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Sublethal Responses of the Tadpoles of the European Frog *Rana temporaria* to Two Tributyltin Compounds

Roy Laughlin and Olof Lindén

Swedish Water and Air Pollution Research Laboratory, Baltic Sea Laboratory, Studsvik, 61182 Nyköping, Sweden

Trialkyltin compounds have been widely used as fungicides (VAN DER KERK & LUYTEN 1954), in agriculture as pesticides (GITLITZ 1976), in public health efforts to control the mollusc hosts of bilharisis (HOPT et al. 1967; DESCHIENS et al. 1966; SMITH et al. 1979), as wood preservatives (CROWE et al. 1978), and more recently, as the active components of marine antibiofouling paints (WIZGIRDA 1972; EVANS & SMITH 1975). As for a number of other compounds, there is now concern that non-target organisms may also be at risk due to environmental contamination by these compounds. (ZUCKERMAN et al. 1978; SHELDON 1975). There is very little information regarding the concentrations of trialkyltin compounds which might cause harm, or the type of damage they would cause. Previous studies have shown that even concentrations between 1 to 10 µg/L (ppb) can cause acute toxicity to some aquatic organisms (LINDÉN et al. 1979; LAUGHLIN & FRENCH 1980). In this paper, we report the effects of two compounds, tributyltin oxide (TBTO) and tributyltin fluoride (TBTF) on survival and growth of developing embryos of the common European frog, Rana temporaria.

MATERIALS AND METHODS

Rana temporaria frog eggs were collected near Tysteberga, Sweden, in late April. The post-gastrula stage of development was chosen for exposure to tributyltin. Exposure to alkyltins lasted only 5 days with all larvae hatching on the last two days of the experiment.

Two groups, each containing 5 eggs, were exposed in glass bowls to 100 mL butyltin solution. Exposure concentrations were 0.3, 3, and 30 µg/L (ppb) of either tributyltin oxide (TBTO) or tributyltin fluoride (TBTF) both from Alpha Products. These were

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prepared immediately prior to use by dissolving µL quantities of an acetone stock solution in aged tap water. There were two control series, one consisting of tapwater, the other an acetone control (1 mL/L tapwater). This concentration of acetone was the highest used to make dilutions of the stock solutions. These solutions were changed every second day.

All larvae hatched within a 2 day period. On the second day, they were collected, individual weights taken, to the nearest 0.01 mg. Subsequently, the tadpoles were dried for 2 days at 60°C and reweighed. Using these two weights, the percent body water was calculated.

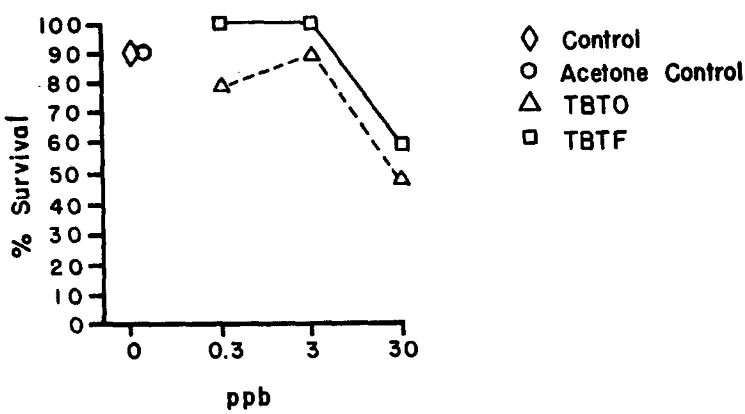

Fig. 1. Survival of frog embryos, Rana temporaria, exposed to tributyltin oxide (TBTO) or tributyltin fluoride (TBTF).

RESULTS

Survival: The survival of the controls and tadpoles in tributyltin concentration of 0.3 and 3 ppb was uniformly at least 80%. Only at trialkyltin concentrations of 30 ppb did much mortality occur: 40 and 50% for TBTO and TBTF, respectively. These data indicate that the toxicant levels were not highly toxic within the time frame of this experiment.

Wet and dry weights for the different groups are shown in Fig. 2. The pattern of the wet weights with respect to toxicant level is similar to that observed for survival. The control, 0.3 and 3 ppb groups have very similar mean wet weights, between 7 and 8 mg.

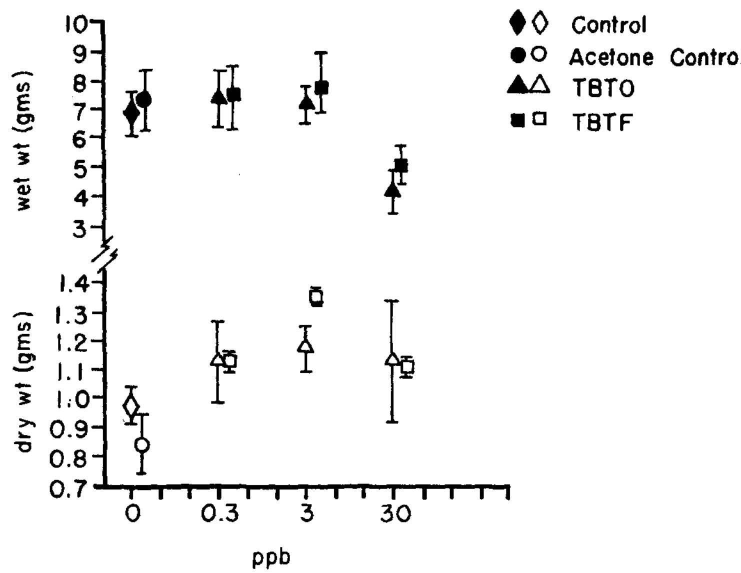

Fig. 2. Wet (closed symbols) and dry (open symbols) weights of tadpoles, Rana temporaria, which were exposed as embryos to tributyltin oxide (TBTO) or tributyltin fluoride (TBTF).

Only in 30 ppb TBTO and TBTF was a noticeable decrease in weight observed with those exposed to TBTF exhibiting a slightly lower mean size than the TBTO group. This decline for the 30 ppb dose of both compounds was significant ($F = 13.546$ $p = 0.00001$), but a comparison of the difference between the compounds showed no statistically significant effect (Contrasts used pooled error variance).

Tadpoles exposed to tributyltin compounds always exhibited higher mean dry weights than the controls (Fig. 2), although increases were not consistently dose- dependent. The control groups had mean dry weights of 0.97 mg (tap water) and 0.84 mg (acetone), while the exposure groups' were consistently above 1.1 mg. Differences due to dose were statistically significant ($F=17.530$, $p = 0.00001$).

Perhaps the most consistent response to alkyltin exposure is shown by the changes in percent body water which is in fact the ratio of wet to dry weights (Fig. 3). A consistent decline from 86 - 88.5% for the controls, mean to a low of 73.8% for that of the group exposed to 30 ppb TBTF is evident. The differences due to tributyltin dose are significant ($F=31.769$, $p = 0.00001$), but comparisons of the effect of each compound at the same dose showed no significant differences. (Contrasts used pooled error variance).

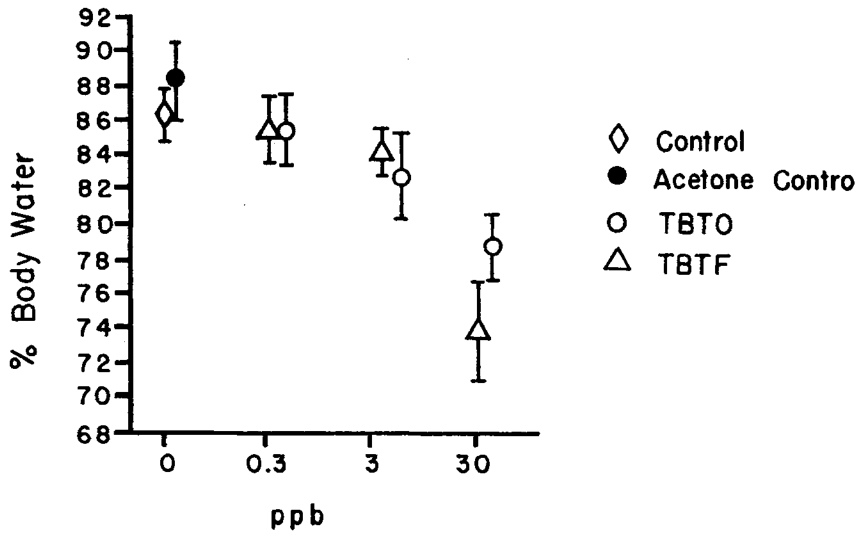

Fig. 3. Percent body water of tadpoles, Rana temporaria, which were exposed as embryos to tributyltin oxide (TBTO) or tributyltin fluoride (TBTF).

DISCUSSION

Of the several indices tested here, the least useful was the wet weight. Significant reductions occured only in 30 ppb exposure, a level which is probably far in excess of ones caused by chronic pollution sources, but not necessarily of water where newly-treated structures are present. On the other hand, the mean dry weights of tadpoles, exposed during embryonic development to butyltin compounds, were always greater than the controls'. However, the differences are not consistently dose-dependent, perhaps due to the protective nature of the jelly coating around the egg. This jelly could either bind, or allow only a limited amount of the available butyltins into the developing eggs. A suitable test of this hypothesis must await development of a sensitive method for the analysis of alkyltins in biological materials. A comparison of the percent body water with the dry weight indicates that yolk utilization was affected. Lipid constitutes a major portion of the yolk initially, but as this hydrophobic material is incorporated into the developing organism, one would expect the water content to increase with the degree of development. During the present study, we did not determine the fate of the yolk material during development, but the question deserves attention in future studies.

These tadpoles are more resistant to the tributyltin compounds tested than are crustaceans. For example, the acute toxicity of TBTO for *Nitocra spinipes* (a copepod), the American lobster, *Homarus americanus*, and larvae of the shore crab, *Hemigrapsus nudus*, were quite low; in the first two cases less than 1 ppb (LINDÉN et al. 1979, LAUGHLIN & FRENCH 1980). This pattern of butyltin toxicity to crustaceans can be extended to other alkyltin compounds. The sensitivity of terrestrial vertebrates is much less, and that of fish slightly less than that of crustaceans (ZUCKERMAN et al. 1979; SMITH, International Tin Research Institute (ITRI) Publication No. 538). The sensitivity of these tadpoles, therefore, resembles that of fish. Mode of action studies may help explain this toxicity pattern. The available data show that trialkyltin compounds are ionophones in mitochondrial membranes for negatively charged ions (ALDRICH 1976). If this occurred in membranes responsible for ionic regulation, several problems could arise, even if only very small amounts of material were present.

Conclusions regarding the actual environmental impact of trialkyltin compounds must await detailed determinations of the amounts and chemical forms in the environment. There have been a few reports of environmental levels of alkyltins (HODGE et al. 1979; BRAMAN & TOMKINS 1979) and these have reported only parts per trillion levels. However, organotin use has been widespread and environmental contamination will increase.

Our investigation of the effects has been motivated by both the the need for comparative data on the toxicity of these compounds, and by published protocols for the use of frog larvae as *in situ* monitors of pollution in freshwaters (COOKE 1981), just as mussels are being used in the marine environment. Furthermore, in some tropical and subtropical areas where rice is grown, frogs are a significant food by-product of this agricultural habitat. The use of agricultural chemicals may be counter-productive in the total scheme even if it increases the production of one of the crops (MOHANTY-HEJMADI & DUTTA 1981).

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REFERENCES

ALDRICH, W.N. : In: Organotin Compounds: New Chemistry and Applications. Edited by J.J. Zuckerman. American Chemical Society, Washington, D.C., p. 186 (1976).

BRAMAN, R.S., AND M.A. TOMPKINS : Anal. Chem. 51, 12 (1979)

COOKE, A.S. : Env. Pollut. 25A, 123 (1981).

CROWE, A.J., R. HILL and P.J. SMITH : Proc. 5th International Symposium on Controlled Release of Bioactive Materials, U.S. National Bureau of Standards, Gaithersberg, Maryland (1978).

DESCHIENS, R., H. BROTTES, AND L. MVOGO : Bull. Soc. Path. Exot. Sec. 968 (1966)

EVANS, C.J., AND P.J. SMITH : Oil Colour Chem. Assoc. 58, 160 (175)

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

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

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

LAUGHLIN, R.B., JR., AND W. FRENCH : Bull Env. Contam. Toxicol. 25, 802 (1980).

LINDÊN, E., O. SVANBERG AND G. SUNDSTROM : Chemosphere 11, 843 (1979)

MOHANTY-HEJMADI, P., AND S.K. DUTTA : Env. Pollut. 24A, 145 (1981).

SMITH, P. : Toxicological data on organotin compounds. International Tin Research Publication No. 538. 21 pp.

SMITH, P. J., A. J. CROWE, V. G. K. DAS AND J. DUNCAN: Pest. Sci. 10: 419 (1979).

VAN DER KERK, G. J. M., AND J. G. LUIJTEN : J. Appl. Chem. 4, 314 (1954).

WIZGIRDA, R. J. : Paint Varnish Prod. 25 (1972).

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