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Toxicity of triorganotin compounds to the brine shrimp, *Artemia salina*

Hai Nguyen, Nwaka Ogwuru, Quyen Duong and George Eng*
Department of Chemistry and Physics, University of the District of Columbia, 4200 Connecticut Avenue, NW Washington, DC 20008, USA

The 14 triorganotin compounds that were screened against the brine shrimp, *Artemia salina*, were least effective against the first nauplii stage (24 h). This was attributed to the presence of a yolk membrane which reduced the contact between the triorganotin compounds and the organism. The data indicated that the species responsible for the toxicity is primarily the hydrated triorganotin cation. However, the anion X group may also play a minor role in the toxicity of these compounds. The observed order of activity for the triorganotins does not parallel their hydrophobicities, indicating that other factors must be involved in the toxicity mechanism.

Keywords: *Artemia salina*; aquatic toxicology; brine shrimp; triorganotins

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INTRODUCTION

Triorganotin compounds (R_3SnX) have a wide range of uses, depending on the R group that is attached to the tin atom.[1–3] The R_3SnX series of organotin compounds are known to be effective biocides. For example, trimethyltin compounds are highly toxic to insects[1–3] whereas tripropyltins are toxic to Gram-negative bacteria.[1–3] On the other hand, tributyltins are toxic to Gram-positive bacteria and fungi.[1–3] The role of the X group is not clear, as reports[4–8] cite both the insignificance and importance of this group in the toxicity of R_3SnX compounds. Increased usage of these compounds has raised their concentrations in the aquatic environment. Because of their toxic behavior, numerous studies have been conducted on various aquatic organisms. For example, extensive literature on the effects of tributyltins on aquatic organisms such as bivalves,[9–11] dogwhelk,[12,13] oyster[9,14,15] and periwinkle[16] have been reported. Although triorganotin compounds are used effectively in the control of target organisms such as barnacles, teredo worms and sea grass,[3] they may also have the ability to affect non-targeted species such as shellfish[17] in the same environment. This is because the non-targeted organisms may have similar physiological or biological systems as the target organism. Triorganotin compounds can pose hazards to the aquatic biota either by direct ingestion of the compounds by organisms or indirectly by their contact with the compounds in the water column or with resuspended sediment containing the triorganotin compound.[18] There have been numerous toxicity studies on targeted aquatic species, but few studies have been performed on aquatic organisms that occupy the lower levels of the food chain. Studies on the lower levels of the food chain would give a more complete picture of the toxicity of triorganotin compounds in the environment, since triorganotins are known to bioaccumulate in aquatic species.[19,20] The marine crustacean *Artemia salina* (brine shrimp) is a good test organism since it is found in bodies of water with a wide range of salinity (10–220 g dm^{-3})[21] and is at the low end of the food chain. Its life cycle from egg to the adult stage is completed within a few weeks. This latter feature allows the testing of the triorganotin compounds on the different developmental stages. The toxicity of several triorganotin compounds at the various nauplii stages of the brine shrimp is reported herein.

* Correspondence to: George Eng, Department of Chemistry and Physics, University of the District of Columbia, 4200 Connecticut Avenue, NW Washington, DC 20008, USA.
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Table 1 LC_{50} values (μM dm^{-3}) of triorganotin compounds after 24 h of exposure against various nauplii stages

Compound[a]	24 h	48 h	72 h	96 h
Cy_3SnOH	2.40 ± 0.49	0.31 ± 0.05	0.26 ± 0.05	0.26 ± 0.05
Cy_3SnCl	1.79 ± 0.15	0.32 ± 0.05	0.30 ± 0.01	0.42 ± 0.05
Cy_3SnBr	1.36 ± 0.31	0.53 ± 0.11	0.27 ± 0.02	0.29 ± 0.02
Cy_3SnF	1.52 ± 0.08	0.57 ± 0.05	0.75 ± 0.02	0.54 ± 0.05
Me_3SnOH	5.36 ± 0.17	3.43 ± 0.33	0.81 ± 0.22	3.13 ± 0.51
Me_3SnCl	5.87 ± 0.07	3.75 ± 1.94	3.68 ± 1.03	3.54 ± 1.17
Me_3SnBr	4.55 ± 0.86	2.01 ± 0.78	2.38 ± 0.16	1.48 ± 0.37
Bu_3SnCl	0.86 ± 0.01	0.34 ± 0.09	0.34 ± 0.01	0.34 ± 0.03
Bu_3SnOAc	2.03 ± 0.05	0.71 ± 0.01	0.52 ± 0.09	0.29 ± 0.03
Ph_3SnOH	3.98 ± 0.33	1.80 ± 0.16	1.62 ± 0.22	1.04 ± 0.05
Ph_3SnCl	2.33 ± 0.16	1.48 ± 0.02	1.30 ± 0.08	1.19 ± 0.02
$(Ph_3Sn)_2O$	1.57 ± 0.36	0.38 ± 0.08	0.32 ± 0.07	0.20 ± 0.01
Ph_3SnOAc	2.00 ± 0.10	1.47 ± 0.22	1.15 ± 0.05	0.66 ± 0.02
Ph_3SnF	4.12 ± 0.30	1.48 ± 0.19	1.52 ± 0.30	1.03 ± 0.05

[a] Cy, cyclohexyl; Me, methyl; Bu, butyl; Ph, phenyl.

EXPERIMENTAL

Hatching conditions

The brine shrimp eggs (*Artemia cysts*) were obtained from Ocean Star International, Haywood, CA, USA. The hatching medium was made by dissolving 34.7 g of synthetic sea salt (Instant Ocean, Aquarium Systems, Mentor, OH 44060, USA) in 1 dm^3 of deionized water. The resultant hatching medium was placed in a 2 dm^3 beaker to which was added 0.2 g of dried *Artemis* eggs. The beaker was then aerated with an airstone for 24 h. The hatching tank (beaker) was placed under a fluorescent lamp or in an incubator to maintain the temperature between 25 and 30 °C. Food for the brine shrimp was obtained from Ocean Star International, Haywood, CA, USA. The shrimp were fed at the 72 and 96 h developmental stages, since the yolk membrane was exhausted after approximately 72 h. The food was sprinkled over the hatching medium twice daily.

Preparation of stock triorganotin solutions

All compounds were obtained commercially and used without further purification. The concentrations of the triorganotin stock solutions ranged from 25 to 1000 mg dm^{-3}, depending on their solubility. Dimethyl sulfoxide (DMSO) or 95% ethanol was used as the solvent. The DMSO was spectrograde quality while the 95% ethanol was reagent grade. Tricyclohexyltin chloride and bromide, triphenyltin chloride, hydroxide and fluoride, bis(triphenyltin) oxide, tributyltin acetate and trimethyltin chloride and hydroxide were dissolved in dimethyl sulfoxide. The remaining compounds, tricyclohexyltin hydroxide and fluoride, triphenyltin acetate, tributyltin chloride and trimethyltin bromide, were dissolved in 95% ethanol.

Toxicity assay

The toxicity assays were performed in plastic disposable Petri dishes (100 mm $\times$ 15mm) using 20 brine shrimps for each concentration of interest. The brine shrimps were transferred employing a micropipette with a volume between 5 and 20 μl of hatching medium, depending on the size of the shrimp. Deionized water and aliquots of the triorganotin stock solution were added to each Petri dish to give the desired concentration. The total volume in the Petri dish was 20 cm^3. Analytical analysis showed that the total tin concentration remained constant during the test period. The initial-dose range was done at concentrations of 5.0×10^{-3} to 2.0 mg dm.$^{-3}$ Each concentration and control was performed in triplicate. The control consisted of brine shrimp, deionized water and the appropriate solvent. The mortality rates for the developmental (nauplii) stages (24, 48, 72 and 96 h after hatching) and the control were determined after a 24-h exposure to the test compound. A 0% mortality was observed for the control. The mean lethal concentration, LC_{50} (the dose which caused 50% mortality), values were calculated by the Reed–Muench analysis method,[22,23] which in-

Table 2 Comparison of the toxicity of R_3SnX as a function of the anionic X group

R	24h	48h	72h	96h
Cy	$Br^- > F^- > Cl^- > OH^-$	$OH^-, Cl^- > Br^- > F^-$	$OH^-, Br^- > Cl^- > F^-$	$OH^-, Br^- > Cl^- > F^-$
Me	$Br^- > OH^- > Cl^-$	$Br^- > OH^- > Cl^-$	$OH^- > Br^- > Cl^-$	$Br^- > OH^- > Cl^-$
Bu	$Cl^- > OAc^-$	$Cl^- > OAc^-$	$Cl^- > OAc^-$	$OAc^- > Cl^-$
Ph	$O^{2-} > OAc^- > Cl^- > OH^- > F^-$	$O^{2-} > OAc^-, Cl^- > OH^- > F^-$	$O^{2-} > OAc^- > Cl^- > F^- > OH^-$	$O^{2-} > OAc^- > OH^-, F^- > Cl^-$

Table 3 Comparison of the toxicity of R_3SnX as a function of the R group

X	24 h	48 h	72 h	96 h
OH^-	Cy > Ph > Me	Cy > Ph > Me	Cy > Me > Ph	Cy > Ph > Me
Cl^-	Bu > Cy > Ph > Me	Bu, Cy > Ph > Me	Cy > Bu > Ph > Me	Bu > Cy > Ph > Me
Br^-	Cy > Me	Cy > Me	Cy > Me	Cy > Me
F^-	Cy > Ph	Cy > Ph	Cy > Ph	Cy > Ph
OAc^-	Bu = Ph	Bu > Ph	Bu > Ph	Bu > Ph

volved two graphs. The first graph comprised two curves obtained by plotting the number of accumulated deaths and the number of accumulated survivors on two different vertical axes against the log concentration of the toxicant. The intersection of the two curves gave the LC_{50} value. The LC_{50} value was confirmed by a second graph, consisting of a plot of the percentage mortality against the log concentration of toxicant. The LC_{50} values obtained using either plot should be identical.

RESULTS AND DISCUSSION

The developmental (nauplii) stages of the brine shrimp were divided into four time periods based on the number of hours (24, 48, 72 and 96 h) after the shrimp eggs were hatched. The mean lethal concentrations (LC_{50}) in μM and their standard deviations for the compounds tested on the various nauplii stages of the brine shrimp are reported in Table 1. Results from Table 1 show that the triorganotin compounds were least toxic to the first nauplii stage (24 h stage). This reduced toxicity was attributed to a yolk membrane present in the first nauplii stage. This membrane serves as a food source for the brine shrimp in its first 72 h of existence. The membrane also acts as a barrier to the triorganotin compounds and prevents them from interacting with the organism. As shown in Table 1, the compounds became more effective as the yolk membrane was lost since, in general, there was a decrease in the LC_{50} values from the 24 h to the 72 h stage. The results also indicate that the trimethyltins were the least effective, with the exception of the 72 h stage for trimethyltin hydroxide. Numerous replications of the 72 h stage have resulted in similar LC_{50} values (0.81 μM dm^{-3}). At present, we cannot explain this feature. The overall trimethyltin results are in agreement with earlier studies establishing that trimethyltins were the least effective compounds within a homologous series when tested against other aquatic species such as the water flea, *Daphnia magna*,[24] and mud crab, *Rhithropanopeus harrisii*.[25]

The biocidal potency of the compounds tested was further evaluated by comparing their toxicity as a function of the X group (Table 2) and R group (Table 3) attached to the tin atom. It was expected that the degree of interaction of the cationic and anionic species as well as the hydrophobicity of the triorganotins would contribute to the overall toxicity of the triorganotin compounds. These two criteria have been cited as the dominant factors in their toxicity against the zoeae of *Rhithropanopeus harrisii*.[25]

As evident from Table 2, there appears to be a minor correlation between the X group attached to the tin atom and its toxicity. For example, triphenyltin acetate is more effective than triphenyltin chloride, while tricyclohexyltin bromide is more effective than tricyclohexyltin fluoride in all nauplii stages. This is in agreement with other studies that indicate that the X group may have some effects on the biological properties of triorganotins within a particular series.[26,27] The occasional reversibility observed for the order of toxicity as a function of the X group may be due to the low toxicity of X itself, the various sizes of the brine shrimp at a given nauplii stage and/or the fluctuations within experimental error. More likely, it is due to a combination of these factors. Thus, it appears that the overall toxicity of the triorganotin compounds is independent of the X group, since no definitive trend was observed. This phenomenon is well cited in the literature.[28] Assuming that the X group plays a minor role in the toxicity, then the species most likely to be responsible for the toxicity is the hydrated R_3Sn^+ species. This type of explanation has been employed in explaining the antifungal activities of various triphenyltin compounds.[29–32]

Examination of Table 3 indicates that, in general, the order of activity for the triorganotins against the brine shrimp is butyl > cyclohexyl > phenyl >

methyl. With the exception of trimethyltin hydroxide, the occasional reversibility may simply be attributable to fluctuations within the parameters of experimental error. The same order of toxicity was observed by Badsha *et al.*[33] when they investigated the toxicity of a series of di- and tri-organotin carboxylates of mefenamic acid and thiophene-2-acrylic acid against the brine shrimp, except that their study did not include the butyltins. The order of toxicity observed by Badsha *et al.*[33] in their study parallels the hydrophobicity of the triorganotin compounds. Hydrophobicity of compounds can be correlated to their log *P* values, where *P* is the partition coefficient of the compound between 1-octanol and water. The average log *P* values calculated for tricyclohexyltins, tributyltins, triphenyltins and trimethyltins using the ClogP program (BioByte Corporation, Claremont, CA, USA) are 5.5, 3.8, 3.3 and −0.51, respectively.

Results in the present study indicate that the hydrated triorganotin cation is the primary species responsible for the toxicity of the compounds tested. This species has been cited as being responsible for the inhibition of *Ceratocystis ulmi*.[32] The ability to form cations is dependent on the type of ligands attached to the tin atom, whereas the efficacy of the compounds is dependent on the transport of the cation through the cell's phospholipid membrane (lipid bilayer) as well as its ability to interact with the mitochondria of the cell and/or other components of the aqueous cytosol. The more hydrophobic compounds are expected to penetrate the lipid bilayer membrane easily but are also more likely to be sequestered in the lipid bilayer membrane, thus reducing their interaction with the mitochondria. Simple partition theory indicates that bioaccumulation is related to the uptake and elimination rates. Bioaccumulation results when the uptake rate is greater than the elimination rate of the compound. Greater bioaccumulation of increasingly hydrophobic organic molecules has been shown experimentally to be related to slower elimination kinetics.

Passage of compounds through the membrane depends not only on the lipophilicity of the compound, but also on molecular charge, size and solubility of the chemical. In terms of lipophilicity, extremely hydrophobic organic compounds ($\log P > 6.0$) bioaccumulate less than predicted,[34,35] but the uptake of moderately lipophilic compounds ($\log P = 3$ or 4) by aquatic species increases somewhat with the partition coefficient,[36] as was observed with the brine shrimp (Bu > Ph > Me). Large organic compounds such as the tricyclohexyltins may be further retarded by their higher molecular weight. Gobas *et al.*[37] suggested that the membrane–water partition coefficient declines in proportion to the partition coefficient of highly lipophilic solutes because of the increased energy required to force the large molecules into the structured phospholipid membrane, as was observed with the tricyclohexyltins ($\log P > 5$). Since the order of toxicity of the compounds tested does not parallel their hydrophobicities, it is clear that the tolerance of the brine shrimp to the triorganotins must depend on additional factors such as the type of anionic substituent, organic ligand, age and size of the nauplii as well as on normal partition theory.

Less hydrophobic molecules such as the trimethyltin compounds do not penetrate the phospholipid membrane as effectively as the other triorganotin compounds and as a result they exhibit a lower potency. Nichols *et al.*[38] observed that hydrophilic compounds ($\log P < 1$) partitioned poorly and had limited uptake by the organism, thus accounting for the high LC_{50} values observed for the trimethyltins.

Triorganotins are believed to bind to the mitochondria and inhibit oxidative phosphorylation.[39] They can also act as Cl^-/OH^- exchangers across the mitochondrial membrane, resulting in a disturbance of the proton gradient.[39] Although the mechanisms of toxicity of triorganotins are not well understood, the triorganotin-induced phenomena such as a decrease in mitochondrial respiration and red blood cell lysis suggest that the triorganotin toxicity is at the membrane level and may involve proteins and/or phospholipids.[39,40]

Our results suggest that the efficacy of the triorganotin compounds towards the brine shrimp is dependent on several factors, two of which are the hydrophobicity of the compounds and the ability of the triorganotins to form their respective cation. Additional factors must be considered since these two alone would make the tricyclohexyl compounds more active. Other factors may include the uptake of the compound by the organism, the transport and distribution of the compound within the organism, and the ability of the organism to metabolize the compound. However, further studies are needed to elucidate the importance of each of these factors.

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