RESPONSE OF BIOLUMINESCENT BACTERIA TO ALKYL Tin COMPOUNDS(U) NAVAL OCEAN SYSTEMS CENTER SAN DIEGO CA

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RESPONSE OF BIOLUMINESCENT BACTERIA TO ALKYLTIN COMPOUNDS.

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ABSTRACT

The reduction of light intensity in bioluminescent bacteria upon exposure to toxic substances can be used for rapid screening of materials. Results are often comparable to more expensive standard bioassays.

A commercially available system was used to determine the relative response of bioluminescent bacteria to a number of alkyltin compounds: R₂Sn, R₂SnX, R₂SnX₂, and R₂SnX₃, where R = alkyl group and X = halide. Within a series of compounds differing only in the number of R groups attached to the central tin atom, the most toxic compound was always the trialkyltin compound. The greatest difference in toxicity was found in the butyltin series of compounds; tributyltin was ~35 times more toxic than dibutyltin and ~750 times more toxic than (mono)butyltin. When trialkyltin compounds were compared, the toxicity to these bacteria increased with the number of carbons in the alkyl chain; the tributyltin compounds are ~150 times more toxic than trimethyltin compounds.

INTRODUCTION

The biocidal effects of alkyltin compounds at part per billion levels make these compounds ideal candidates for such uses as antifouling ship paints, fungicides, and wood preservatives. This paper compares the relative toxicities of a number of alkyltin compounds using a highly reproducible, simple and rapid bioassay using bioluminescent bacteria (Microtox®). To our knowledge there is no published information on the relative toxicity of the alkyltin compounds to these microbes.

The Microtox® system can produce a bioassay within 30 minutes and has been shown to have similar sensitivity and generally a good correlation to more time consuming and difficult bioassays. DeWart and Slooff (1) demonstrated that Microtox® sensitivity compared favorably with 20 other aquatic bioassays. Lebsack et al. (2) tested fossil fuel process water and pure phenolic compounds, which are constituents of this water, and found that Microtox® results were comparable to both static and flow-through acute fish bioassays. Qureshi et al. (3) found, in most cases, that Microtox® exhibited similar levels of sensitivity as did fish, crustacea, and other bacterial bioassays, particularly toward organic compounds and complex effluents. Likewise, Bulich et al. (4) showed good correlations between Microtox® and fish bioassays for pure compounds and complex effluents. The Microtox® bioassay is a convenient way to compare the relative toxicity of compounds and to demonstrate degradation of compounds as a decrease in toxicity (5) and a method to study synergistic and antagonistic effects of compounds (6).

Ribó and Kaiser (7) used Microtox® to calculate structure-activity correlations with physico-chemical parameters. A good correlation existed between Microtox® toxicity and the molar refractivity parameters of para-substituted phenols and also the octanol/water partition coefficients of chloro-substituents in the benzene ring of chlorobenzenes. Curtis et al. (8) demonstrated a high correlation between Microtox® toxicity and fish bioassays in semihomologous series of alcohols, ketones and ethane derivatives. Toxicity for Microtox® and fish increased as the number of carbons increased for
**METHODS AND MATERIALS**

Tri-n-butyltin chloride, tri-n-buty- 
lin bromide, triethyltin chloride, trime-
thylin chloride, dimethyltin dichloride,
 methyltin trichloride, diethyltin dichlo-
ride, di-n-butyltin dichloride, tetra-
butylin and tetraallyl tin were obtained
 from Alfa Products (Danvers, MA). Di-
 propyltin dichloride, tri-n-propyltin 
chloride, n-butylin trichloride and tri-
phenyltin chloride were obtained from 
Facts and Bauer, Inc. (Waterbury, CT).

The chemicals were used without fur-
ther purification. Tetrapentyltin was pre-
pared by reaction of SnCl₄ (Alfa Prod-
ucts, Danvers, MA) with n-pentylmag-
nesium bromide (Alfa Products, Dan-
vers, MA). Tetrabutyl-
eryl, tributyl-
eryl, and tribut-
3-ethyltin, and tetra-
butylin bromide, tribut-
3-ethyltin bromide and dibut-
3-ethyltin di-
bromide were prepared by bromina-
tion of the symmetrical tetraorganotin compound.

These compounds were purified by vacuum distillation and column chromatography on Florisil (Supelco, Inc., Bellefonte, PA).

For Microtox® testing, stock solu-
tions of the compounds were prepared in 95% ethanol at 1-2 mg/ml. Appropriate a-
mounts of the ethanol solutions were added to 2% aqueous NaCl to achieve a workable concentration while keeping the ethanol concentration as low as possible. Typ-
cally the ethanol concentration was about 0.05%. Where higher concentrations of ethanol were required owing to solubility problems with the stock solution, an ethanol blank was also prepared for the Micro-
tox® control.

The Microtox® Toxicity Analyzer Model 2055, manufactured by Microbics Corpora-
tion, Carlsbad, CA, is basically a photo-
tometer with variable temperature control

(9,10). The bioassay measures the re-
lative reduction in light output by a lum-
inescent bacterium, *Photobacterium pho-
phomaeum NRC 9-111577* when exposed to a 
toxicant. The light output is a normal 
metabolic function of these bacteria once 
rehydrated with distilled water. The 
bacteria are provided in a convenient 
freeze-dried form by Microbics Corpora-
tion and are immediately activated by the addi-
tion of 1 ml distilled water. Upon rehy-
dration, the bacterial suspension is used 
as a reagent by adding 10 ul to each 
sample. Since the bacteria are marine, 20 
NaCl is used to provide osmotic protection 
to the bacterial cells.

Serial dilutions of each compound for 
measurement are performed in the Microto-
photometer/incubator at 15 °C. Controls 
consist of triplicate 1 ml portions of 20 
NaCl and candidate toxics are prepared in 
and subsequently serially diluted in 20 
NaCl, with a final volume of 1 ml for each 
dilution. After a 5 minute period for 
temperature equilibration, 10 ul of re-
gent (rehydrated bacteria) is added to 
each of the controls and the serial dilu-
tions of the test compound. Measurements 
in the photometer are made at 5 and 15 
minutes after addition of the reagent. 
This procedure is repeated at least four 
separate times for each compound to pro-
vide four independent toxicity values.

The toxicity value is expressed as an 
EC50 concentration, which is the concen-
tration of a compound which caused a 50% 
reduction in light output. The EC50 con-
centrations were determined by graphic 
interpolation on log-log paper, plotting the 
gamma function against concentration. 
The gamma function is the ratio of the 
amount of light lost to the amount of 
light remaining. A gamma value of 1 
corresponds to a 50% reduction in light, 
or EC50. The EC50 values at 5 and 15 
minutes are reported here since these 
times are most commonly reported in the 
for 
literature when Microtox® is compared to 
fish and other bioassays. A low EC50 
value indicates high toxicity.

...
RESULTS

The EC50 values at 5 and 15 minutes for the methyl-, ethyl-, propyl- and butyltinns are shown in Figures 1, 2, 3, and 4, respectively, as a function of the number of alkyl groups (R) attached to the tin atom. In each case, the EC50 decreases and, hence, toxicity increases, from (mono)alkyl to trialkyltin. Toxicity decreases from trialkyltin to tetraalkyltin compounds. The largest increase in toxicity was found for the butyltin compounds, where tributyltin was found to be ~780 times more toxic than the monobutyltin. The greatest difference in toxicity was also found between dibutyl- and tributyltin compounds among the series tested. The dialkyl- to trialkyltin difference in toxicity increased as the number of carbons in the R groups increased.

Methyltin

Figure 1. EC50 values for methyltin trichloride, dimethyltin dichloride and trimethyltin chloride.

Figure 2. EC50 values for diethyltin dichloride and triethyltin bromide.

Figure 3. EC50 values for dipropyltin dichloride, tripropyltin chloride and tetrapropyltin.
Figure 4. EC50 values for butyltin tri-chloride, dibutyltin dichloride tributyltin chloride and tetrabutyltin.

Figures 5, 6, 7 and 8 show the EC50 at 5 and 15 minutes for mono-, di-, tri- and tetraalkyltin, respectively, as a function of the number of carbons in the alkyl chain. As seen in Figure 5, the toxicity of methyl and butyltin is approximately the same. For the dialkyltin compounds, shown in Figure 6, toxicity increased dramatically from dimethyl- to diethyltin and was approximately the same for diethyl-, dipropyl- and dibutyltin. The greatest range in toxicity is shown in Figure 7 for the trialkyltin compounds, where tributyltin was found to be ~150 times more toxic than trimethyltin. Toxicity decreased again from tributyltin to tripentyltin. The greatest toxicity was found for butyltin species in the tetraalkyltin compounds, shown in Figure 8, as well. The EC50 for tetrabutyltin could only be determined as a "greater than value" owing to solubility problems.
Trialkyltin compounds show considerable variation in species response (11,12). Maximum toxicity in a homologous series of compounds differing only in carbon chain length was shown to be trimethyltin for insects, triethyltin for mammals, and tripropyltin for gram-negative bacteria. Gram-positive bacteria, fish and fungi all show maximum response to tributyltins. The bioluminescent bacteria, Photobacterium phosphoreum, although gram-negative, showed maximum sensitivity to triethyltin compounds. The quantitative results are very similar to LC50 data measured by primary productivity in freshwater diatoms (13) and to LC50 determined by viable cell counts in gram-negative bacteria associated with fecal pollution (14). As also reported by Sieststein et al. (12), the toxicity decreased with increasing carbon chain length beyond the point of maximum toxicity.

Bioaccumulation and biochemical activity have been shown to be dependent on octanol/water partition coefficients (15,16,17). Although data on water-octanol partition coefficients and on correct values for the metal-substituent bonds to calculate the Hansch pi parameters are not available Lauhlin et al. (18) showed correlation between toxicity to mud crab larvae and the Hansch pi parameter for the carbon chain fragment. Using bioluminescent bacteria, we could show correlation only for the trialkyltins from methyl through butyl only. Pentyltin compounds, triphenyltin and the experimental compounds mentioned below showed anomalous behavior.

The Microtox® system was also used to determine the toxicities of some experimental compounds. Table 1 shows the EC50 for some symmetrical unsaturated tetraorganto compounds and their saturated analogues. Tetraallyltin shows greater toxicity than tetrapropyltin. Tetrabutenyltin with double bonds in the 1, 2 and 3 positions exhibit EC50s different from each other and generally lower than tetrabutyltin. These results are contrary to expectation, since the polarity induced by the double bond should cause a lower

![Figure 7. EC50 values for trialkyltins.](image1)

![Figure 8. EC50 values for tetraalkyltins.](image2)
partition obligatorily and result in a lower toxicity [17]. Mallyldibutyltin
palliates as BCSO intermediate between tetrabutyltin and dibutyltin, as might
be suggested.

| TABLE 1 |
|-----------------|-----------------|
| COMPARISON OF SATURATED AND UNSATURATED TRIORGANOTINS |
| Compound | 5 min BCSO (µM) | 15 min BCSO (µM) |
| Pr₂Sn | 41.10 + 5.14 | 30.14 + 4.79 |
| All₂Sn | 19.37 + 3.29 | 14.79 + 3.27 |
| All₂Bu₂Sn | 6.65 + 1.14 | 4.11 + 1.20 |
| BuSn | 4.31 + 1.70 | 2.10 + 0.95 |
| Bu(3)Sn | 0.81 + 0.10 | 0.64 + 0.04 |
| Bu(2)Sn | 4.71 + 1.50 | 2.59 + 0.68 |
| Bu(1)Sn | 1.74 + 0.38 | 0.97 + 0.11 |
| Bu = butyl, CH₃CH₂CH₂CH₂- |
| Bu(3) = but-3-etyl, CH₃CH=CHCH₂- |
| Bu(2) = but-2-etyl, CH₃CH=CHCH₂- |
| Bu(1) = but-1-etyl, CH₃CH₂CH=CH- |
| Pr = propyl, CH₃CH₂CH₂- |
| All = allyl, CH₂=CHCH₂- |

Data for tri- and diorganotins are shown in Table 2. The two tributenyltin
compounds are both more toxic than tributyltin and exhibit toxicities different
from each other. Dibutenyltin is less toxic than dibutyltin. These compounds do
show the expected trend of decreasing toxicity with double bond-induced
increasing polarity.

| TABLE 2 |
|-----------------|-----------------|
| COMPARISON OF SATURATED AND UNSATURATED TETRORGANOTINS |
| Compound | 5 min BCSO (µM) | 15 min BCSO (µM) |
| R₂SnCl | 0.06 + 0.03 | 0.02 + 0.01 |
| R₂SnBr | 0.13 + 0.01 | 0.06 + 0.03 |
| R₂((3)SnBr | 0.64 + 0.05 | 0.27 + 0.03 |
| R₂((1)SnBr | 0.82 + 0.16 | 0.44 + 0.04 |
| R₂SnCl₂ | 1.41 + 0.05 | 1.09 + 0.23 |
| R₂((3)SnBr₂ | 5.13 + 0.69 | 2.82 + 0.22 |
| R₂SnCl | 0.36 + 0.08 | 0.14 + 0.02 |
| R = butyl, CH₃CH₂CH₂CH₂- |
| R(3) = but-3-etyl, CH₂=CHCH₂CH₂- |
| R(1) = but-1-etyl, CH₃CH₂CH=CH- |
| Ph = phenyl |

The reversal in toxicity response in the tetraorganotin and triorganotin com-
ounds when unsaturation is introduced would seem to indicate that either steric
or electronic effects may be at least as important as hydrophobicity as a predictor
toxicity. Although the possible effect of having a mixture of stereoisomers pre-
sent in compounds with the double bond in the 1 or 2 position has not been taken
into account, the variation in toxicity shown with placement of the double bond
would seem to corroborate this observation. Hansen and Lea (19) noted that
while highly specific biological response does show dependence on relative hydro-
phobicity, it is difficult to analyse because specificity arises out of the additional
Bacterial luminescence and electron effects. 

Aequorin (pH 7.0). Sound molecular size to be a better predictor of toxicity than simple fragment correlations. This approach might better explain the reversal in toxicity found in unsaturated organotin compounds.

CONCLUSIONS

BCS0 data from bioluminescent bacteria bioassays correlates both qualitatively and quantitatively with most other bioassay procedures that have been used for organotin compounds. The method is rapid and relatively inexpensive. Data obtained on experimental compounds indicates that the method is valuable for assessing the relative toxicities of similar compounds.

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REFERENCES


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