

Toxicity and genotoxicity of the fungicide triphenyltin hydroxide

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ABSTRACT

A study was made on the effect of triphenyltin hydroxide (TPTH) on the reproductive success of the snail *Biomphalaria tenagophila* and its genotoxicity in rat bone marrow cells *in vivo* and in human lymphocytes *in vitro*. TPTH significantly decreased the production of eggs per egg mass, the number of egg masses per snail and the percentage of viable embryos per egg mass in *B. tenagophila*. The frequency of inviable embryos per egg mass, however, was not affected. TPTH was also assessed through an *in vivo* clastogenicity test in rat bone marrow cells and, at higher dosages of TPTH (150 and 225 mg/kg). There was a significant induction of chromosomal aberrations. Due to its insolubility in water and in dimethyl sulfoxide, *in vitro* tests in human lymphocytes were conducted with plasma of rats treated with TPTH to determine clastogenic effects of possible TPTH metabolites. There were no such effects.

INTRODUCTION

In Brazil, triphenyltin hydroxide (TPTH) is applied to several crops, such as rice, potatoes, onions, tomatoes, cocoa, peanuts, beans and wine grapes as a fungicide. Other applications include the control of algae and freshwater snails in rice fields (Wensen *et al.*, 1991, Becker *et al.*, 1992; Andrei, 1993). The toxicity of triphenyltins is probably a result of inhibition of oxidative phosphorylation, thus acting as metabolic inhibitors (Snoei *et al.*, 1987). Genotoxicity studies of some organotin compounds have been reported using various test systems, such as the SOS chromotest, Chinese hamster ovary cells (CHO) and human lymphocytes. Organotin compounds such as butyltin, phenyltin and methyltin have shown varying degrees of genotoxicity, such as weak mutagenicity for eucaryotic systems and possible mutagenicity in procaryotic systems (Li *et al.*, 1982; Ghosh *et al.*, 1990; Hamasaki *et al.*, 1993).

Trimethyltin compounds increase the frequency of chromosomal aberrations in male Swiss albino mouse bone marrow cells, at different doses and treatment times (Ganguly, 1994). According to Yamada and Sasaki (1993), organotins can also act as co-clastogens in mammalian systems.

Freshwater gastropods can be used as indicators of water quality (Harman, 1974). According to Camey and Paulini (1964) and WHO (1980), tin-based fungicides are very toxic to mollusks.

MATERIAL AND METHODS

TPTH was obtained from HOECHST Company of Brazil, with 97.3% purity. Water solubility is 4.3 ppm at 22°C, pH 5, molecular mass 367.03, batch ZD97003.

Wistar rats test

A cytogenetic test for chromosomal aberration was performed using rat bone marrow cells. TPTH was homogenized in water with a magnetic stirrer in order

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to ensure a homogeneous suspension at concentrations of 75, 150 and 225 mg/kg, which represents 25, 50 and 75% of LD 50% (300 mg/kg), respectively. The suspensions were immediately injected via *ip* into the animals. Six animals (three males and three females) were used for each treatment group and 100 metaphase cells were analyzed for each animal. The Wistar rats came from the Central Animal Facility, Universidade de Brasília, they were fed Purina mice chow and filtered water *ad libitum*. All animals were given 0.5 ml of colchicine at 0.16%, 90 min before being killed by asphyxia with ether. Chromosomal preparations were obtained by treating the femur marrow cells with a hypotonic 0.075 M KCl solution; fixation was done in methanol-acetic acid (3:1) according to Ford and Hamerton (1956), modified. Structural chromosomal aberrations were investigated in coded slides. Cyclophosphamide (Aldrich) at 20 mg/kg body weight was used as the positive control.

Genotoxicity testing *in vitro*

TPTH was administered in rats by intraperitoneal injection in water suspension at a dose of 225 mg/kg body weight (2 x 225 mg with a 24-h interval). Homogeneity of the TPTH water suspension was maintained with a magnetic stirrer during application. Two hours after the last treatment, blood was drawn by cardiac puncture with a heparinized needle. The blood was centrifuged for 10 min at 2000 rpm, plasma was collected and 0.5 ml was added into 5 ml of RPMI 1640 lymphocyte culture medium. The *in vivo* metabolic activation/deactivation processes were studied according to the protocols developed by Natarajan *et al.* (1983). Five independent cultures of human lymphocytes were set up for each experimental *in vitro* test. Blood samples were obtained from healthy non-smoking adults not on medication (three males and two females, 22-37 years old). Each blood sample was cultivated with: 1) control, 2) plasma from untreated animals, 3) plasma from TPTH-treated rats and 4) plasma from animals treated with 20 mg/kg cyclophosphamide (positive control). The test compound (rat plasma) was added 6 h after lymphocyte stimulation (G_1 phase) and the cultures were incubated for 48 h at 37°C. One hundred metaphases were examined for structural and numerical chromosomal aberrations per treatment group. Lymphocyte cultures followed the technique of Moorhead *et al.* (1960), modified, using 80% RPMI medium (GIBCO) with 20% normal serum, phytohemagglutinin (WELCOME, 0.02 ml/ml medium), 0.01 mg/ml streptomycin (CEME) and 0.005 mg/ml penicillin (Fontoura-Wyeth SA). The mitotic

index was obtained by counting 2000 cells of each culture. Both chromosomal aberrations and mitotic index data were obtained by a blind test with coded slides.

Reproductive studies in *Biomphalaria*

Wild type *B. tenagophila* from the State of Santa Catarina (Southern Brazil) have been bred and kept in the Laboratório de Malacologia da Universidade de Brasília for more than four years. The animals, sized between 8 and 12 mm in diameter, were four to six months old. They were kept isolated in 125-ml vials. Egg masses were collected daily. A lethal concentration (LC 50%) of 0.050 mg/l was calculated according to the Spearman-Kärber method (Hamilton *et al.*, 1977), with snails treated at concentration levels of 0.01, 0.02, 0.05, 0.10, 0.20 and 0.50 mg/l of TPTH.

Experimental procedure

The snails were randomly placed in four groups of 16 specimens, in which reproductive performance was assessed through the following criteria: 1) number of egg masses, 2) viable embryos, 3) inviable embryos and 4) number of eggs per egg mass, for a period of 30 days pre-treatment and 30 days post-treatment. After the 24-h treatment at concentration levels of 0, 0.005, 0.010 and 0.050 mg/l of TPTH at room temperature (21-23°C), the snails were washed in distilled running water before being returned to their original flask, where they remained for 30 days. Pre- and post-treatment reproductive performances were statistically compared by the Wilcoxon test (GBSTAT, 1988).

RESULTS AND DISCUSSION

Dosages of 150 and 225 mg/kg TPTH significantly increased the frequency of chromosomal aberrations in rat bone marrow cells ($P = 0.0339$). Excluding chromatid and chromosomal gaps, and considering only the frequency of chromatid and chromosomal breaks, the results still showed clastogenicity (Table I).

For the tests on human lymphocytes *in vitro*, it was not possible to directly treat lymphocytes due to the high insolubility of TPTH in water, DMSO and in oil. So, TPTH was injected into rats before treating human lymphocytes with rat plasma. This allowed possible genotoxic activities of TPTH metabolites on human lymphocytes in cultures to be observed. The plasma of TPTH-treated rats had no clastogenic effects. However, plasma of cyclophosphamide-treated rats was clastogenic, showing the sensitivity of this methodology. On

Table I - Results of the experiment by analyzing the frequencies of structural and numerical chromosomal aberrations in rat bone marrow cells treated with triphenyltin hydroxide.

Dose mg/kg	G'	G''	B'	B''	% of C.A.	% of C.A. without GAPS	Polyp. %	M.I.
Control	5	0	5	0	1.67	0.83	0	2.9
75	5	1	2	0	1.33	0.33	1	2.1
150	11	0	19	4	5.67*	3.83*	2	1.7
225	12	3	12	4	5.17*	2.66*	0	2.5
CP	19	55	21	27	20.33*	13.66*	8	2.1

*P < 0.05, Wilcoxon test; C.A. = chromosomal aberrations; G' and G'' = chromatid and isochromatid gap, respectively; B' and B'' = chromatid and isochromatid break, respectively; CP = cyclophosphamide, MI = mitotic index.

Table II - Structural and numerical chromosomal aberrations in human lymphocytes treated *in vitro* with rat plasma. The rats were given 225 mg/kg triphenyltin hydroxide.

Samples	Cells	G'	G''	B'	B''	% of C.A.	Aneupl.	Polyp.	MI %
Control Untreated rat plasma	600	5	1	3	0	1.5	5	3	6.12
TPTH-treated rat plasma	520	6	1	10	2	3.6	2	3	2.75*
CP-treated rat plasma	468	4	1	8	1	2.9	4	2	1.88*
CP-treated rat plasma	560	8	11	13	15	8.3*	2	1	2.18*

*P < 0.05, Wilcoxon test. Abbreviations as in Table I.

the other hand, a significant decrease in the lymphocyte mitotic index was observed indicating a possible cytotoxic effect (Table II). As we had insufficient information on the rate and pathway of metabolism of TPTH under these conditions, a treatment of 2 x 225 mg/kg rat body weight in 48 h was chosen to avoid excessive cytotoxicity. Darroudi and Natarajan (1985) also used an *in vivo* activation system to assess a possible genotoxic activity of several chemical agents. TPTH was found to be clastogenic in rat bone marrow cells *in vivo*, but not in human lymphocytes after rat metabolism (Tables I and II), which probably means an *in vivo* deactivation process. The mitotic index values of plasma-treated cultures were significantly lower than those found in the control group. This reveals some antiproliferative activity of plasma *per se*, while plasma of TPTH-treated rats was not significantly different from rat plasma control (P > 0.05). Therefore, the observed effect was due to some component of rat plasma and probably not to metabolites of TPTH.

TPTH interfered in the reproductive success of *B. tenagophila*. It did not affect the production of inviable embryos (Figure 1). However 0.005, 0.010 and 0.050 mg/l had a significant effect on number of egg masses and eggs per egg mass. In the dose-response study, 0.005, 0.010 and 0.050 mg/l affected the number of egg masses, eggs and at the higher

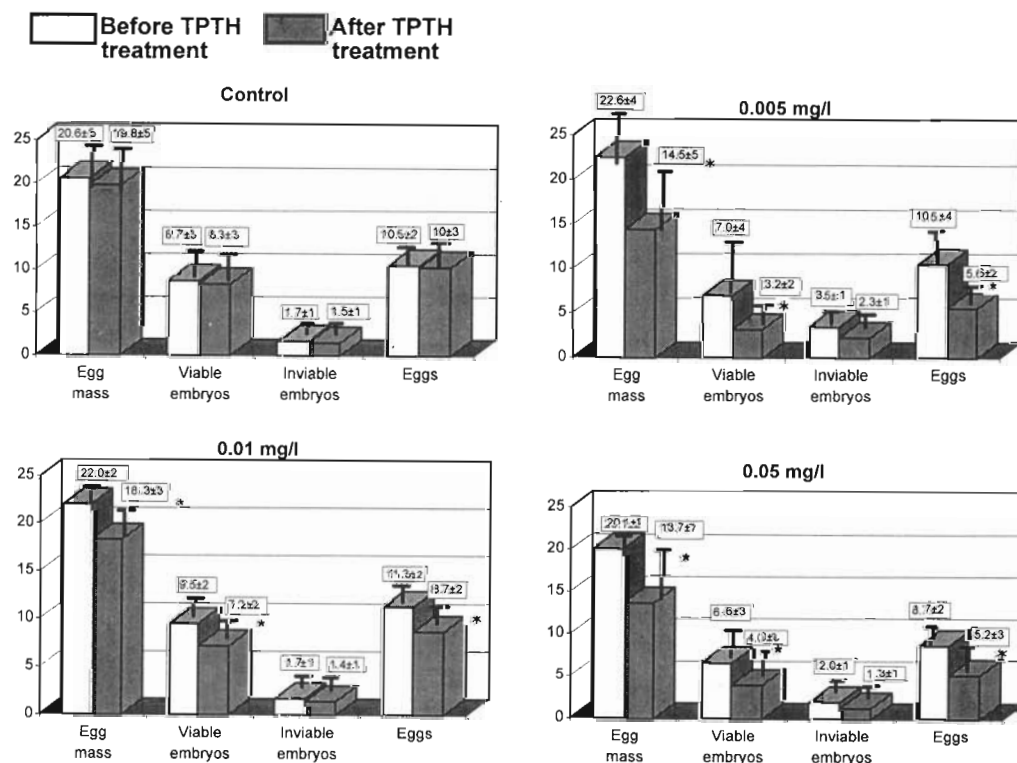


Figure 1 - The effect of triphenyltin hydroxide (TPTH) at different concentrations on *Biomphalaria tenagophila*. N = 16, per group treatment. *P < 0.05, Wilcoxon test.

dosage (0.05 mg/l, LC 50%) some snails died in the post-treatment period. Obviously, at the LC 50% dosage a decrease in fertility is expected. However, even at the lower dosage of 0.005 mg/l, deleterious effects of TPTH on the reproductive success of *B. tenagophila* were observed.

TPTH may act as a clastogen in rat bone marrow cells. After an *in vivo* metabolization process in mammals, it probably became non-genotoxic. It was found to be highly toxic to snails, especially mature animals, embryos and eggs. We found a strong molluscicidal effect of TPTH under our test conditions, as animals died at a concentration of 0.05 ppm after a 24-h exposure to the active ingredient.

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RESUMO

Os compostos organo-estânicos apresentam efeitos clastogênicos em diferentes sistemas biológicos; além disso, são muito tóxicos para organismos aquáticos, especialmente moluscos. O trifetil hidróxido de estanho (TPTH) foi avaliado quanto a sua toxidez para *Biomphalaria tenagophila*, através de ensaios sobre sua performance reprodutiva. Os resultados demonstraram que, mesmo em doses extremamente baixas como 0,005, 0,01 e 0,05 ppm, o TPTH afetou drasticamente as frequências de desovas, o número de embriões viáveis por desova e o número de ovos por desova. Nos ensaios de genotoxicidade, o TPTH apresentou efeitos clastogênicos sobre as células de medula óssea de ratos Wistar nas doses de 150 e 225 mg/kg peso. No teste de mutagenicidade indireta, o soro de ratos tratados com o TPTH não induziu aberrações cromossômicas em linfócitos humanos *in vitro*.

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