

Acetyl Tributyl Citrate and Dibutyl Sebacate Inhibit the Growth of Cultured Mammalian Cells

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Acetyl tributyl citrate (ATBC) and dibutyl sebacate (DBS) plasticizers are components of polyvinylidene chloride. These compounds can pass from the polyvinylidene chloride packing films to the enclosed food (Baba et al. 1988). Therefore, there may be a potential hazard to health. Toxicity of these compounds in laboratory animals is well known (Mallette and Haam 1952; Smith 1953; Finkelstein and Gold 1959). As the *in vitro* toxicity of these compounds to various mammalian cells, determined using the same cell culture systems has apparently not been documented, we examined the toxicity of ATBC and DBS on human, monkey and dog cells grown in culture systems.

MATERIALS AND METHODS

The following compounds were used: acetyl tributyl citrate (ATBC) (Tokyo Chemical Ind. Co., Tokyo, Japan) and dibutyl sebacate (DBS) (Wako Pure Chemical Ind. Co., Osaka, Japan). ATBC and DBS were dissolved in ethyl alcohol, then diluted in Eagle's minimum essential medium (final concentration of ethyl alcohol was under 0.5 %).

Human KB cells, monkey Vero cells and dog MDCK cells and the method of cultivation was as described (Mochida et al. 1988) and the toxicity test methods were described in the same report. The ID₅₀ values (50% inhibitory dose to growth of cells) served as an index of toxicity of the compound.

RESULTS AND DISCUSSION

Figure 1 shows dose-response curves obtained with ATBC and DBS. Human KB cells exposed to these compounds for 72h showed a dose dependent decrease in growth, as compared to controls. Monkey Vero and dog MDCK cells also showed a dose-dependent inhibition of growth, to both compounds.

Table 1 shows the ID₅₀ values obtained with these compounds. We noted that ATBC was more toxic than DBS to human KB, monkey Vero and dog MDCK cells (Table 1), a finding in agreement with the results of Ekwall et al. (1982) who used human HeLa cells. As the values we obtained, that is 72h-ID₅₀

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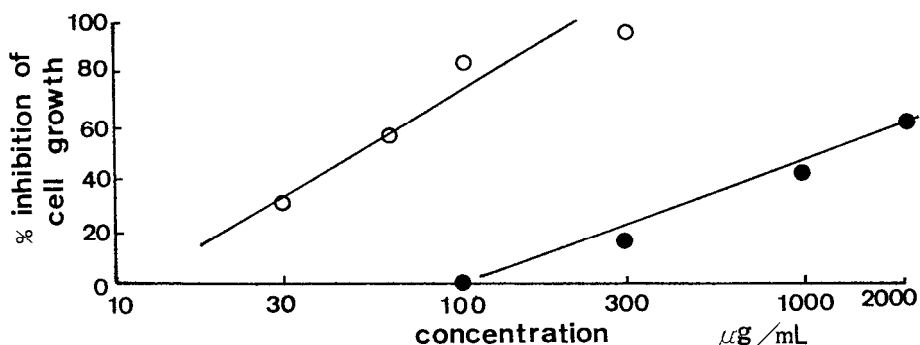


Figure 1. The dose-dependent inhibition of human KB cell growth caused by ATBC(○) and DBS(●).

Table 1. ID₅₀ values for human KB, monkey Vero and Dog MDCK cell lines exposed to ATBC and DBS.

compound	72h-ID ₅₀ valuens (ug/mL) ¹		
	KB cells	Vero cells	MDCK cells
ATBC	44.7 ± 2.99	39.9 ± 2.02	42.1 ± 2.02
DBS	1549 ± 4.99	1510 ± 3.81	1529 ± 4.29

¹ID₅₀ : the concentration of each compounds that reduced cell growth to 50% of control culture during 72h period of exposure. Values are the mean ± S.D.

values(ATBC:44.7ug/mL,DBS:1549 ug/mL) to human KB cells are lower than 7days-IC₅₀ values(ATBC:14000 ug/mL, DBS:2600 ug/mL) reported by Ekwall et al.(1982) who used human HeLa cells, our present cell culture system seems to be more sensitive.

The ID₅₀ values of tricresyl phosphate(TCP) and triphenyl phosphate(TPP) used as plasticizers, obtained using the same human KB cell culture systems were 70 ug/mL and 200 ug/mL, respectively (Mochida et al. 1988). Our present results shows that ATBC is more toxic than TCP to human KB cells, however, ATBC is less toxic than TPP to human KB cells. DBS is less toxic than TCP to human KB cells, however, DBS is more toxic than TPP to human KB cells.

The ID₅₀ values for butylated hydroxyanisole(BHA) and butylated hydroxytoluene(BHT) used as antioxidants for plastic, and tested using the same human KB cell culture system, were 12.5 ug/mL and 43.0 ug/mL, respectively(Mochida et al. 1985). Our present results shows that ATBC is about 3.6 times less toxic than BHA to human KB cells, however, ATBC has a toxicity close to that of BHT. DBS is about 36 times and 124 times less toxic than BHT and BHA to human KB cells, respectively (Table 1).

Our findings suggest no remarkable difference in sensitivity to ATBC and DBS since all had similar ID₅₀ values for human KB, monkey Vero and dog MDCK cell lines. We recommend that the sensitive system we used be adopted for further related studies.

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