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Tolerance of *Aedes aegypti* larvae to triorganotins

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

The effects of triorganotins on the 4th instar stage of *Aedes aegypti* larvae were evaluated. The most effective of the 15 triorganotins used in the study was tributyltin chloride with an LC_{50} value of 0.57 ± 0.07 mg dm^{-3}. The low LC_{50} values obtained indicated that this class of compounds is an effective larvicide against the larvae of this species of mosquito. The toxicity of the compounds was found to be primarily dependent on the R group attached to the tin atom. The order of activity for the triorganotins tested was Bu > Ph > Cy > Me. However, a limited order, based on two series of the compounds tested, was also observed for the anionic X group on the tin atom.

Keywords: *Aedes aegypti*; dengue fever; larvicide; mosquitoes; toxicity; triorganotins; yellow fever

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INTRODUCTION

It is well established that the toxicity of organotin compounds depends on the number of organic groups attached to the tin atom as well as on the nature of the group. The biological activities of organotins increase with the number of organic substituents bound to the tin atom, reaching a maximum activity with three organic substituents.[1,2] In addition, the nature of the organic group is important in the kind of biocidal activity that the organotin compound possesses. For example, triphenyltins are known to have a broad range of biocidal activities,[1–3] and they are therefore used to control various pests on agricultural crops.[3–5] While there is a host of information on the use of organotins as fungicides, there are few studies on organotins as larvicides.[6–9]

As tolerance is built up against current insecticides, there is a need for the development of new classes of insecticides/larvicides. For example, the *Anopheles* mosquito, the vector for the transmission of human malaria, has shown resistance to major insecticides.[10] By 1976, 42 species of *Anopheles* mosquitoes were resistant to one or more insecticides.[10] Permethrin has been used to control *Aedes aegypti* (*Ae. aegypti*) mosquitoes, the vector for the transmission of Yellow and dengue hemorrhagic fever. However, Mebrahtu *et al.*[11] reported larvae resistance to permethrin in the Couva (R) strain of *Ae. aegypti*. In view of the serious problems of insecticide resistance and recurrence of outbreaks of these infectious diseases, the development of better insecticides/larvicides to combat these mosquitoes would be of worldwide interest. The biocidal activities of triorganotins suggest that these compounds may be effective insecticides/larvicides. The results are reported here of the screening of 15 triorganotins against the 4th instar of *Aedes aegypti* larvae.

EXPERIMENTAL

Hatching of the mosquito larvae

Dried *Ae. aegypti* eggs were obtained from Mr Steve Sackett, Kenner, LA 70065, USA. A bovine liver solution was used as the nutrient source for the growing larvae. The liver stock solution was made by adding 3.0 g of bovine liver powder (ICN Biomedicals, Aurora, OH 44202, USA) to 50.0 cm^3 of deionized water. The hatching medium was made by adding 10 cm^3 of the stock liver solution to

* 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 After 24 h for various triorganotins on the 4th instar of *Ae. aegypti*

Compound	LC_{50} (mg dm^{-3})	LC_{50} (μmol dm^{-3})
Trimethyltin hydroxide	1.23 ± 0.10	6.80 ± 0.56
Trimethyltin bromide	1.54 ± 0.37	6.32 ± 1.51
Trimethyltin chloride	2.39 ± 0.20	11.90 ± 1.67
Tributyltin acetate	1.40 ± 0.20	4.01 ± 0.57
Tributyltin chloride	0.57 ± 0.07	1.75 ± 0.23
Tricyclohexyltin hydroxide	2.21 ± 0.31	5.74 ± 0.86
Tricyclohexyltin bromide	2.49 ± 0.98	5.55 ± 2.18
Tricyclohexyltin chloride	2.53 ± 0.29	6.27 ± 0.71
Tricyclohexyltin fluoride	3.35 ± 0.29	8.65 ± 0.75
Triphenyltin hydroxide	1.49 ± 0.35	4.06 ± 0.96
Triphenyltin chloride	2.53 ± 0.29	6.56 ± 0.75
Triphenyltin acetate	2.30 ± 0.76	5.79 ± 1.85
Triphenyltin fluoride	1.50 ± 0.09	4.06 ± 0.23
Bis(triphenyltin) oxide	0.84 ± 0.21	1.17 ± 0.29
Tris(*p*-tolyl)tin chloride	2.34 ± 0.67	5.47 ± 1.57

1 dm^3 of deionized water. Approximately 0.02 g of dried *Ae. aegypti* eggs were placed in the hatching medium and the container was then kept at 25–29 °C with a humidity of 80%. The eggs hatched into larvae after two days.

Preparation of the test compounds

All the organotin compounds were obtained commercially and used without further purification. The organotin stock solutions, which ranged from 25 to 1000 mg dm^{-3}, were prepared by dissolving the compound of interest in an appropriate solvent to a volume of 10 cm^3. The solvents used included dimethyl sulfoxide (DMSO) and 95% ethanol. The DMSO was spectrograde quality and 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.

Larvae toxicity studies

The toxicity studies were performed in plastic disposable Petri dishes using 20 *Ae. aegypti* 4th instar (4–5 days old) stage larvae. The *Ae. aegypti* were transferred into the Petri dishes from the hatching medium using a micro-pipetter set at 100 μl. Aliquots of deionized water and the triorganotin stock solution were then added to the Petri dish to give the desired concentration of organotin. The total volume in the Petri dish was made up to 20 cm^3 with deionized water. Analytical analysis showed that the total tin concentration remained constant during the test period. Initial dose ranges for the compounds tested were between 5.0×10^{-3} to 2.0 mg dm^{-3}. A Petri dish containing *Ae. Aegypti*, deionized water and the solvent was used as the control. Three triplicates were used for each concentration tested. The mortality rate of the mosquitoes was determined after 24 h. The mean lethal concentration of the toxicant, or LC_{50} value (the dosage which caused 50% mortality), was calculated by the Reed–Muench analysis method,[12,13] which involved 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 the second graph, which consisted 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 LC_{50} values and their standard deviations for the triorganotin compounds tested on the 4th instar larvae stage of the *Ae. aegypti* are reported in Table

Table 2 Comparison of the toxicity of R_3SnX as a function of the anionic X substituent based on the LC_{50} (mg dm^{-3}) values

R[a]	X
Ph	$O^{2-} > F^-$, $OH^- > OAc^- > Cl^-$
Cy	$Br^- > OH^- > Cl^- > F^-$
Bu	$Cl^- > OAc^-$
Me	$Br^- > OH^- > Cl^-$

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

Table 3 Comparison of the toxicity of R_3SnX as a function of the R group based on the LC_{50} (mg dm^{-3}) values

X	R
Hydroxide	Ph > Cy > Me
Fluoride	Ph > Cy
Chloride	Bu > Ph, Cy > Me
Bromide	Cy > Me
Acetate	Bu > Ph
Oxide	Ph

1. The LC_{50} values are expressed in mg dm^{-3} as well as in μM so that compounds with dissimilar anions may be compared directly. The data in the table indicate that triorganotin compounds are good larvicides against the *Ae. aegypti* mosquito, since the LC_{50} values for the triorganotins tested ranged from 0.57 mg dm^{-3} to 3.4 mg dm^{-3}. The data in Table 1 are further summarized in Tables 2 and 3. Table 2 lists the potency of the compounds as a function of the anionic X group attached to the tin atom while Table 3 gives the potency as a function of the different R groups attached to the tin atom. Using the μM values from Table 1, there appears to be a limited correlation between the X group attached to the tin atom and its toxicity. The observed order for the limited set is $Br^- > OH^- > Cl^-$ based on the trimethyltin and tricyclohexyltin compounds. However, a larger data set must be evaluated before this conclusion can be accepted. While it is generally accepted that the anionic X group on triorganotin compounds exerts little influence on their activity,[5,14,15] there have been reports in the literature where the investigators have concluded that the X group does have some effects on the biological properties of organotins within a particular series.[16,17] This observation would support our conclusion. Since a more general correlation was not obtainable, the overall data would suggest that the anionic group X plays a minor role in the activity of these compounds.

In general, the LC_{50} results from the present studies are higher than those observed in a previous study[6] in which 25 organotins were screened against the larvae of the *Ae. aegypti* mosquito using a Liverpool red-eye and a local dichlorodiphenyltrichloroethane (DDT) strain. Also, with us the trio(*p*-tolyl)tin chloride was not the most effective, although it was in the previous study.[6] These differences can be attributed to the fact that a different strain of *Ae. aegypti* mosquitoes was used. In the present study an Orlando strain was used.

Assuming that the anionic group did not play a major role in the toxicity, the species that must be responsible for the toxicity was the trioganotin cation or its hydrated species. Triorganotin cations can be obtained by the dissociation of the parent compound according to Eqn [1]. This type of dissociation for triorganotins is well documented in the literature[18–20] and has been proposed as the species responsible for the inhibition of *Ceratocystis ulmi*, the fungal agent of Dutch elm disease.[18–20] Examination of Table 3 indicates that the activity for the triorganotins against *Ae. aegypti* is Bu > Ph > Cy > Me. A similar order was observed in a structure–toxicity study of organotin compounds on algae.[21]

$$R_3SnX \rightleftharpoons R_3Sn^+ + X^- \qquad [1]$$

The most active compound against *Ae. aegypti* larvae is tributyltin chloride (TBTCl), with an LC_{50} of 0.57 mg dm^{-3}. Its activity is better or comparable to the LC_{50} values reported for the natural product, dioncophylline A, a naphthylisoquinoline alkaloid, which was tested against the 1st to the 4th larval stages of the *Anopheles stephensi* mosquito.[22] Thus, TBTCl should be considered as a potential larvicide.

In summary, our study indicated that triorganotin compounds, in general, are good candidates for controlling *Ae. aegypti* larvae, as is evident from their low LC_{50} values. The toxicity of the compounds is found to be primarily dependent on the R group attached to the tin atom, although a limited effect was observed for the anionic X group within two series of compounds. Therefore, any future work in this area should concentrate on the synthesis of triorganotins in which the X group is known to have biological activity. The hope would be that by incorporating a biologically active ligand on the triorganotin molecule, a more active compound could be attained through a synergistic effect. It may also be of interest to modify the organic portion of the molecule since Kumar Das *et*

al.[6] indicated that tris(*p*-tolyl)tin chloride was the most active of all the organotins they screened.

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