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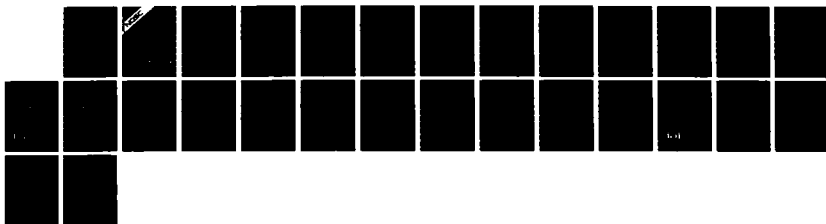
BUTYLtin COMPOUNDS IN TISSUES(U) NAVAL OCEAN SYSTEMS  
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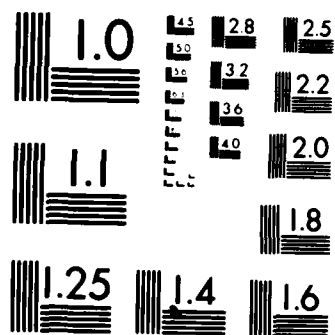
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NOSC TR 1089

**Technical Report 1089  
January 1986**

# **Butyltin Compounds in Tissues**

Carol A. Dooley

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## **ADMINISTRATIVE INFORMATION**

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## SUMMARY

Marine organisms exposed to organotin antifoulants accumulated solvent extractable tin in their tissues. Accumulation in oysters did not appear to have reached an equilibrium after 60 days of exposure; while fish appeared to be able to deal with tributyltin fairly efficiently. Depuration of tributyltin in oysters occurred at ~5 percent/day to give a calculated half-life of about 2 weeks.

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## BACKGROUND

Marine organisms exposed to butyltin compounds have been shown to accumulate tin in their tissues (Dooley & Homer, 1983). Whether the toxic tributyltin ( $\text{Bu}_3\text{Sn}$ ) is accumulated as such or whether the various marine organisms have enzyme systems to degrade or detoxify it is uncertain. The rate and extent of the accumulation and the degradation/detoxification are also not known.

Extraction, separation, and identification techniques were required to investigate this issue. A literature search and personal communications yielded likely extraction procedures, and a gas chromatograph/mass spectrometer (GC/MS) system provided identification as well as separation.

## METHODS AND MATERIALS

### Instrumentation

Total solvent extractable tin was estimated with a Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer (AAS) equipped with an HGA-500 graphite furnace and an AS-40 autosampler. Aliquots of tissue extracts were diluted as necessary with methylisobutyl ketone (MIBK), and the quantity of total tin was determined using standard additions of a freshly prepared tin AAS standard (5000-ppm organic tin salt in an organic matrix, Chemplex) in MIBK to prepare the calibration curve.

Derivatized butyltin compounds were determined, qualitatively and quantitatively, with a Varian 2100 Gas Chromatograph equipped with a hydrogen flame ionization detector (FID). Samples were injected directly onto a 6-ft glass column packed with 3-percent OV-17 on Chromosorb W(HP) 80/100 mesh. Injector and detector temperatures were 220°C and 250°C, respectively. Gas flow rates used were 30 ml/min for the helium carrier and hydrogen gas and 300 ml/min for the air. The oven was programmed from 100°C to 150°C at 10°C/min, after an initial 0.5-min hold. For analysis of tissue extracts, the oven temperature was increased to 200°C after 20 min. A Shimadzu CR3A Chromatopac was used for data collection.

A Finnigan Model 4000 Mass Spectrometer (MS) interfaced with a glass jet separator to a Finnigan Model 9610 Gas Chromatograph (GC) was used to obtain electron impact mass spectra of the butyltin compounds. Retention times and mass spectra verified the identity of the extracted compounds and standards. Initially the column and temperatures were as described for the Varian 2100. The separator and transfer line were maintained at 160°C. Trace levels of butyltin-containing species were determined by the multiple ion detection (MID) mode to obtain the necessary sensitivity. Four ions in three clusters may be monitored in this technique, and the sensitivity approximates the FID response. At a later stage, the packed column was replaced with a 30-m SPB-1 wide-bore (0.75 mm) capillary column that operated isothermally at 170°C with a carrier gas flow rate of 10 ml/min. Injector, separator, and transfer lines were operated at 220°C. A Finnigan Model 6115 Data System was used for data collection and analysis.

Thin-layer chromatography of butyltin compounds was done on Whatman LK50 silica gel plates with preadsorbent layer. Plates were developed with 9:1 hexane/acetic acid (v/v) for approximately 30 minutes. Dithizone, 0.1 percent in acetone containing 10-percent water (v/v), was used for detection of the compounds. For further GC/MS analysis, bands were scraped off the plates, the substances were redissolved in hexane, and the solvent was evaporated to give an appropriate amount of compound.

### Butyltin Compounds

Tri-*n*-butyltin chloride, di-*n*-butyltin dichloride, *n*-butyltin trichloride, and tetra-*n*-butyltin were obtained from Aldrich Chemical Company. Samples of beta-hydroxybutyldibutyltin bromide and delta-hydroxybutyldibutyltin chloride in diisopropyl ether were supplied by Richard Lee, Skidaway Institute of Oceanography, Savannah, Georgia.

The Grignard reagent, 1.8M *n*-pentylmagnesium bromide (PeMgBr) in ether, was obtained from Alfa Ventron and was used to prepare pentylated derivatives of the butyltin compounds. A measured excess of Grignard reagent and butyltin halide or appropriately diluted tissue extracts in hexane were shaken together at room temperature for 20 minutes. Excess Grignard reagent was destroyed by shaking with 1N H<sub>2</sub>SO<sub>4</sub>. The hexane layer was recovered and stored in the refrigerator in a capped vial containing anhydrous sodium sulfate. One- to three- $\mu$ l aliquots were used for GC/MS analysis.

Trisilyl Z, consisting of trimethylsilylimidazole in dry pyridine (manufactured by Pierce Chemical Company) was used to prepare the trimethylsilyl ether derivatives of the hydroxylated butyltins. Aliquots of the hydroxylated compounds in ether were evaporated to dryness in reactivials. An excess of Trisilyl Z was added, the vials were sealed and heated for an hour at 60°C. The solution was then analyzed directly for the proposed derivative.

### Sample Collection and Treatment

Oysters (*Crassostrea virginica*) and fish (*Citharichthys stigmaeus*) were exposed to a level of 0.7- $\mu$ g/l tributyltin originating from an antifouling coating (SPC 954, International Paints) for 64 days in a flowthrough system. At intervals of 0, 1, 2, 4, 7, 14, 30, and 64 days of exposure, 3 fish and 10 oysters were removed. Ten oysters were also collected after 7 and 14 days of a depuration phase.

The fish were pooled and homogenized in 1 to 2 volumes of distilled water in a blender. Oysters were shucked, pooled, blotted dry, and blended without additional water in a Tissumizer (Tekmar Instruments). Aliquots of the homogenized tissues were taken to determine dry weight; average dry weights of 4.5 and 14.7 percent were found for fish slurry and oysters, respectively. Samples were stored frozen before further treatment. In addition, fish exposed for 64 days to 0.5- $\mu$ g Bu<sub>3</sub>Sn/l were dissected to obtain their livers and digestive organs. This material was ground without additional water using the Tissumizer and was extracted with chloroform/methanol/water.

In a separate experiment, designed to look only at the depuration phase, oysters were exposed to 0.5- $\mu$ g/l  $\text{Bu}_3\text{Sn}$  for 75 days. The oysters were then transferred to clean tanks and were sampled weekly for 4 weeks. These bivalves were processed immediately, and the extracts were stored in a dark freezer prior to analysis.

### Tissue Extractions

a. Ethyl acetate. Accurately weighed 10- to 15-gram portions of homogenized wet tissue were stirred 10 minutes with an equal weight of 1:1  $\text{HCl}/\text{H}_2\text{O}$  (v/v). Ten grams of  $\text{NaCl}$  were dissolved in the mixture before it was extracted for 20 minutes with 5-ml ethyl acetate in a shaker. The organic layer was collected after centrifugation at 3,500 rpm for 10 minutes and was reduced to approximately 1 ml in a 60°C water bath under a stream of argon. The residue was extracted with 10-ml hexane, which was recovered after centrifugation and evaporated to 1 ml for analysis. The redissolution of the extracted compounds in hexane was necessary as a cleanup step and was required for alkylation procedures and GC and GC/MS analysis.

b. Chloroform/methanol/water. Accurately weighed 10- to 15-g portions of homogenized wet tissue were extracted for 8 hours with 50ml of a one-phase mixture of 1 part chloroform, 1 part water, and 2.5 parts methanol by volume. Ten ml of chloroform and 10 ml of water then were added, and the separated phases were allowed to settle overnight. The organic layer was recovered after centrifugation, evaporated to near dryness, and the residue was redissolved in 1-ml hexane.

c. Tetramethylammonium hydroxide (TMAH). Accurately weighed 10- to 15-g portions of homogenized wet tissue were heated at 60°C in 25 ml of 25-percent aqueous TMAH, obtained from Alfa Ventron, until a clear yellow solution was obtained. After cooling, the solution was adjusted to pH 1 with concentrated  $\text{HCl}$ , and the mixture was extracted with 5-ml hexane containing 0.1-percent tropolone. The hexane volume was reduced to 1 ml for analysis.

## RESULTS

### Tissue Extractions

Table 1 shows the results of spiked recoveries obtained in one extraction from oyster tissue for the ethyl acetate/ $\text{HCl}$  and for the  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  extraction systems. The TMAH procedure was not tested for spiked recovery because we had observed that recovery was low, the results were not improved, and the reagent was hazardous to handle. The oysters were obtained from a clean commercial source; the amount of tin was determined by graphite furnace atomic absorption spectrophotometry (GFAAS). The validity of these results for naturally accumulated butyltin compounds as opposed to spiked tissues is uncertain, but the results do support the observation (mentioned below) that  $\text{EtOAc}/\text{HCl}$  is a better extraction system by a factor of more than two for naturally exposed tissues.

Table 1. Spiked recoveries from oyster tissue with two extraction systems.

Compound	Extraction System	
	EtOAc/HCl	CHCl <sub>3</sub> /MeOH/H <sub>2</sub> O
Bu <sub>3</sub> Sn	85.0 ± 2.1%	31.5 ± 1.8%
Bu <sub>2</sub> Sn	87.0 ± 2.9%	58.2 ± 7.4%
BuSn	118.0 ± 2.8%	15.6 ± 2.9%

Based on personal communications and a search of the pertinent literature, three extraction techniques were tested for their effectiveness on tissues of marine organisms. In summary, butyltin compounds were extracted as their chlorides with ethyl acetate, in the presence of HCl and NaCl (Arakawa et al., 1981). This extraction technique is important because it removes the uncertainty about the anion. Grignard derivatization requires that the anion be either chloride or bromide. Richard Lee (personal communications, 1983) suggested the use of a one-phase mixture of chloroform, water, and methanol to disrupt tissues and to release endogenous butyltin compounds. Finally, Chau et al. (1984) used 25-percent aqueous TMAH to solubilize tissues prior to extraction with benzene containing sodium diethyldithiocarbamate for recovery of alkyllead species. This technique was modified by using hexane with tropolone as the extractant (Chau et al. 1984; Meinema et al., 1978).

With oyster tissue, all three extraction methods were moderately successful. The ethyl acetate extraction gave the highest recovery of the three methods, by more than double, as determined by GFAAS. Recoveries were similar, though lower, with the other two extraction techniques. The TMAH had, in addition, a high-tin background that interfered with the analysis.

With whole fish tissue, the ethyl acetate could not be used because it formed an unbreakable emulsion; but both the chloroform/water/methanol and TMAH techniques were successful and gave similar values for estimated tin content. The results with fish tissue are uncertain because of the expected lower recovery of solvent extractable tin with these extractants and a suspected inhomogeneity of the tissue.

### Metabolites of Tributyltin

Two extraction systems were used because of the reported lability of suspected metabolites of butyltin compounds. Fish et al. (1975) and Kimmel et al. (1977) showed that mammals possess enzyme systems that can hydroxylate butyl groups in alpha, beta, gamma, and delta positions to the central tin atom and some of the hydroxylated products are unstable at physiological pH or upon acidification. Their data are summarized in figure 1 with the anion shown as chloride, and the acid was assumed to be HCl for clarity. Two of the metabolites, alpha- and beta-hydroxybutyldibutyltin chloride, give rise to dibutyltin dichloride upon acidification. The beta-hydroxy compound is said to be unstable at physiological pH 7.4. Acid

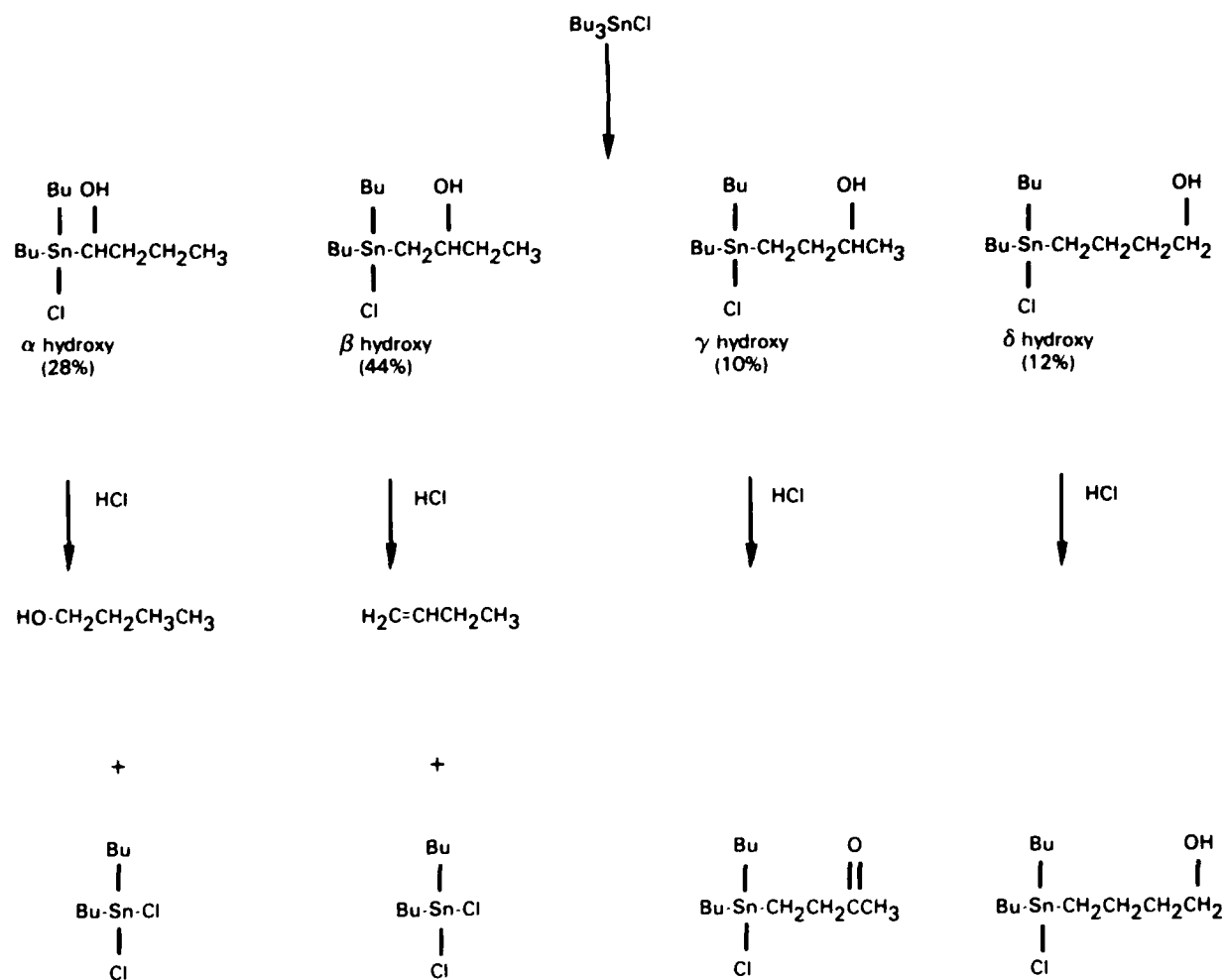


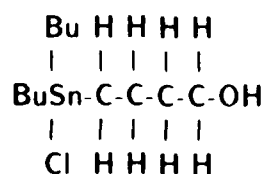
Figure 1. Potential metabolites of tributyltin chloride.

degradation of the alpha- and beta-hydroxylated metabolites would occur with the EtOAc/HCl extraction system; dibutyltin dichloride would appear as an artifact in the extract. The fate of potential metabolites in the  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$  extraction system is unknown, and there is uncertainty regarding the anion.

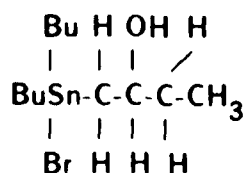
Two of the hydroxylated butyltins were synthesized (Fish et al., 1976; Lahournere & Valade, 1971) at Richard Lee's lab and were donated for study. As discussed, the delta-hydroxy compound is stable; on the other hand, the beta-hydroxy compound is unstable. Therefore, the experiments described here could be performed with more certainty on the delta-hydroxy compound and inferred for the beta-hydroxy compound.

Hydroxylated  $\text{Bu}_3\text{Sn}$  species chromatograph with lower retention times than their unhydroxylated analogues. The mass spectra resemble those of tetraalkyltins (Chambers et al., 1967, 1968; Dibeler, 1952) and trialkyltin compounds (Gielen & Mayence, 1968). Briefly, all alkyltin spectra are characterized by a distinctive, though complex, ion abundance pattern for each tin-containing fragment and a low abundance, or nonexistent, molecular ion, so that the molecular weight of the compound cannot be determined. Fragments of greatest abundance are tin-containing and are formed by successive loss of alkyl groups. Those fragments where tin is mono- or trisubstituted, i.e.,  $\text{RSn}^+$  or  $\text{R}_2\text{SnCl}^+$ , are in greatest abundance. There is a very low abundance of ions formed from  $\text{CH}_2$  elimination from an alkyl chain. Figure 2 shows the electron impact mass spectrum of tributyltin chloride. Salient features are: (1) no molecular ion at  $m/z$  326 and (2) major fragment ions at  $m/z$  269 ( $\text{Bu}_2\text{SnCl}^+$ ),  $m/z$  177 ( $\text{BuSn}^+$ ),  $m/z$  155 ( $\text{SnCl}^+$ ), and  $m/z$  120 ( $\text{Sn}^+$ ). Smaller abundance fragments appear at  $m/z$  212 ( $\text{BuSnCl}^+$ ),  $m/z$  234 ( $\text{Bu}_2\text{Sn}^+$ ),  $m/z$  291 ( $\text{Bu}_3\text{Sn}^+$ ), and  $m/z$  135 ( $\text{MeSn}^+$ ) from  $\text{CH}_2\text{CH}_2\text{CH}_3$  elimination from  $\text{BuSn}^+$ .

The mass spectrum obtained on a sample of delta-hydroxybutyldibutyltin chloride is shown in figure 3. A family of fragment ions appears to arise from the primary loss of  $\text{CH}_2\text{OH}$  from the molecular ion



since such species as  $m/z$  255 ( $\text{BuPrSnCl}^+$ ),  $m/z$  198 ( $\text{PrSnCl}^+$ ), and  $m/z$  163 ( $\text{PrSn}^+$ ) appear. The mass spectrum obtained on a sample of beta-hydroxybutyldibutyltin bromide (figure 4) shows similar behavior with the loss of  $\text{CH}_3\text{CH}_2\text{CHOH}$  from



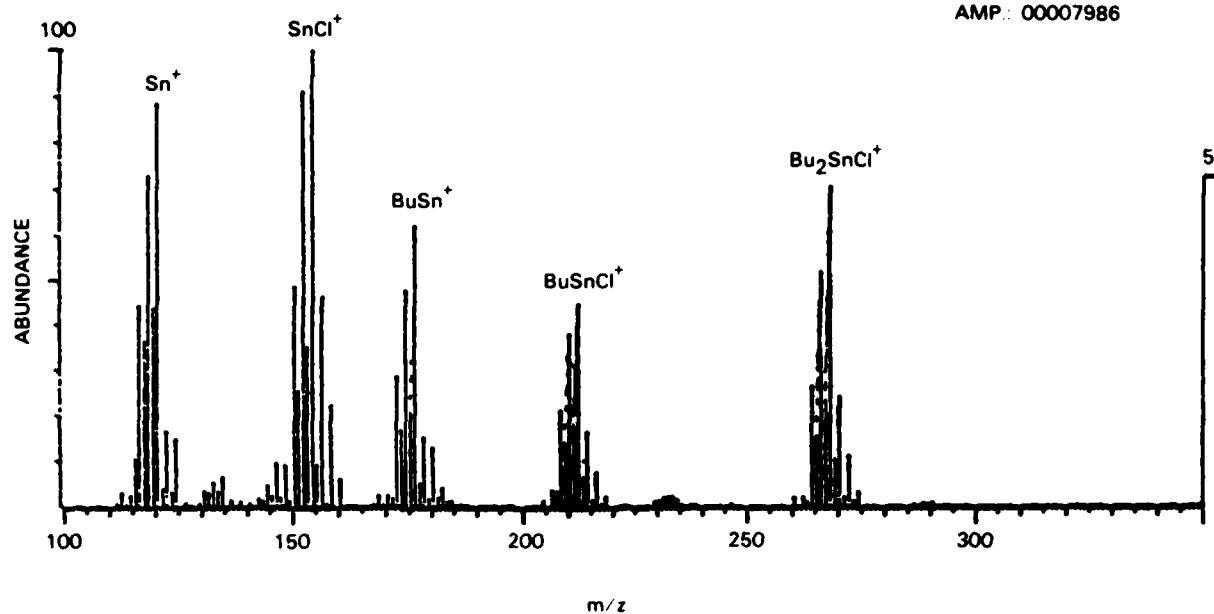


Figure 2. Electron impact mass spectrum of tributyltin chloride.

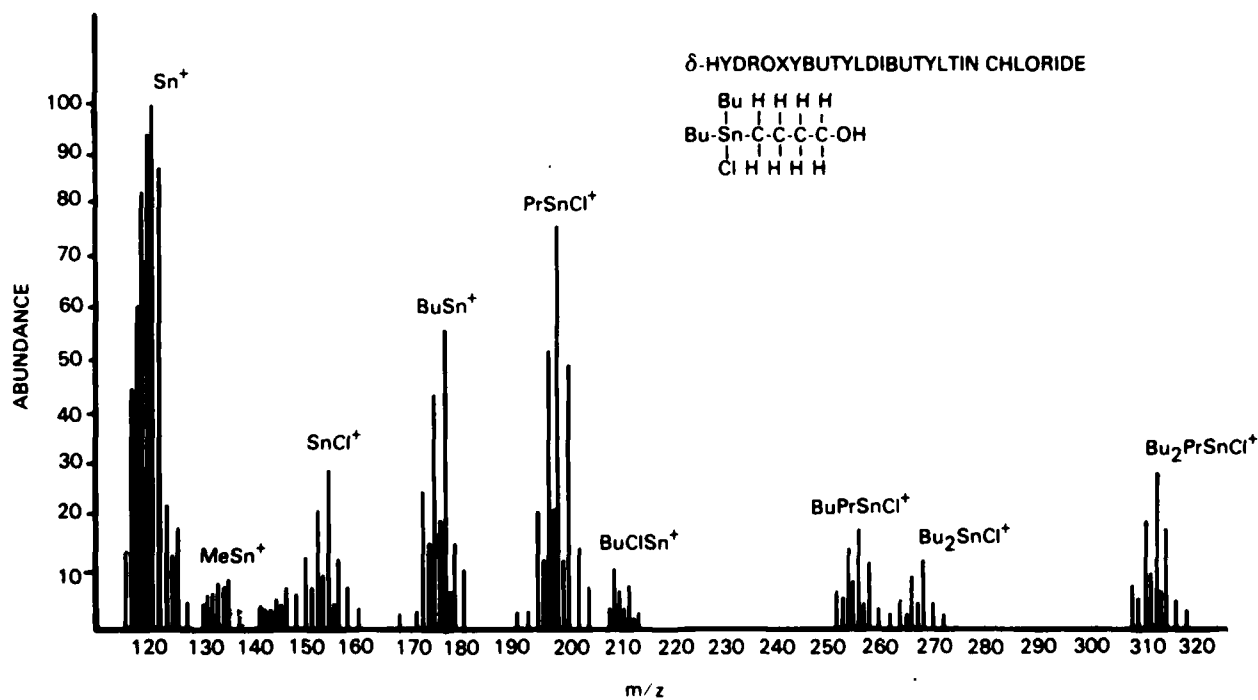


Figure 3. Mass spectrum of  $\delta$ -hydroxybutyldibutyltin chloride.

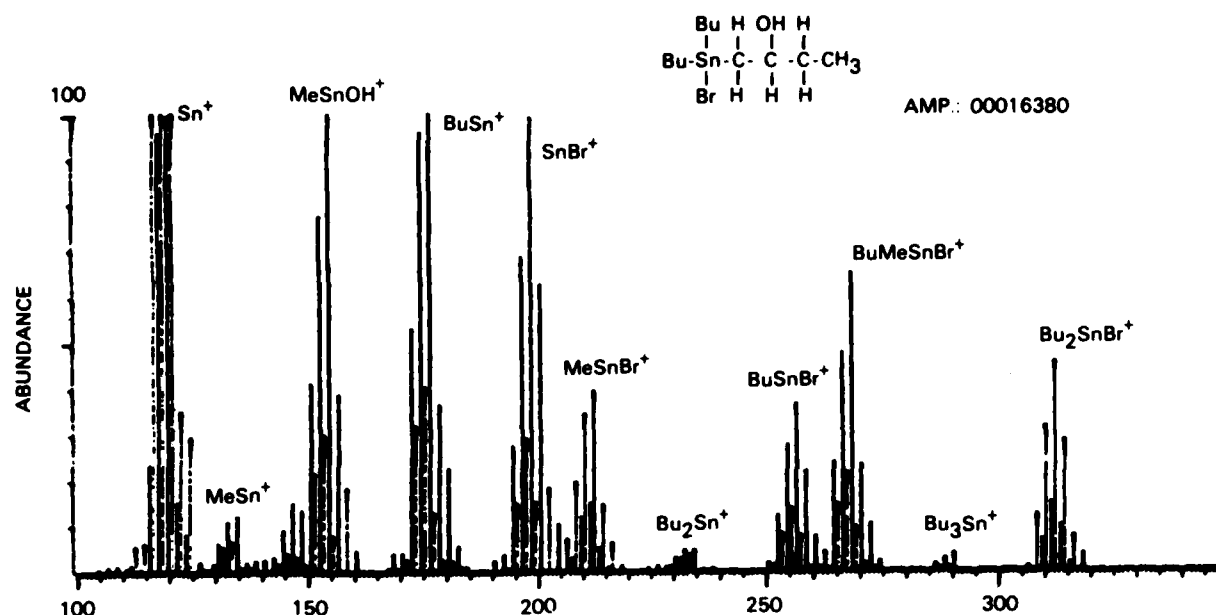
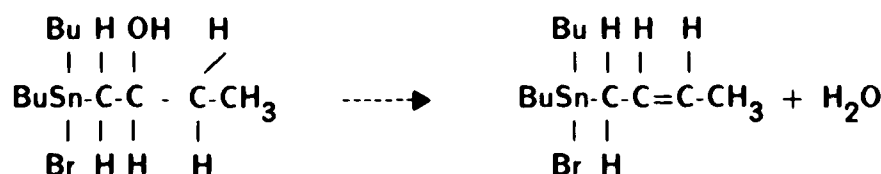


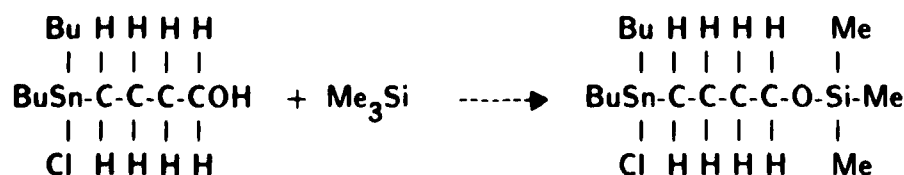
Figure 4. Mass spectrum of  $\beta$ -OH hydroxybutyldibutyltin bromide.

producing fragment ions, such as  $m/z$  271 ( $\text{BuMeSnBr}^+$ ) and  $m/z$  214 ( $\text{MeSnBr}^+$ ). These results contrast with those of Fish et al. (1974, 1977), who saw fragments associated with hydroxyl groups when chemical ionization techniques were used. Kingston et al. (1974) noted a tendency for tetrabutyltin alcohols to dehydrate in the ion source and suggested lowering instrument temperatures to prevent this. The mass spectrum obtained for beta-hydroxybutyldibutyltin bromide could also originate in such a process with fragmentation occurring between carbons 1 and 2



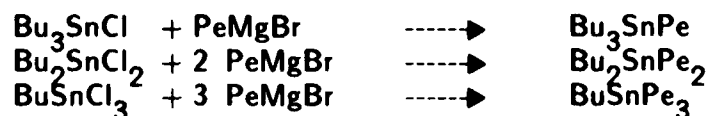
however, the delta-hydroxybutyldibutyltin chloride mass spectrum cannot be reconciled this way.

The hydroxylated butyltins were derivatized with trimethylsilylimidazole (Trisilyl Z) to form trimethylsilyl ethers, for example,

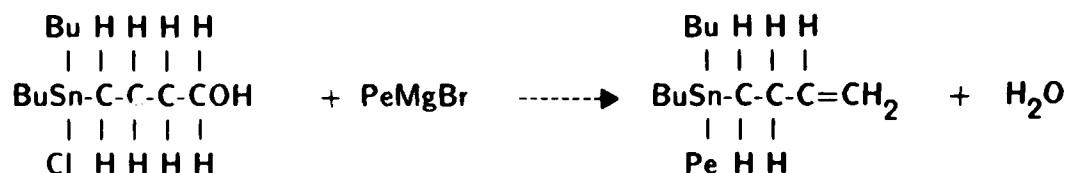


The derivatives produced had greatly increased retention times but the mass spectra obtained were similar to those of the alcohols, except for low  $m/z$  fragments attributable to the  $\text{Me}_3\text{Si}$  group. Apparently, any functional group in an analogous position directs a similar fragmentation pattern.

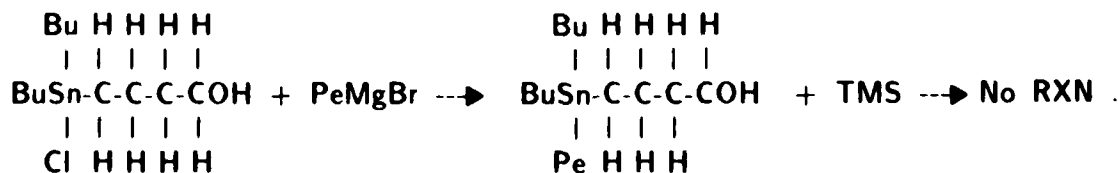
Butyltin chlorides (or bromides) react with the Grignard reagent pentylmagnesium bromide (Maguire & Huneault, 1981) to form mixed tetraalkyls that show good gas chromatographic behavior:



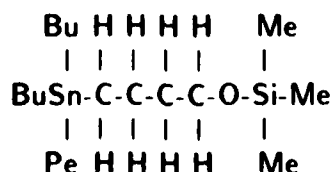
When hydroxylated butyltins were reacted with  $\text{PeMgBr}$ , large amounts of both  $\text{Bu}_2\text{SnPe}_2$  and  $\text{Bu}_3\text{SnPe}$  were formed. The rationale for this result may be that, for beta-hydroxy compound,  $\text{Bu}_3\text{SnPe}$  arises from the side product  $\text{Bu}_3\text{SnBr}$  and, possibly, from dehydration of the tributylpentyl alcohol (Kingston et al., 1974), and the  $\text{Bu}_2\text{SnPe}_2$  arises from conversion of the labile parent compound to  $\text{Bu}_2\text{SnBr}_2$ . The delta-hydroxy compound mixture can produce  $\text{Bu}_2\text{SnPe}_2$  from the contaminant  $\text{Bu}_2\text{SnCl}_2$  and  $\text{Bu}_3\text{SnPe}$  from the dehydration of the alcohol under the conditions of the reaction or under conditions of the measurement.



Such a product might be virtually indistinguishable from the saturated tetraalkyl owing to the overlapping spectra produced by the polyisotopic tin (Carrick & Glocking, 1967; Dibeler, 1952). Circumstantial evidence for the occurrence of dehydration during pentylation, a highly exothermic reaction, is that the following double derivatization did not occur:



The product



did not form. Based on these experiments, we believe the labile metabolites, if present, will form  $\text{Bu}_2\text{SnPe}_2$  upon derivatization because the extraction process has converted them to  $\text{Bu}_2\text{SnCl}_2$  and the stable metabolites will form a derivative indistinguishable from  $\text{Bu}_3\text{SnPe}$ . These experiments and previously reported data (Dooley, 1986) enabled the selection of common, intense ion fragments for use in the MID mode GC/MS necessary to achieve sensitivity for trace compounds.  $\text{BuSn}^+$  ( $m/z$  177),  $\text{Sn}^+$  ( $m/z$  120),  $\text{PeSn}^+$  ( $m/z$  191), and, in some cases,  $\text{MeSn}^+$  ( $m/z$  135) were used to identify solvent extractable, tin-containing compounds in tissue. In any case, all three extraction methods gave the same qualitative information for oysters; the two methods for fish gave approximately the same result.

### **Uptake of Tributyltin**

The total ethyl acetate extractable tin in oysters and chloroform extractable tin in fish is shown as a function of exposure and depuration time in figure 5. If we assume that equilibrium concentrations were reached in each case after 64 days of exposure, bioconcentration factors (BCF) of at least 3500 and 700 for oysters and fish, respectively, were reached. In fact, the accumulation by oysters does not seem to have leveled out, and the apparent plateau in fish may be an artifact of the extraction procedure. Furthermore, experiments in this lab have shown that the total extractable tin value is dependent upon the compounds of tin actually present and the standard used to measure it (Dooley & Vafa, 1986). The dependency cannot be corrected by standard addition unless there is prior knowledge of the sample composition. The total extractable tin value is probably too low, although, as explained below, the BCF values for oysters may not be too far off, assuming equilibrium concentrations were attained.

These extracts were derivatized by reaction with  $\text{PeMgBr}$ . Figure 6 compares the amount of  $\text{Bu}_3\text{SnPe}$  found relative to the amount of total extractable tin; the numerical comparison is not accurate (for the total tin) but the results are parallel until the depuration phase. Small and variable amounts of  $\text{Bu}_2\text{SnPe}_2$  were also found that could be the result of independent accumulation or the result of  $\text{PeMgBr}$  reacting with labile metabolites. Both  $\text{Bu}_3\text{SnPe}$  and  $\text{Bu}_2\text{SnPe}_2$  found in the tissue had mass spectra matching those of prepared pure compounds. The results with whole fish were unsatisfactory owing to the much lower concentration of recovered organic extractable tin.

Underivatized samples produced similar gas chromatograms for both extraction methods, namely, three butyltin species at retention times of 5.5, 5.6, and 6.1 minutes in addition to  $\text{Bu}_3\text{SnCl}$  at 9.2 minutes; so extraction with the chloroform/methanol/water system must equally affect the additional species. Delta-hydroxybutyldibutyltin chloride has a retention time of 6.0 minutes.

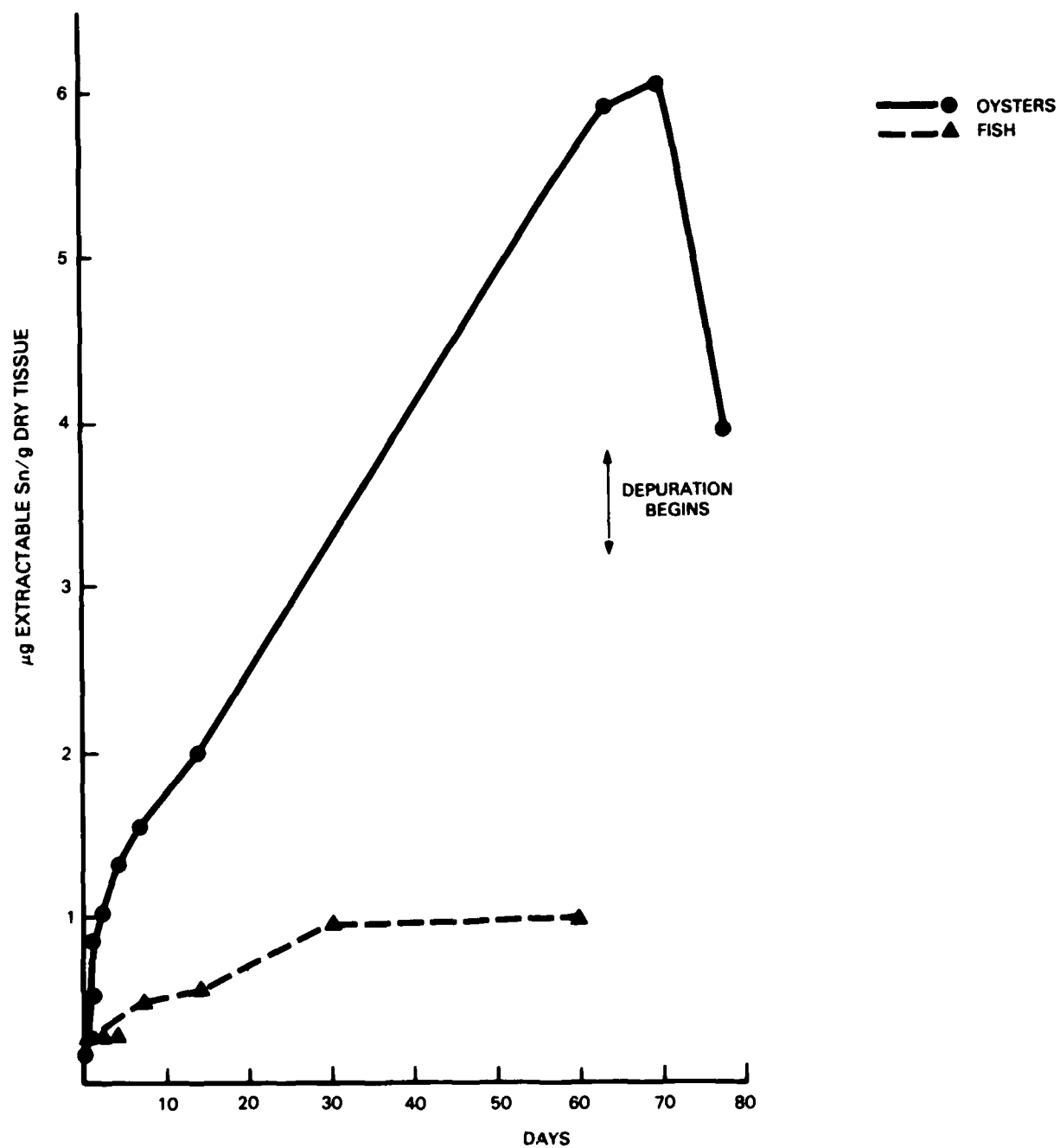


Figure 5. Bioaccumulation of organotin in oysters and fish.

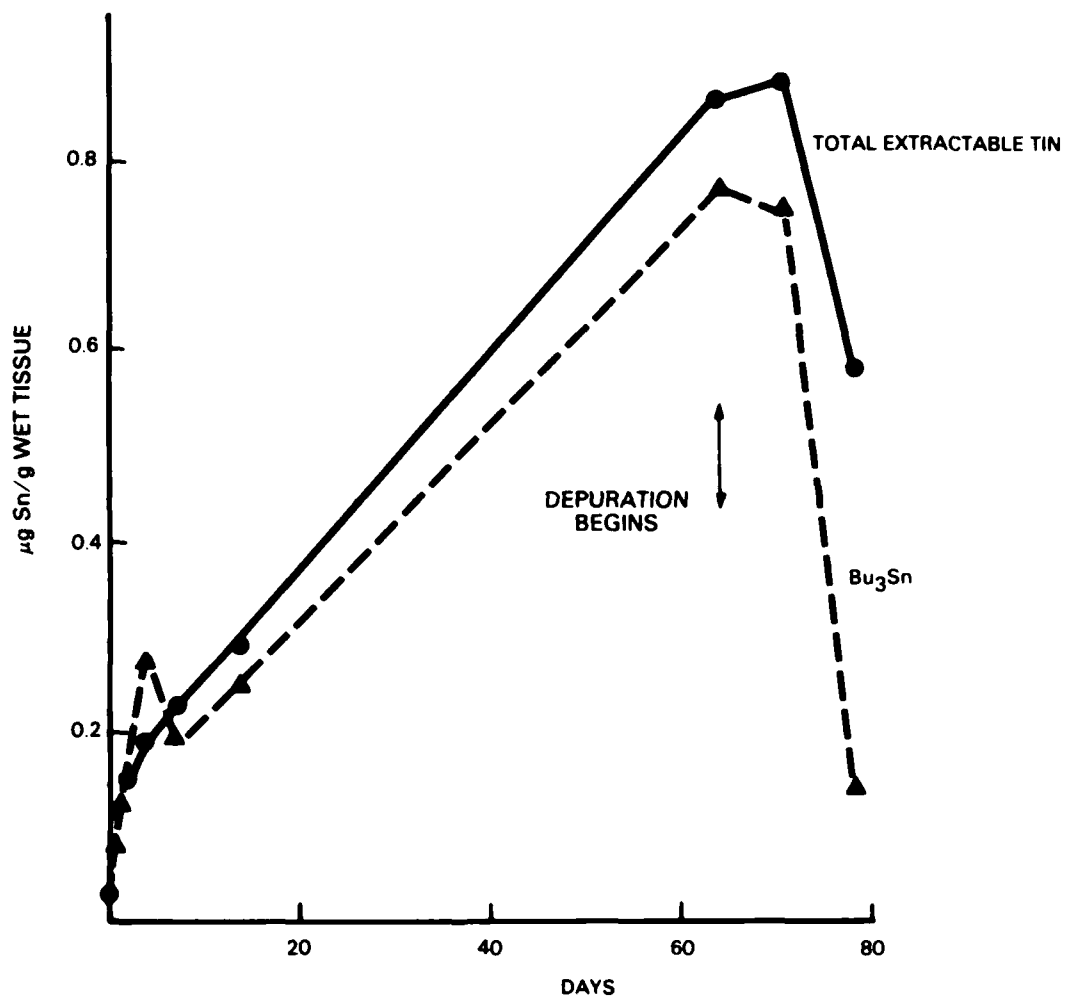


Figure 6. Accumulation of solvent extractable tin by oysters.

## Thin-layer Chromatography (TLC)

Butyltin chlorides can be easily separated on silica gel plates in about 30 minutes with a 9:1 hexane/acetic acid developing solvent. Detection can be achieved at the 5- $\mu$ g-tin level using dithizone. Rf values of ~0.51, ~0.28, ~0.01, and 0 are found for tributyltin, dibutyltin, butyltin, and inorganic tin, respectively. Reaction with dithizone produces a fast-fading yellow compound with tributyltin, an orange compound with dibutyltin and butyltin, and a pink compound with inorganic tin. Although the migration of authentic compounds was not retarded in the presence of heavy loads of tissue extract, either insufficient reacting compound was present in unspiked tissue extracts or color formation was inhibited. Nevertheless, TLC can serve as a cleanup technique for tissues prior to mass spectroscopy.

A separate batch of oyster tissue extract and fish liver (and viscera) extract was run with TLC. The butyltin compounds from the tissues were recovered by scraping bands of silica gel and extracting overnight in a large volume of hexane. The hexane was then reduced in volume under argon. The various band fractions were run with GFAAS to locate the tin-containing regions, and these fractions were derivatized with  $\text{PeMgBr}$  and run with GC/MS using the MID mode at  $m/z$  120 ( $\text{Sn}^+$ ), 177 ( $\text{BuSn}^+$ ), and 191 ( $\text{PeSn}^+$ ). Oysters showed only the recovery of  $\text{Bu}_3\text{SnPe}$  in fraction #4, corresponding to an Rf of 38 to 50 (see figure 7). The amount of  $\text{Bu}_2\text{SnPe}_2$  was low and variable. The situation with fish extract, shown in figure 8, is more complex. The greatest amount of  $\text{Bu}_3\text{SnPe}$  was found in fraction #5 (Rf 50-65). A nonpentylated butyltin also was found in that fraction.  $\text{Bu}_2\text{SnPe}_2$  and two more  $\text{BuSn}$  species, both greater in quantity, occurred maximally in fraction #4 (Rf 40-50). Fraction #3 covers Rf 25-40. Although this information is qualitative, it indicates a more active mobilization, perhaps metabolic, system in fish than in oysters.

These results plus the parallel tracking of  $\text{Bu}_3\text{Sn}$  and total extractable tin in oysters suggested that a low, continuous depuration of  $\text{Bu}_3\text{Sn}$  was taking place. Experiments with fish were temporarily abandoned because oysters are more relevant from a human standpoint because the whole tissue is consumed; whereas the greatest concentration of butyltins is found in the fish liver, which is normally discarded.

## Depuration Results

The total extractable tin is shown in figure 9 relative to the measured  $\text{Bu}_3\text{SnPe}$  and  $\text{Bu}_2\text{SnPe}_2$  for oysters during a depuration period of 4 weeks. The maximum quantity of  $\text{Bu}_3\text{Sn}$  measured at 0 days of depuration was ~1.7  $\mu\text{g}$   $\text{Bu}_3\text{Sn/g}$  wet tissue. Assuming an exposure level of ~0.4  $\mu\text{g}$   $\text{Bu}_3\text{Sn/l}$  and that equilibrium concentrations were attained, a BCF of 4500 was reached in 75 days of exposure. This can be considered a minimal value, since optimal recovery was not attempted.

The amount of  $\text{Bu}_2\text{SnPe}_2$  measured was low and variable, typically around 0.2- $\mu\text{g}$   $\text{Bu}_2\text{Sn/g}$  wet tissue. The source of this compound is not known but it may be either separately accumulated from polyvinyl chloride (PVC) pipes, for example, or derived from an acid-labile metabolite, as discussed previously.

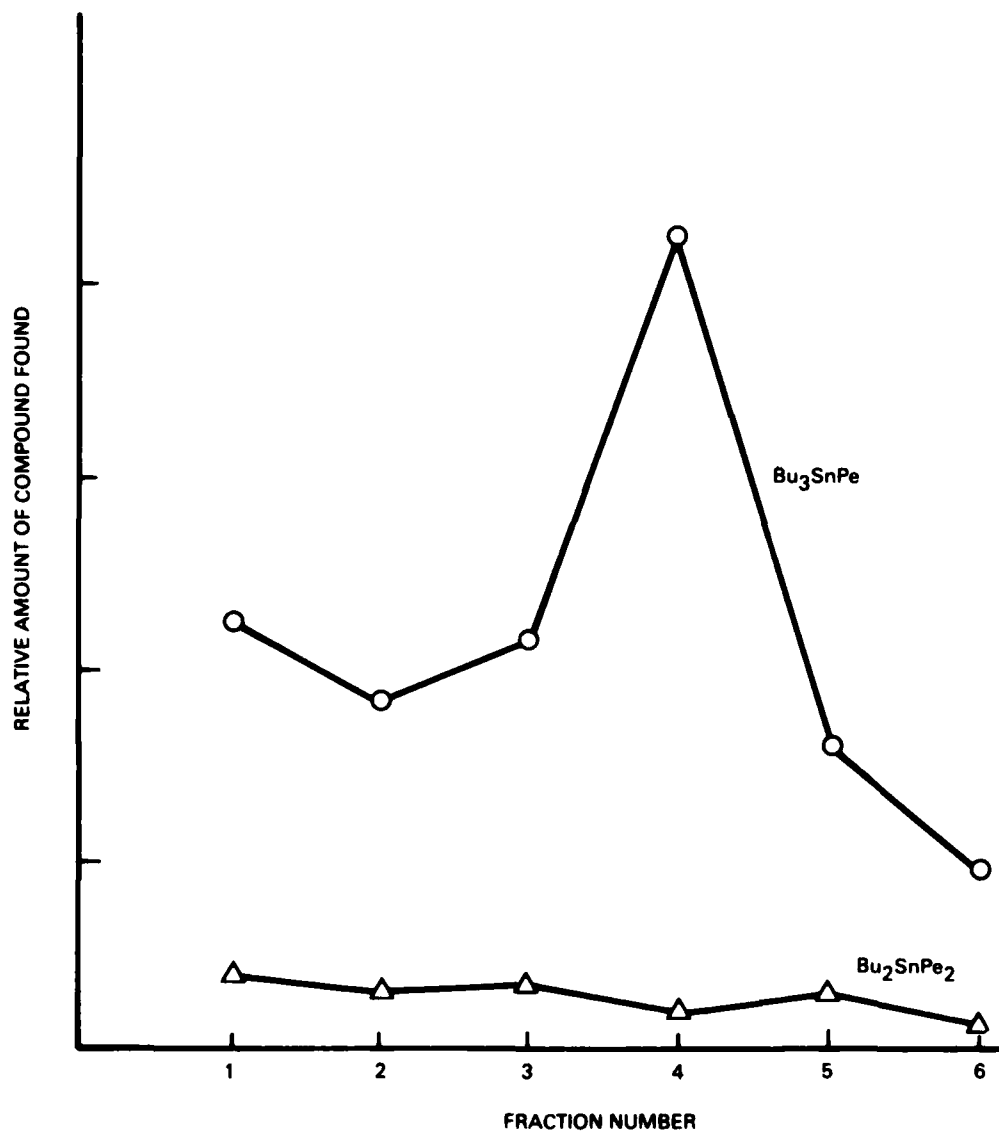


Figure 7. TLC fractions of oyster tissue extract.

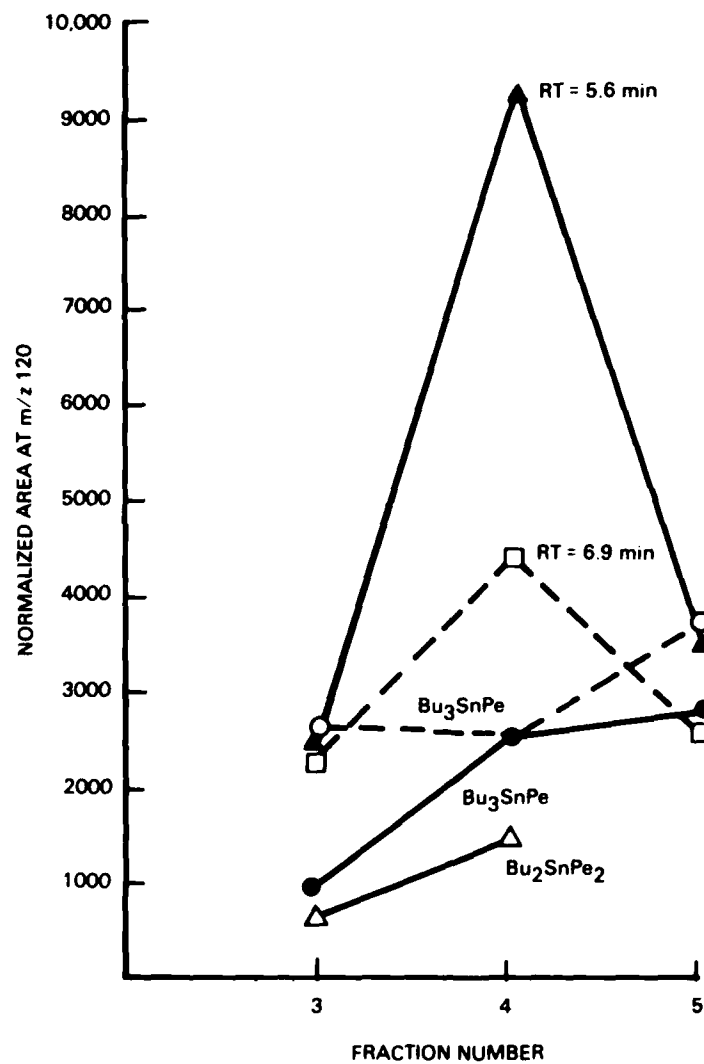


Figure 8. TLC fractions of fish liver extract.

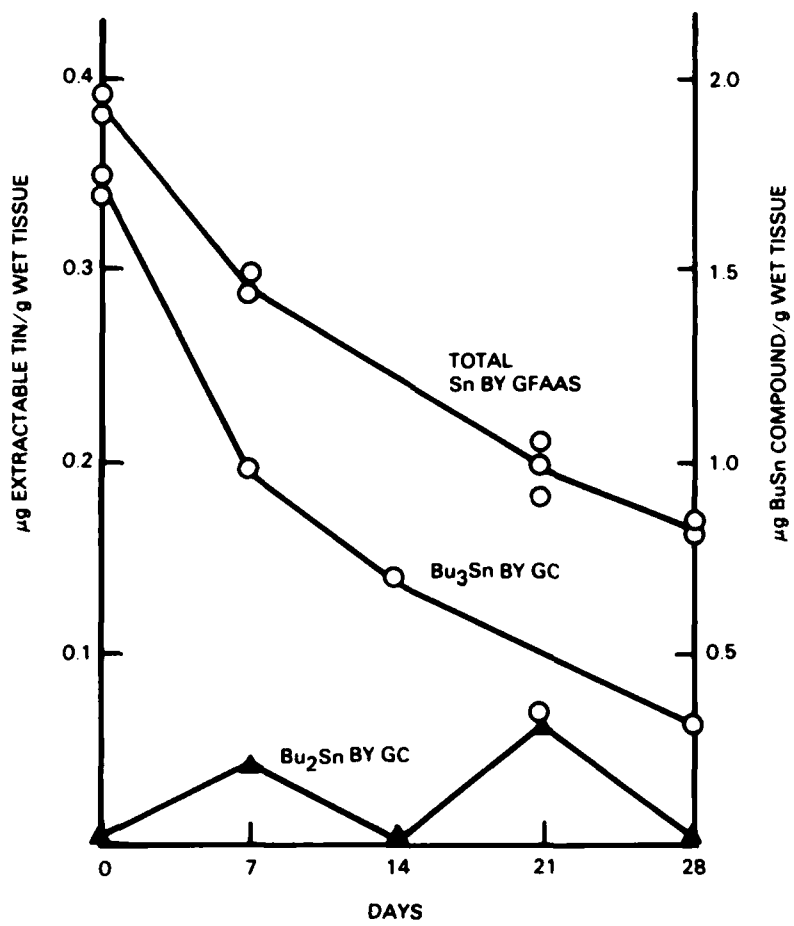


Figure 9. Depuration in oysters.

GC/MS analysis using the MID mode and a wide-bore capillary column at  $m/z$  120, 135, 177, and 191 was performed on a series of the depuration phase replicates. The results, sample weight and background corrected (shown in figure 10), are constructed from the  $m/z$  177 response using tetrabutyltin as an internal standard. Substance A has a retention time (5.7 min) and ion abundance ratios characteristic of  $Bu_3SnPe$ ; and substance B has those of  $Bu_2SnPe_2$  (7.7 min). Substance C, with a retention time of 2.4 minutes, does not pentylate and has a large  $m/z$  135 ( $MeSn^+$ ) response; Substance D, with a retention time of 4.6 minutes, pentylates and has a large  $m/z$  135 fragment. The absence of pentylation may indicate either a blocked site or nonreactive anion. The large  $m/z$  135 fragments may indicate either a methylated species or a species whose fragmentation is directed to yield a large  $m/z$  135 fragment, as discussed previously.

The important point is that  $Bu_3Sn$  steadily declines (see figure 11). If a first-order rate constant is assumed, a decay rate of ~5 percent/day can be calculated from the equation  $\ln 2 = kt$ . A half-life for  $Bu_3Sn$  in oysters of ~14 days can also be calculated. The source of the additional  $BuSn$  species is uncertain. In addition to authentic metabolites produced by the action of oyster enzymes on the  $Bu_3Sn$ , other possibilities include compounds or their degradation products that were separately accumulated (i.e., contaminants in the paint system), or compounds produced by bacteria in the oyster gut. The compounds are not present in sufficient quantity to obtain full mass spectra on them. Since no authentic compounds are available, the quantities cannot be accurately determined.

## CONCLUSIONS

Oysters and marine fish accumulate solvent extractable tin in their tissues during exposure to organotin antifoulants. Accumulation in oysters did not appear to have reached an equilibrium after 60 days of exposure; fish, on the other hand, appeared to be able to deal with tributyltin fairly efficiently. A large portion of the solvent extractable tin is recoverable as tributyltin in oysters, but not in fish. A minimum BCF for oysters was found to be ~4500; this is more difficult to determine in metabolically active fish. Fish show a whole range of  $BuSn$ -containing species in their livers and guts. However, oysters are more important from a human standpoint since the whole animal is consumed.

Depuration of tributyltin from oysters occurs at ~5 percent/day and is mainly characterized by loss of tributyltin. Variable amounts of at least three other  $BuSn$  species are also present. The source of these compounds is uncertain. Possible sources include metabolic breakdown of accumulated tributyltin by either oyster enzymes or bacteria or separately accumulated contaminants and their possible degradation products. A half-life of about 2 weeks for tributyltin in oysters was found. Fish showed a more complex range of  $BuSn$  species in their organic extracts, and relatively little of the material was found as tributyltin.

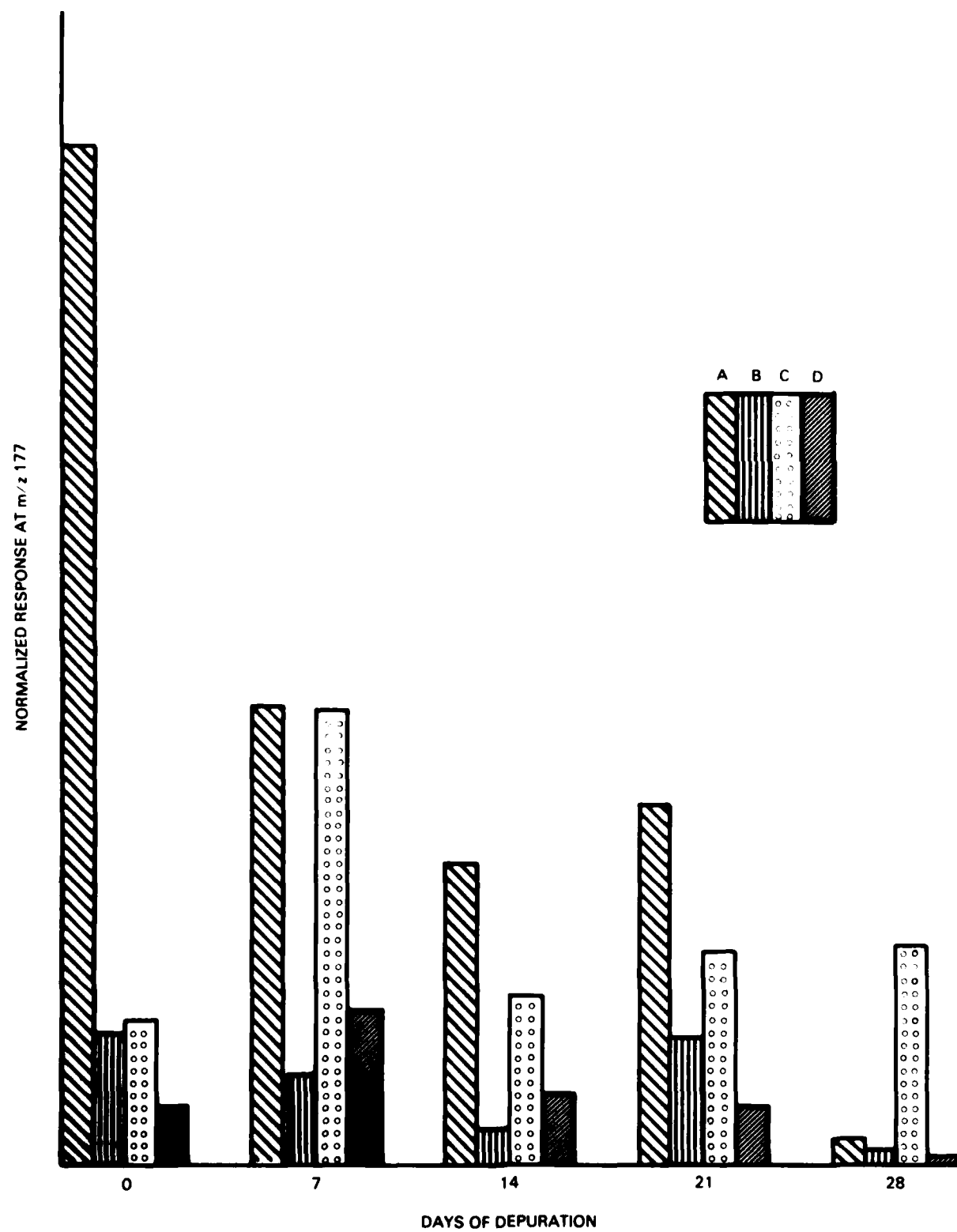


Figure 10. Butyltin compounds recovered from oyster tissues.

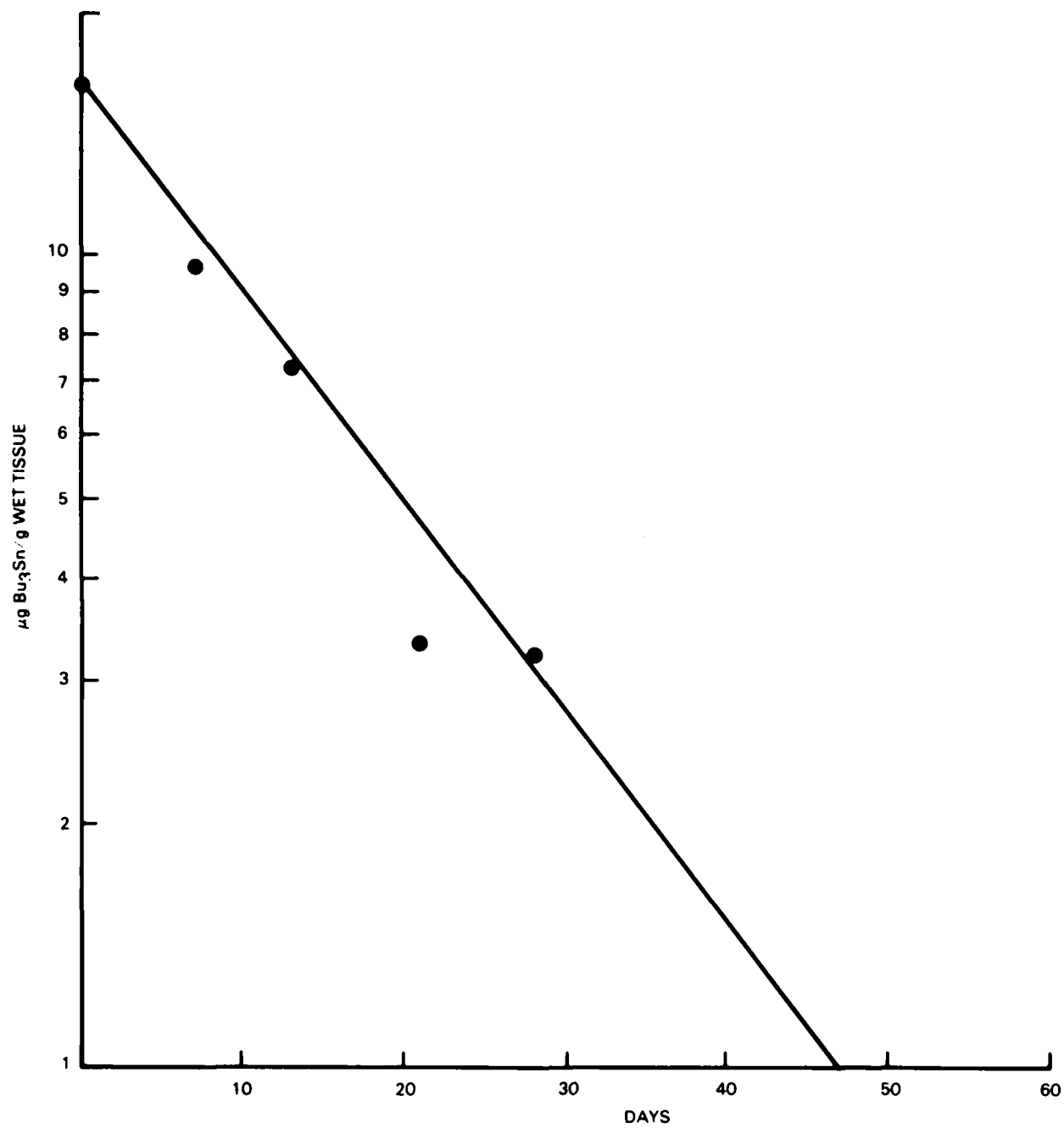


Figure 11. Depuration of  $\text{Bu}_3\text{Sn}$  from oysters.

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