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# Comparative Study of the Acute Toxicity of a Homologous Series of Trialkyltins to Larval Shore Crabs, *Hemigrapsus nudus*, and Lobster, *Homarus americanus*

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In 1976, over 2 x $10^6$ lbs of alkyl tins, predominantly bis-(tributyltin) oxide (TBTO) were produced in the U.S., primarily for use as the active component in marine antifouling paints (ZUCKERMAN et al. 1978). It is expected that the use of antifouling paints, including formulations containing aklyltins, will increase, because of the energy saving advantages of a ship's clean hull. This paper presents preliminary information on the acute toxicity of a homologous series of alkyl tins to zoeal shore crabs, Hemigrapsus nudus, and for TBTO to lobster larvae, Homarus americanus.

## MATERIALS AND METHODS

Gravid female shore crabs, Hemigrapsus nudus, were collected intertidally from Bodega Bay, California (USA), and maintained in 20 cm finger bowls containing approximately 300 ml 35 o/oo S artificial sea water (Instant Ocean) at 15° C until the zoeae hatched. Exposure to toxicants began 2-3 days after hatching to insure that only the most robust zoeae were used.

For the crab zoeae bioassay, 30 larvae from each of the hatches used were divided into groups of 10, and reared in 8 cm finger bowls containing 50 ml 32 o/oo S artificial seawater-toxicant solution. The substances tested, source, and the initial concentrations are as follows: Tributyltin oxide (TBTO) (Biomet®, M&T Chemicals, Inc.) and tripropyltin hydroxide (TPTO)(K & K, ICN Pharmaceuticals, Inc.): 25, 50, 75, 100, 500 and 1000 ppb; Triethyltin hydroxide (TETO) and trimethyltin hydroxide (TMTO) (ICN Pharmaceuticals): 50, 75, 100, 150, 500 and 1000 ppb. TETO was synthesized from triethyltin bromide, (Alpha Products) according to INGRAM et al. (1960).

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Stock solutions were prepared in acetone so that less than 100 µl of stock would be required to make the exposure solution. A seawater control was run, along with an acetone control (90 µl acetone/l artificial seawater). The zoeae were censused every second day, for living and dead individuals, removed to clean bowls containing freshly-prepared exposure solution and given freshly-hatched Artemia nauplii as food. Temperature and photoperiod were respectively, 15° C and 12:12 L:D.

The data have been derived from two sets of experiments using larvae from 5 different females. In the first experiment, zoeae from three different females were exposed to the three highest concentrations of each alkyltin except TETO. Subsequently, a second series, employing the larvae of two additional females, and testing a lower series of all four alkyltins, including TETO, was initiated. The second series continued for 14 days, which was the time period during which all but one alkyltin-exposed larva died. The controls were in the second zoeal stage by this time.

Lobster larvae (Homarus americanus) were obtained from the Aquaculture Program at the Bodega Marine Laboratory, and reared in 32 o/oo S, 20° C artificial sea water until metamorphosis, 22 days for the controls. They were tested in groups of 5 in 12 cm finger bowls containing 80 ml of toxicant solution. Initial TBTO exposure concentrations were 0 (80 µl acetone/l artificial seawater), 1, 5, 10, 15, or 20 ppb. The larvae were censused daily, removed to clean bowls containing freshly-prepared solution, and given Artemia nauplii as food. All the larvae used in the lobster experiment were the progeny of one female.

## RESULTS

The survival for the two Hemigrapsus nudus control groups is shown in Fig. 1a. During the 14 day period, 80% of the seawater controls and 84% of the acetone-exposed zoeae survived. At this time, almost all these larvae were in the second stage.

All alkyltin concentrations tested were acutely toxic to zoeae. Fig. 1b shows the toxicity of TBTO. Zoeae in 1000 and 500 ppb did not survive 2 days. With decreasing concentrations between 100 and 25 ppb, survival time increased but most larvae were dead by the end of the eighth day of exposure. No LC 50 estimations have been calculated for these data for two

reasons. In no test did toxicity become asymptotic with time. Thus, LC 50 estimations would be of doubtful interpretive value (STANDARD METHODS 1975). Secondly, a crustacean's susceptibility to toxicants seems to be drastically increased at ecdysis, especially the first one in many brachyuran larvae (LAUGHLIN & NEFF 1979). Under the conditions used here, most control larvae molted between the 7th and 10th days. A rough estimation of the time for 50% mortality can be derived from the graphs. In both 1000 and 500 ppb, this estimation method yields a 50% survival rate of 1 day. However, casual inspection of this and the other highest alkyltin bioassays concentrations during the first day indicated that the mortality actually occurred during the second day of exposure. These times increased with decreasing TBTO levels. In 100, 75, 50 and 25 ppb, respective values were 3.4, 4.8, 5.8 and 6.2 days. It should be noted that one larva in 25 ppb did survive for the 14 day exposure period, but did not appear to have molted to the second zoeal stage.

Tripropyltin hydroxide (TPTO) toxicity is shown in Fig. 1c. Like TBTO, 1000 and 500 ppb TPTO killed all larvae in 2 days. In lower TPTO concentrations, most of the mortality occurred between the second and sixth day with a dose-dependent increase in survival time with decreasing TPTO levels. The graphically-interpolated mean survival times are 3.5 days for both 100 and 75 ppb, 4.6 days for 50 ppb and 5.1 days for 25 ppb. The longest-lived larva survived 10 days exposure to 25 ppb.

TETO was the most acutely toxic alkyltin tested (Fig. 1d). Fifty percent of the larvae in 150 ppb TETO lived 2.8 days. Values for 100 and 75 ppb were very similar, 3.2 and 3.5 days, respectively, and that for 50 ppb increased slightly to 4.8 days. Even in relatively low doses, between 50 and 150 ppb almost all larvae died during the first six days of exposure and none survived longer than 8 days. Thus, it is apparent from this and a comparison of the estimates for the survival times of 50% of the test organisms, that TETO was the most toxic alkyltin tested.

The toxicity of the homologous alkyltins increases as the carbon number of the alkyl moiety decreases between $C_4$ and $C_2$, but the trend reverses for TMTO (Fig. 1e). Even in 150 ppb, 50% of the larvae survived 3 days. In 100, 75, and 50 ppb, survival times for half of the zoeae increased to respective values of 4.4, 5.2 and 6.6 days.

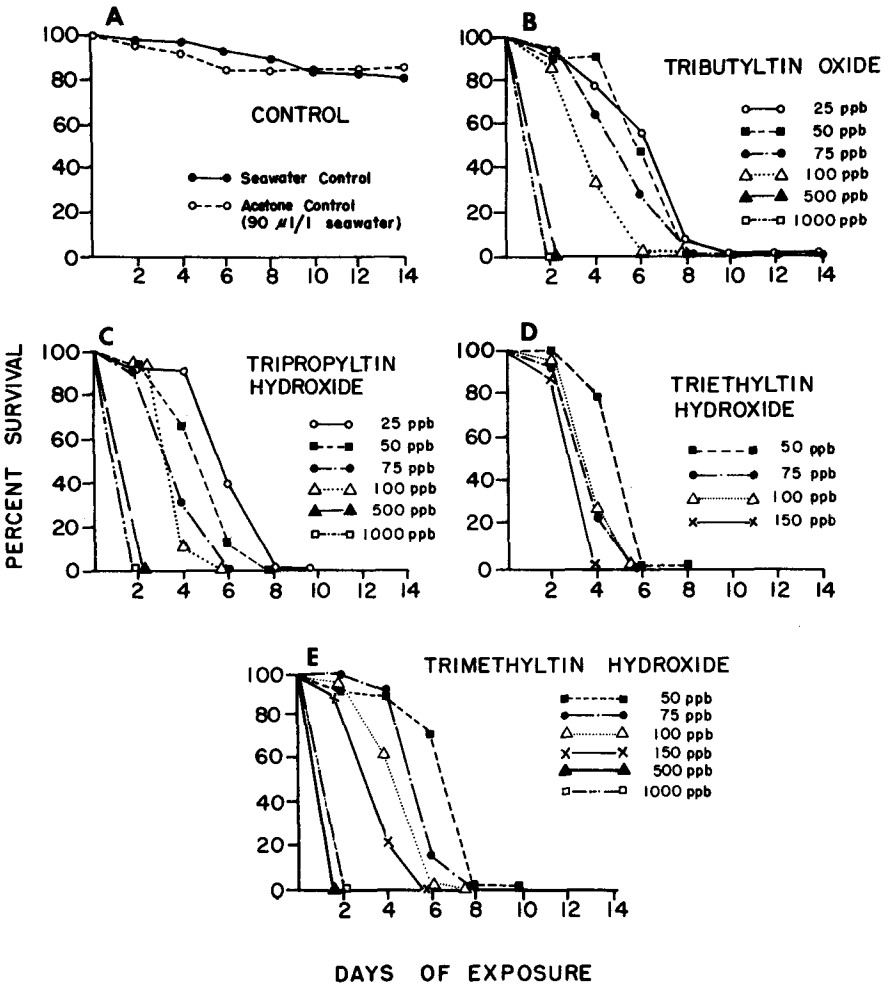


Fig. 1. Acute toxicity of trialkyltins to crab larvae, Hemigrapsus nudus. No TETO concentrations greater than 150 ppb were tested.

Lobster larvae, Homarus americanus, were much more sensitive to TBTO than Hemigrapsus nudus larvae (Fig. 2). The highest TBTO concentration tested, 20 ppb, killed all larvae in 24 hr. Concentrations between 15 and 5 ppb were acutely toxic within 6 days, with the length of survival times increasing slightly with decreasing TBTO concentrations. Survival patterns of lobster larvae in 1 ppb TBTO and the acetone control showed a typical decapod crustacean pattern. Even in the controls, there was a high mortality associated with the first ecdysis, occurring here between 3 and 5 days following hatching. Survival remained fairly high subsequently with any ensuing mortality usually associated with ecdysis of later stages. Six controls (43%), but only one TBTO-exposed larva metamorphosed successfully. For controls, the mean development time to fourth instar was 19.2 $\pm$ 1.8 days (mean $\pm$ 1 standard deviation). The one animal in 1 ppb TBTO metamorphosed on day 21, which is within the 95% confidence interval of the control group, indicating that TBTO did not significantly affect the development rate of Homarus americanus. A comparison of dry weights indicated a significant effect on growth. The controls had a mean dry weight of 8.230 $\pm$ 0.54 mg (range: 2.436 - 3.775 mg) while the one TBTO-exposed survivor weighed 1.132 mg. The lower bound of the 95% confidence interval for the controls was 2.605 mg, indicating that 1 ppb TBTO significantly decreased larval growth.

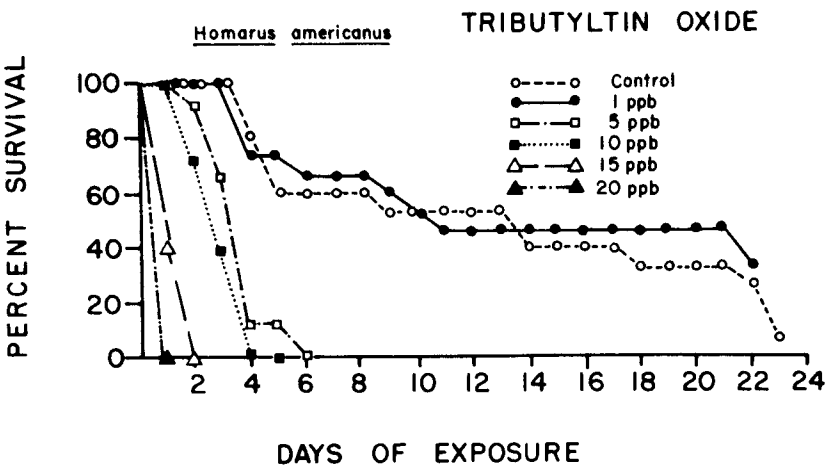


Fig. 2. Toxicity of TBTO to lobster larvae, Homarus americanus exposed throughout larval development.

## DISCUSSION

The data indicate that the trialkyl tin oxides are acutely toxic to non-target marine organisms at low ppb concentrations. Even nominal TBTO concentrations of 1 ppb killed more than 90% of developing lobster larvae when control survival was about 50% during the same period.

Invertebrates seem to be much more sensitive to trialkyl tin compounds than mammals on the basis of comparisons of environmental exposure of the former and oral dosing of the latter (ZUCKERMAN et al. 1978). In aquatic systems, trialkyl tins are used, as mentioned earlier, for biofouling control where concentrations between 0.05 and 0.1 ml/l produce acute toxicity to barnacles and other organisms in several days (ZUCKERMAN et al. 1978; EVANS & SMITH 1975). A LC(I) of 0.002 $\pm$ 0.001 mg/l TBTO has recently been reported for the copepod Nitocra spinipes (LINDEN et al. 1979). The results of tests with TBTO to determine its effectiveness as a molluscicide have shown that molluscs are about as sensitive as the crustaceans tested here (HOPF et al. 1967; FRICK & DeJIMENEZ 1964). Although the sensitivity of crab and lobster larvae is not markedly greater than that for any other tested species, the rather low concentrations at which trialkyl tins cause toxicity may make them particularly hazardous to non-target organisms because sublethal effects may occur at concentrations approaching those expected in the environment. To our knowledge, there are no published values for environmental levels of trialkyltins in marine waters. However, n-alkyl- and dialkyltin levels as high as 45 ng/l have been reported in San Diego Bay, California (HODGE et al. 1979). These substances are probably degradation products of trialkyl tins. Experiments on sublethal effects of alkyltins are in progress.

There are many published comparisons of the toxicity of structurally different trialkylated tins to diverse phyla. Mammalian toxicity, although relatively low, as noted above, peaks with ethyl substituted tins, and drops off with increasing side-chain length (ZUCKERMAN et al. 1978). Insects are most sensitive to the methyl tins while fungi, on the other hand, seem to be maximally inhibited by tributyl and tripropyl isomers (GITLITZ 1976; VAN DER KERK & LUYTEN 1954; BENNETT & ZEDLER 1966; ZEDLER & BEITER 1962; HEDGES 1960). Our data for crab larvae indicate that this species pattern of sensitivity to substituents resembles that of mammals rather than

insects to which they are more closely related phylogenetically. It should be noted that trimethyl- and triethyltin compounds are not used as biocides because they are generally considered too toxic for general dispersal (GITLITZ 1976).

Definitive statements regarding the environmental hazards of alkylated tins can only be made when concentrations in ecosystems and organisms have been accurately determined. However, further attention is warranted given the extremely low levels which are acutely toxic to non-target organisms and the uses of these compounds in the aquatic environment.

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## REFERENCES

BENNETT, R.F., and ZEDLER, R.J.: J. Oil Colour Chem. Assoc. 49, 928 (1966).

EVANS, C.J., and SMITH, P.J.: Oil Colour Chem. Assoc. 58, 160 (1975).

FRICK, L.P., and DEJIMENEZ, W.Q.: Bull. WHO 31, 429 (1964).

GITLITZ, M.H.: In: Organotin Compounds: New Chemistry and Applications. Edited by J.J. Zuckerman. American Chemical Society, Washington, D.C., p. 167 (1976).

HEDGES, E.S.: Research (London) 13, 449 (1960).

HODGE, V.F., SEIDEL, S.L., and GOLDBERG, E.D.: Anal. Chem. 51, 1256 (1979).

HOPF, H.S., DUNCAN, J., BEESLEY, J.S.S., WEBLEY, D.J., and STURROCK, R.F.: Bull. WHO 36, 955 (1967).

INGRAM, R.K., ROSENBERG, S.D., and GILMAN, H.: Chem. Rev. 60, 459 (1960)

LAUGHLIN, R.B., and NEFF, J.M.: Mar. Biol. 53, 281 (1979).

LINDEN, E., SVANBERG, O., and SUNDSTROM, G.: Chemosphere 11, 843 (1979).

PHILLIP, A.T.: Aust. Oil Colour Chem. Assoc. Proc. News, 58, 160 (1975).

Standard Methods for the Examination of Water and Wastewater. 14th ed. American Public Health Association, Washington, D.C. (1975).

VAN DER KERK, G.J.M., and LUYTEN, J.G.A.: J. Appl. Chem. 4, 314 (1954).

ZEDLER, R.J., and BEITER, C.B.: Soap & Cosmet. Chem. Spec. 38(2), 75 (1962).

ZUCKERMANN, J.J., REISDORF, R.P., ELLIS, H.V. III, and WILKINSON, R.R.: In: Organometals and Organometalloids. Occurrence and Fate in the Environnment. F.E. Brinckman and J.M. Bellama, eds. American Chemical Society, Washington, D.C., p. 388 (1978).