

Effect of Diphenyl, o-Phenylphenol and 2-(4-Thiazoyl) benzimidazole on Growth of *Tetrahymena pyriformis*

Kimihito Otsuka,¹ Hiroshi Yoshikawa,² Akira Sugitani,¹ and Makoto Kawai¹

¹Gifu Prefectural Institute of Public Health, 6-3, Noishiki 4 Chome, Gifu, Japan
and ²Gifu University School of Medicine, Tukasamachi 40, Gifu, Japan

Diphenyl (DP), o-phenylphenol (OPP), 2-(4-thiazoyl) benzimidazole (TBZ), are used as fungicides for citrus fruits. The acute toxicity of these compounds has been established for whole animals (DEICHMAN et al. 1947, HODGE et al. 1952, ROBINSON et al. 1965). However, additional information as to the cytotoxic effect of these fungicides is required.

The ciliated protozoan, *Tetrahymena pyriformis*, is a single cell organism (constituting homogeneous culture) easily grown in an axenic medium, and has nutritional requirements similar to those of higher animals (HILL 1972). Therefore, *T. pyriformis* has been used as a tool for the determination of the toxicity of food colors (YONEYAMA and NAGATA 1976) and heavy metals (HUKUDA et al. 1981; HOUBA et al. 1981). We now report the toxicity of DP, OPP and TBZ using *T. pyriformis* as a model test system.

MATERIALS AND METHODS

Tetrahymena pyriformis strain W was grown without shaking at 28°C in 100 ml erlenmeyer flasks with 20 ml autoclaved medium containing 2 % proteose-peptone (Difco Laboratories, Detroit, Mich.) for 96 h. Cell growth of *T. pyriformis* was measured by counting the numbers of cells with a Burkert-Turk counter.

The following fungicides were used: diphenyl ($C_6H_5C_6H_5$; Wako Pure Chemical Ind. Co., Osaka, Japan), o-phenylphenol ($C_6H_5C_6H_4OH$) and 2-(4-thiazoyl) benzimidazole ($C_{10}H_7N_3S$; Tokyo Chemical Ind. Co., Tokyo, Japan). These compounds were dissolved in

Send reprint requests K.Otsuka at the above address.

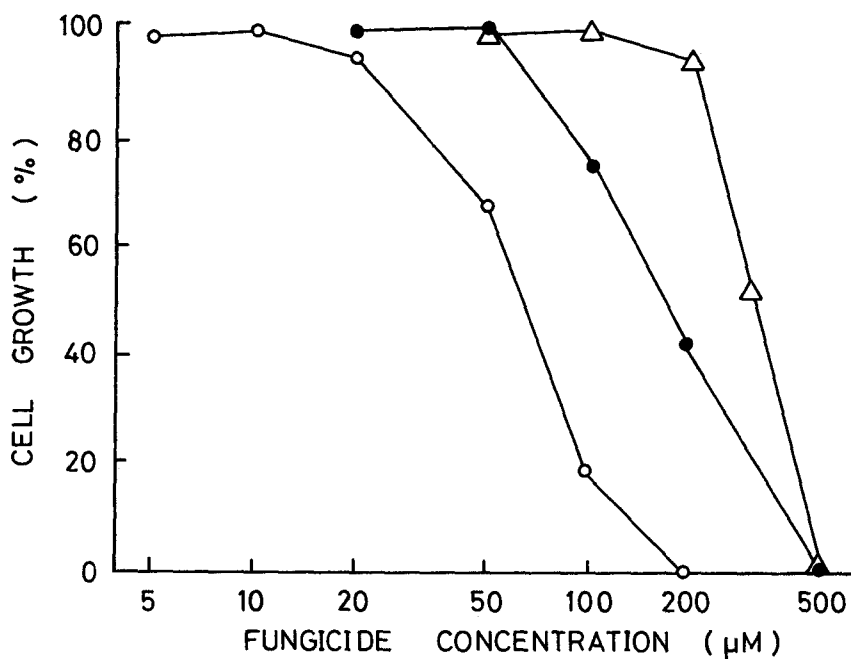


Figure 1. Dose-response curves obtained after 96 h exposure of *Tetrahymena pyriformis* cells in culture to various concentrations of fungicides. The compounds were DP(), OPP() and TBZ().

dimethyl sulphoxide (DMSO). DMSO decreased the cell number by 1 % but had no apparent effect on cell growth at 0.5 %. Based on these results, DMSO which was used as the carrier for fungicides, was added not so as to exceed 0.5 % as the final concentration.

An equivalent amount of DMSO was added to control flasks. The cells were inoculated at a concentration of 1×10^4 per ml and incubated at 28°C for 96 h. Aliquots of the cultures were diluted in water and one or two drops of 3.75 % formaldehyde solution were added to stop the behavior of cells. The inhibition of cell growth induced by fungicides, was determined by comparing the cells in cultures treated with fungicides with the total cell number in cultures that had been treated with DMSO (control). The resultant inhibition, as related to the fungicide concentration, was then plotted on log-prohibit paper. The fungicide concentration reducing a 50 % of cell growth (ID_{50}) was obtained from the dose-response curve. Each point on the resulting curves represents the average of three replicates.

RESULTS AND DISCUSSION

Dose-response curves obtained with DP, OPP and TBZ for T.pyriformis cells are shown in Figure 1. The growth cell number of T.pyriformis decreased in proportion to the amount of fungicides increased. The ID₅₀ values for the fungicides are presented in Table 1. Of the three fungicides, OPP was the most toxic followed by TBZ and DP.

Table 1. Inhibitory effects of fungicides on growth of cultured Tetrahymena pyriformis cells.

compounds	ID ₅₀	(μ M) ¹
o-phenylphenol	64	
2-(4-thiazoyl)benzimidazole	176	
diphenyl	300	

¹ Concentration of fungicide in growth medium that caused a 50 % reduction in viable cell number after 96 h of incubation.

When toxicities of OPP, TBZ and DP were estimated by oral LD₅₀ to rats (Hodge et al. 1952; Robinson et al. 1965; Deichman et al. 1947), OPP was the most toxic followed by TBZ, DP. Our results were in agreement with the results to rats in relation to the order of toxicity of these fungicides. These findings suggest that the ID₅₀ values in T.pyriformis should be used as an index of the toxicity of fungicides. Therefore, T.pyriformis was seemed to be useful to assess the potential hazard of compounds to higher animals and to study those effects at the cellular level. These data should serve as a basis for developement of toxicity tests using T.pyriformis.

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