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Toxicity of Halomethanes to Cultured Human and Monkey Cells

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Halomethanes (trichloromethane, bromodichloromethane, dibromochloromethane, and tribromomethane) are contaminants of drinking water (Bellar et al. 1974; Dowty et al. 1975; Rook 1974). The toxic effects of these halomethanes were noted in mice (Bowman et al. 1978).

We now report the toxicity of trichloromethane, bromodichloromethane, dibromochloromethane, and tribromomethane on mammalian cells grown in culture systems, assessed to determine possible hazards to the human population.

MATERIALS AND METHODS

The human established cell line KB (human oral carcinoma) was provided by Dr. G. Kimura (Kyushu University). African green monkey kidney (AGMK) cells was purchaused from Flow Laboratories, Inc., Rockville, U.S.A. Both lines were cultivated in Eagle's minimal essential medium (MEM) supplemented with 10% newborn calf serum. In all tests, the medium contained 100 units/ml penicillin G, 100 µg/ml streptomycin sulfate and 292 µg/ml L-glutamine. All cells were cultured under conditions of 37°C.
The following halomethanes were used : trichloromethane (Tokyo Chemical Ind. Co., Ltd., Tokyo, Japan), bromodichloromethane, dibromochloromethane and tribromomethane (Kanto Chemical Co., Inc, Tokyo, Japan). These compounds were dissolved in ethyl alcohol and diluted in MEM medium immediately before use. Ethyl alcohol decreased the total cell number by 1% but had no apparent effect on culture growth at 0.5%. Based on these results, ethyl alcohol was used as the carrier for all halomethanes used in later experiments. The cells were suspended in growth MEM medium at a concentration of 1×10^5 cells per ml and 1 ml volumes were seeded in Leighton tubes. After 24 h of incubation at 37°C, the MEM medium was replaced with

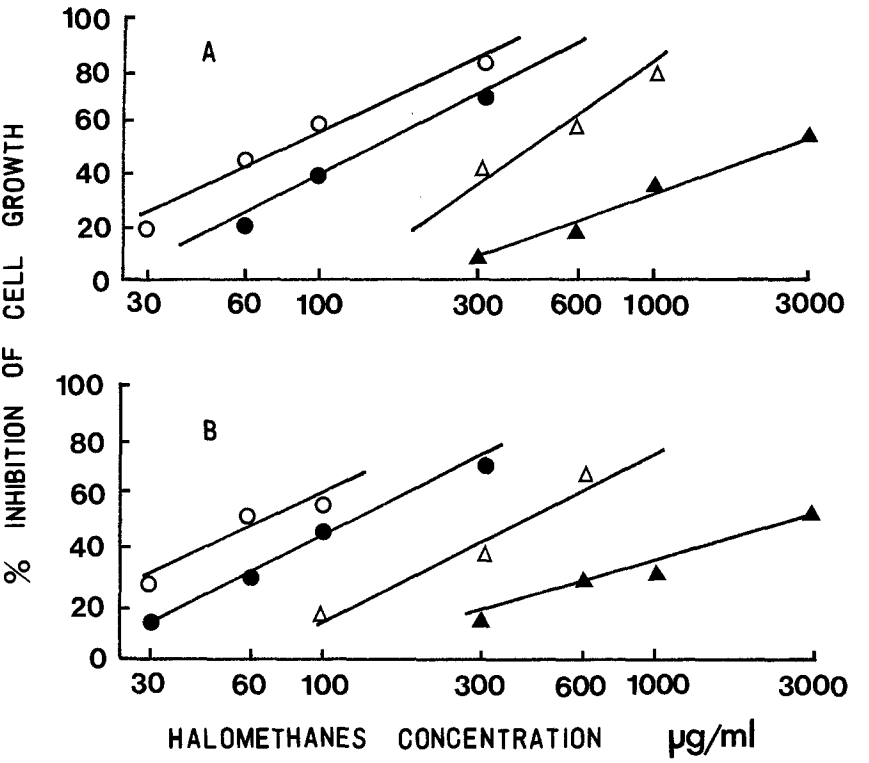

Figure 1. Dose-response curves obtained after 72 h exposure of mammalian cells in culture to various concentrations of halomethanes. The compounds were tribromomethane(O), dibromochloromethane(●), bromodichloromethane(Δ) and trichloromethane(▲). KB cells(A) and AGMK cells(B).

MEM medium-containing compounds. To minimize loss of halomethanes, test cultures were placed in Leighton tubes closed with silicone rubber. After 72 h of additional incubation, the viable cells (numbers determined by nigrosin exclusion method) were determined using a Bürker-Türk cell counter. Halomethane-induced inhibition of cell growth was then determined by comparing the total number of viable cells in halomethane-treated cultures with the total viable cell number in cultures that been treated with only ethyl alcohol (control). The dose-response curve obtained when the halomethane concentration caused a fifty per cent inhibition of cell growth (ID50) was determined. Each point on the resulting curves represents the average of three replicates.

RESULTS AND DISCUSSION

Figure 1 shown dose-response curves obtained with

trichloromethane, bromodichloromethane, dibromochloromethane, and tribromomethane for KB cells and AGMK cells. Inhibition of growth of KB and AGMK cells was dose-dependent.
Table 1 shows the 50% inhibition levels (ID50) obtained with the four compounds used. Tribromomethane was more toxic than dibromochloromethane, bromodichloromethane, and trichloromethane, to both lines of cells. In the case of trichloromethane, the 50% inhibition concentration of cell growth was over 1000 µg/ml. This is in a aggreement with results of Elias et al (1981) who exposed mouse L cells to trichloromethane.
Our findings suggest no remarkable difference in sensitivity to these four compounds since all had the same ID50 values for KB cells and AGMK cells.

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

compounds	ID50 (µg/ml)[1]	
	KB cells[2]	AGMK cells[3]
trichloromethane	2200	2150
bromodichloromethane	420	405
dibromochloromethane	140	130
tribromomethane	80	70

1 Concentration of halomethane in growth medium that caused a 50% reduction in cell number after 72 h of incubation.
2 KB cells : Human established cell line.
3 AGMK cells : African green monkey kidney.

Some halomethanes of organohalides in drinking water were tested. The acquisition of data on toxicity effects of other organohalides (Dowty et al. 1975) in drinking water is the subject of ongoing studies in our laboratory.

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