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Toxicity Assessment of Bis(tri-n-butyltin) oxide (TBTO) Using Yeast *Saccharomyces cerevisiae* and Human KB Cells

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Bis(tri-n-butyltin) oxide (TBTO) is used as a biocide in marine antifouling paints. TBTO have been detected in sea water (harbors, marinas, river) (Cleary and Stebbing 1985) and in fish and shellfish (Takeuchi *et al.* 1987).

The toxic effects of TBTO was noted in rats (Klimmer 1969).
We report here the toxicity of TBTO for *Saccharomyces cerevisiae* and human KB cells.

MATERIALS AND METHODS

Bis(tri-n-butyltin) oxide (TBTO) was obtained from Wako Pure Chemical Industries, Ltd., Japan. The TBTO was dissolved in acetone and diluted in Eagle's minimum essential medium immediately before use (final acetone concentrations was under 0.5%).

Saccharomyces cerevisiae (IFO 2260) was obtained from the Institute for Fermation, Osaka (IFO), Japan and was used throughout. This strain was cultivated in yeast extract - malt extract (YM) medium (3 g yeast extract, 3 g malt extract, 5 g peptone and 10 g glucose in 1 liter of distilled water) at 25°C.
The inhibition of growth of the yeast was estimated in YM liquid culture media. After 48-h of incubation, the cells were suspended 1 X 10^5 cells per 1 mL of YM medium containing serial dilutions of compound and 5 mL volumes of this YM medium were added to the test tubes (18 X 150 mm). Cultures were incubated at 25°C on a shaking incubator. After 72-h of additional incubation, the viable cells (numbers determined by methylene blue exclusion test) were determined using a Bürker-Türk cell counter.

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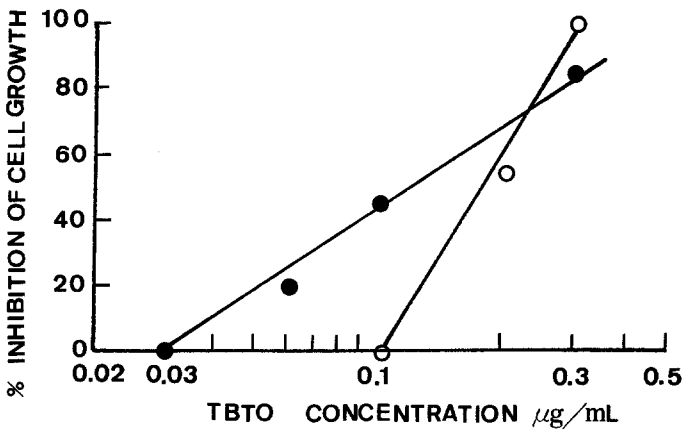

Figure 1. Dose-response curves obtained using *Saccharomyces cerevisiae* (O) and human KB cells (●) to various concentrations of TBTO.

Table 1. ID50 values for TBTO.

chemical	72-h ID50 (μg/mL)[a]	
	Saccharomyces cerevisiae	KB cells
TBTO	0.18	0.12

a 72-h ID50 : 50% inhibitory dose to growth of KB cells and *Saccharomyces cerevisiae* after 72-h of incubation.

The inhibition of cell growth was determined by comparing the total number of viable cells in TBTO-treated cultures with the total number of viable cells in cultures that had been treated only with acetone (control). The resultant inhibition, as related to the TBTO concentration, was then plotted on log-probit paper. The dose-response curve obtained when the TBTO caused a fifty per cent inhibition of cell growth (ID50) was determined.

The KB cells were used throughout this work (Mochida *et al.* 1983). Methods for toxicity testing were as described (Mochida *et al.* 1983). The ID50 values was used as an index of the toxicity of the TBTO.

RESULTS AND DISCUSSION

Figure 1 shows dose-response curves obtained with TBTO for *Saccharomyces cerevisiae* and human KB cells. Cells exposed to TBTO for 72-h showed a decrease in growth, as compared to controls and dose dependently.

Table 1 shows the ID50 values obtained with the TBTO compound. Our findings suggest no remarksble difference in sensitivity to this TBTO compound since all had the same ID50 values for *Saccharomyces cerevisiae* and KB cells.

Liu and Tomson (1986) reported a toxicity test based on the inhibition of enzyme activity of bacteria *Bacillus cerus*. TBTO causing 50% inhibition of dehydrogenase activity was 3.2 μg/mL. Thus, the *Saccharomyces cerevisiae* test (ID50 values : 0.12 μg/mL) used in our study are more sensitive to TBTO than is the bacterium assay.

Studies on TBTO revealed that the 6 day-IC50 value (50% cloning efficiency inhibition concentration) to BHK-21 cells was 0.3 μg/mL (Reinhardt *et al.* 1982) that is more than the ID50 value of TBTO to KB cells (0.13 μg/mL). Hence TBTO is more toxic to KB cells than to BHK-21 cells.

Walsh *et al.* (1985) described the toxicity of TBTO on marine diatoms. The 72h-EC50 values (calculated concentrations that would inhibit growth of diatoms by 50%) were 0.33 $\mu g\ 1^{-1}$ (*Skeletonema costatum*) and 1.03 $\mu g\ 1^{-1}$ (*Thalassiosira pseudonana*). These results show the different of various species to TBTO.

These findings should aid in the development of toxicity tests using yeast and cultured human cells.

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