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Toxicity of 1,1-Dichloroethane and 1,2-Dichloroethylene Determined Using Cultured Human KB Cells

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Nationwide surveys on toxic chemicals in the environment were made by the Environmental Agency of Japan in 1987 (Office of Health Studies, Environment Agency of Japan 1988). *cis*-1,2-dichloroethylene (*cis*-1,2-DCE), *trans*-1,2-dichloroethylene (*trans*-1,2-DCE) and 1,1-dichloroethane (1,1-DCA) were detected in samples of water and sediment. Yoshikawa *et al.*(1992) also found signs of toxicity in ground water in Japan. Effects of these compounds on rodents are well known (Freundt *et al.* 1977; Barnes *et al.* 1985;Mitoma *et al.* 1985).

We estimated quanititave differences in the toxicity of *cis*-1,2-DCE, *trans*-1,2-DCE and 1,1-DCA on animal cells in culture and we found that 1,1-DCA is more toxic than *cis*-1,2-DCE and *trans*-1,2-DCE .

MATERIALS AND METHODS

The cells used throughout this work were the KB cells of human oral carcinoma (Mochida and Yamasaki 1984). 1,1-dichloroethane (1,1-DCA) was obtained from Tokyo Chemical Industry Co.,Ltd., Tokyo,Japan. *cis*-1,2-dichloroethylene (*cis*-1,2-DCE) was obtained from Aldrich Co., Inc., Wisconsin, USA. *trans*-1,2-dichloroethylene (*trans*-1,2-DCE) was obtained from Wako Pure Co., Osaka, Japan. The water solubility of 1,1-DCA, *cis*-1,2-DCE and *trans*-1,2-DCE were 0.55 g/100 g, 0.35 g/100 g and 0.63 g/100 g, respectively (Office of Health Studies, Environment Agency of Japan 1988). These compounds were dissolved in ethyl alcohol (final concentration was under 0.5%). Methods for toxicity testing were as described (Mochida and Yamasaki 1984). The 72h-ID50 values (50% inhibitory dose to growth of cells) served as an index of the toxicity.

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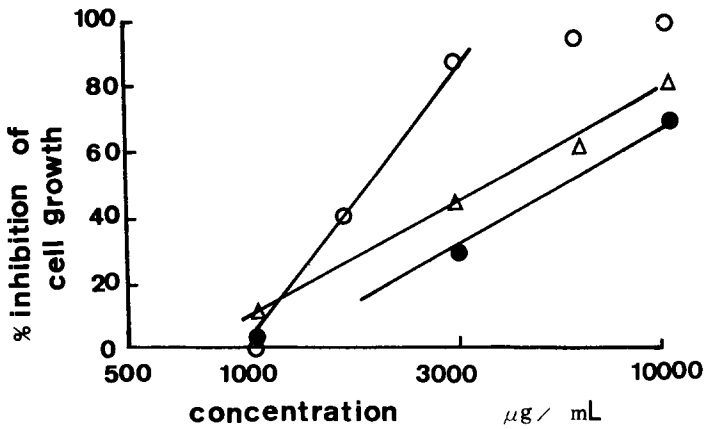

Figure 1. Dose - respons curves obtained after 72h exposure of KB cells to various concentrations of 1,1-DCA (o), *cis*-1,2-DCE (●) and *trans*-1,2-DCE (Δ).

RESULTS AND DISCUSSION

Figure 1 shows dose-response curves obtained from 1,1-DCA, *cis*-1,2-DCE and *trans*-1,2-DCE to KB cells. Growth of KB cells was decreased with increasing concentrations of these compounds, as indicated by cell counts. Therefore, 1,1-DCA, *cis*-1,2-DCE and *trans*-1,2-DCE are toxic to KB cells.

Table 1 shows the 72h-ID50 values obtained with 1,1-DCA, *cis*-1,2-DCE and *trans*-1,2-DCE to KB cells. 1,1-DCA seems to be more toxic and the *trans*-isomer proved to be more toxic than *cis*-isomer to KB cells.

The acute toxicity of 1,2-DCE in experimental animals appears to be low. The oral LD50 for the *trans*-isomer has been reported to be 1260 mg/kg for the rat (Freundt *et al.* 1977) and 2120~2390 mg/kg for the mouse (Barnes *et al.*1985). There are no available data on oral LD50 values for the *cis*-isomer. Thus, there is no relationship between this cell culture assay (ID50) and the acute toxicity (LD50) in rats and mouse.

We reported that the ID50 values of 1,2-DCA (1,2-dichloroethane) obtained using the same KB cell culture system were 1500 μg/mL (Mochida *et al.* 1986). Our present results show that 1,1-DCA (ID50 values:1950 μg/mL) is less toxic than 1,2-DCA to KB cells. The environmental Agency of Japan reported the LD50 values of 1,1-DCA and

Table 1. ID50 values for KB cells exposed to DCA and DCE.

compounds	72h-ID50 values (μg/mL)	oral LD50 values (mg/kg)	
	KB cells [1]	rat	mouse
1,1-DCA	1950	725[2]	-
1,2-DCA[3]	1500	670[2]	-
cis-1,2-DCE	5800	-	-
trans-1,2- DCE	3900	1260[4]	2120 ~ 2390[5]

[1] ID50 values; the concentration of each compound that reduced cell growth to 50% of control cultures during a 72h of exposure.
[2] Office of Health Studies (1988)
[3] Mochida *et al.* (1986)
[4] Freundt *et al.* (1977)
[5] Barnes *et al.* (1985)

1,2-DCA in rats to be 725 mg/kg and 670 mg/kg, respectively (Office of Health Studies, Environment of Japan, 1988), a value in aggreement with results of toxicity (LD50) in rats and this cell culture assay (ID50).

We found that 1,2-DCA (Mochida *et al.*1986) and 1,1-DCA are more toxic than 1,2-DCE of *trans*-isomer to KB cells (Table1) findings in aggreement with results of toxicity (LD50) in rodents (Freundt *et al.* 1977; Barnes *et al.* 1985). The acquisition of data on toxicity (LD50) in experimental animals and this cell culture assay (ID50) are the subject ongoing studies in our laboratory.

We estimated the toxicity of chlorinated organic compounds of ground water contaminations using the same cell culture assay (Mochida and Yamasaki 1984; Mochida and Saito 1985; Mochida *et al.* 1986). We found that 1,1-DCA, *trans*-1,2-DCE and *cis*-1,2-DCE proved to be less toxic than 1,1,1-trichloroethane (ID50 values : 420 μg/mL), trichloroethylene (ID50 values : 630 μg/mL), tetrachloroethylene (ID50 values : 195 μg/mL), dibromochloromethane (ID50values: 140 μg/mL) and bromodichloromethane (ID50 values : 420 μg/mL) to KB cells.

The ID50 value of trichloromethane obtained is 2200 μg/mL (Mochida and Yamasaki 1984) to KB cells. Trichloromethane has about 2 ~ 3 times the toxicity of *trans*-1,2-DCE and *cis*-1,2-DCE to KB cells, however, trichloromethane is less toxic than 1,1-DCA and 1,2-DCA (Mochida *et*

*al.*1986). The differences in toxicity to cultured human KB cells probably can be explained by chemical structure of the compound.

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