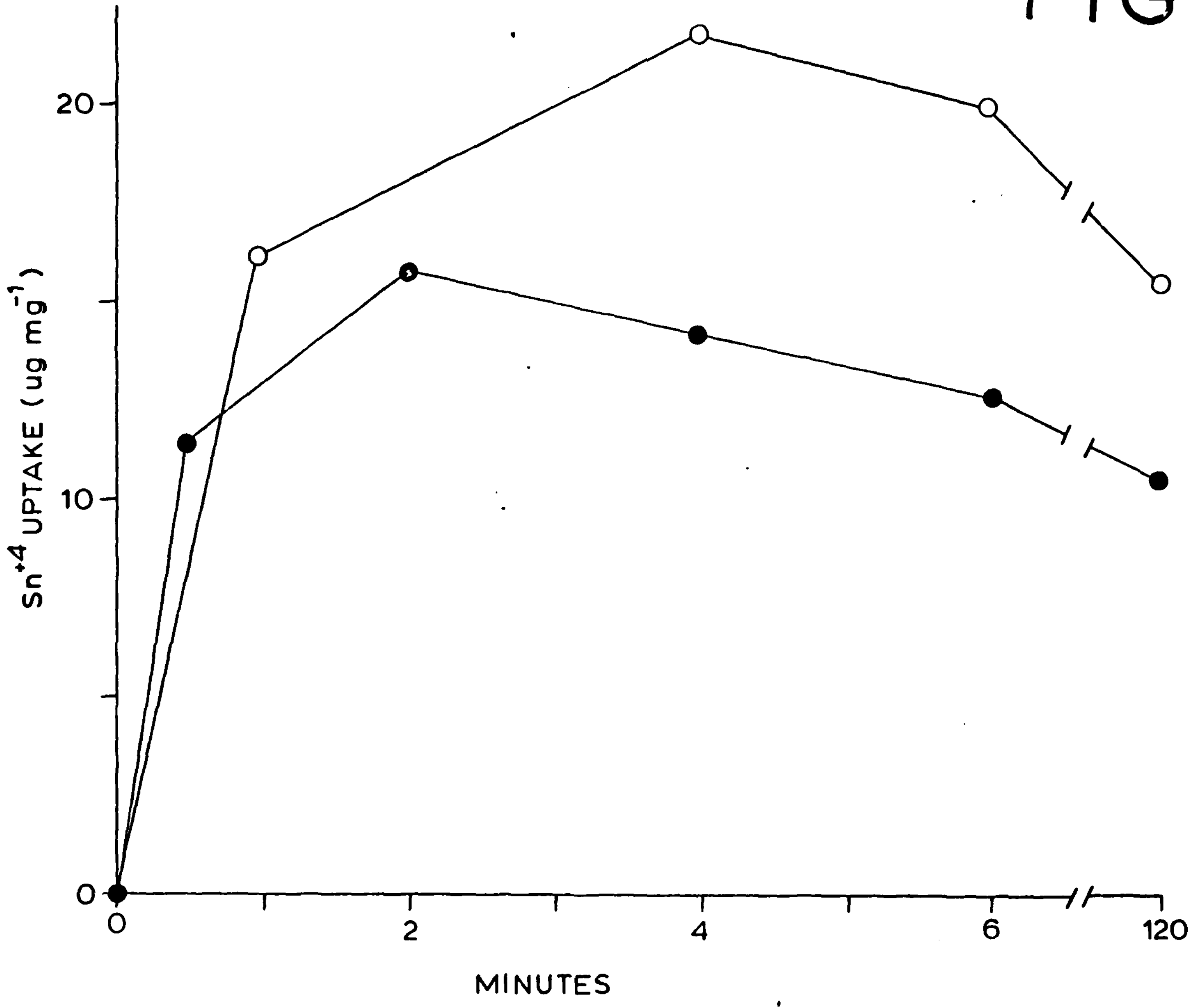


FIG 3
Sn^{+4} UPTAKE (ug mg^{-1})
20
10
0
0
2
4
6
120
MINUTES

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REPORT DOCUMENTATION PAGE

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18. SUPPLEMENTARY NOTES

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Bioaccumulation; Biocides; Biodegradation; Cell membranes; Chromatography Environment; Estuarine bacteria; Organotins; Speciation; Tin-resistance; Tin-specific analysis; Tributyltin.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The uptake and possible metabolic transformation of tri-n-butyltin cation by tin-resistant estuarine bacteria was studied. The bacterial isolates accumulated tributyltin to 3.7 to 7.7 mg tin per g dry weight of cells by a non-energy requiring process, probably by adsorption to the cell envelope. Chemical speciation of cell extracts and culture media by combined liquid chromatorgaphy-atomic absorption spectrophotometry and tin-selective purge and trap flame photometric gas chromatography for possible tributyltin degradation products revealed no signifigant biotransformations of tributyltin cation by the

tributyltin-resistant isolates. Apparently the isolates accumulate, but do not metabolize, tributyltin. Research into the persistance of tributyltin cation in natural esturine waters and sediments is needed.

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