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Effects of Diphenyl, o-Phenylphenol and 2-(4'-Thiazolyl)benzimidazole on Growth of Cultured Mammalian Cells

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Diphenyl (DP), o-Phenylphenol (OPP), 2-(4'-Thiazolyl)benzimidazole (TBZ), are among the pesticides used as fungicides for lemons, oranges, grapefruit and bananas.

The acute toxicity of these compounds has been established for whole animals (DEICHMANN 1947, HODGE et al 1952, ROBINSON et al 1965). However, additional information is required as to the toxic effect on individual mammalian cells.
Tissue culture as a tool for the determination of toxicity of pesticidal chemicals was first reported by LEWIS and RICHARDS (1945) who used the chick embryo. Studies of various pesticides have been reported by GABLIKS and FRIEDMAN (1965) who used both HeLa cells and Chang liver cells, CHUNG et al (1967) who used HeLa cells, and LITTERST et al (1969, 1971) who used HeLa cells and human skin fibroblasts.
We now report the effects of DP, OPP and TBZ on mammalian cells grown in culture systems.

MATERIALS AND METHODS

The human established cell line KB (human oral carcinoma) was provided by Dr. G. Kimura (Kyushu University). AGMK (African green monkey kidney) was purchused from Flow Laboratories, Inc., Rockville, U.S.A.

KB and AGMK cells were cultured in Eagle's minimal essential medium (MEM ; Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10 % newborn calf serum (Flow Laboratories, Inc., Stanmore, Australia), L-glutamine (2.92 µg/ml), penicillin G (100 U/ml), kanamycin (60 µg/ml), and streptomycin sulfate (100 µg/ml). All cells were cultured under the same atmospheric conditions in a humidified 5 % CO_2-95 % air incubator at 37°C, using 50 mm plastic Petri dishes (Nunc, Roskilde, Denmark).

The following fungicides were used:diphenyl ($C_6H_5C_6H_5$; Wako Pure Chemical Ind. Co., Ltd., Osaka, Japan), o-phenylphenol ($C_6H_5C_6H_4OH$) and

2-(4'-Thiazolyl)benzimidazole ($C_{10}H_7N_3S$; Tokyo Chemical Ind. Co., Ltd., Tokyo, Japan). These compounds were first dissolved in dimethyl sulphoxide (DMSO) and then diluted in MEM medium. When fungicides are insoluble in water, an appropriate solvent is required and it must be one that in itself dose not affect cell growth or alter the properties of the solute. Low doses of DMSO tested in vivo and in vitro have little or no biological effect (BROWN 1963, KLIGMAN 1965, HELLMAN and MARTIN 1967). DMSO produced some decrease in the total viable cell number at 1 % but had no apparent effect on growth of the cultured cells, in a dose of 0.5 %. Based on these results, DMSO was selected as the carrier for all fungicides used in later experiments.

To determine the effects of fungicides on cell growth, 0.5 X 10^6 cells in 5 ml of MEM medium were seeded in 50 mm new plastic Petri dishes (Nunc, Roskilde, Denmark). After 24 h of incubation, the medium was replaced with 5 ml of MEM containing serial dilutions of the test compounds and which had been added to the MEM medium in an DMSO solution in such a manner that the concentration of the solvent did not exceed 0.5 %. After 72 h of additional incubation of the viable cells (numbers determined by nigrosin exclusion method), the cells were harvested from Petri dishes by trypsinization, and the viable number was determined using a Bürker-Türk cell counter.
Fungicide-induced inhibition of cell growth was then determined by comparing the total number of viable cells in fungicide-treated cultures with the total viable cell number in cultures that been treated with only DMSO (control). The resultant inhibition, as related to the fungicide concentration, was then plotted on log-probit paper. The dosage-response curve obtained when the fungicide concentration caused a fifty per cent inhibition of cell growth (ID50) was determined. Each point on the resulting curves repesents the average of three replicates.

RESULTS AND DISCUSSION

Dosage-response curves obtained with DP, OPP and TBZ for both cells types are shown in Figure 1. The growth of KB and AGMK cells was not inhibited with these compounds during 24 h, but after 72 h of incubation, KB cells showed the same dose dependent inhibition of growth as the AGMK cells.

The ID50 values for the fungicides are presented in Table 1. DP was more toxic than either OPP or TBZ, to both types of cells. The results obtained revealed no remarkable difference in sensitivity to these three compounds since all had the same ID50 values for AGMK

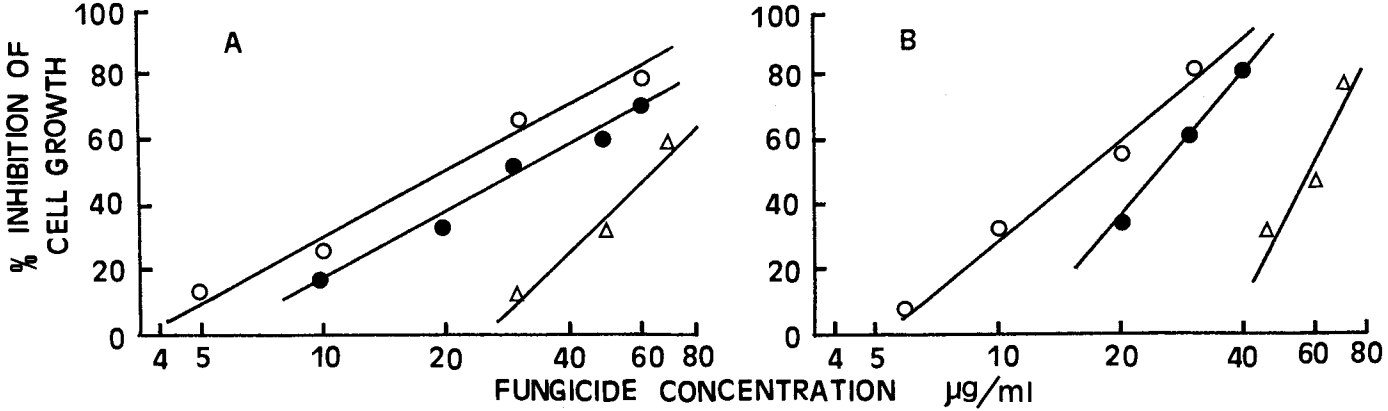

Figure 1. Dosage-response curves obtained after 72 h exposure of mammalian cells in culture to various concentrations of fungicides. The compounds were DP(o—o), OPP(●—●) and TBZ(△—△). KB cells(A) and AGMK cells(B).

Table 1. Inhibitory effects of fungicides on growth of cultured mammalian cells.

compounds	ID50 (µg/ml)[1]	
	KB cells[2]	AGMK cells[3]
diphenyl	20.5	17.0
o-phenylphenol	30.0	26.0
2-(4'-Thiazolyl)benzimidazole	64.0	59.0

1 Concentration of fungicide in growth medium that caused a 50 % reduction in viable cell number after 72 h of incubation.
2 KB cells : human established cell lines.
3 AGMK cells : african green monkey kidney.

cells as those for KB cells.

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