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Aquatic Toxicity Evaluated Using Human and Monkey Cell Culture Assays

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Bacteria, aquatic invertebrates, algae and fish are used as test organisms in studies on aquatic toxicology. A new bioassay using cultured mammalian cells was reported by Kfir and Prozesky (1981, 1982), who used HeLa cells, Buffalo green monkey cells and mouse cells, by Maruoka (1978) and Richardson et al. (1977) who used mouse cells, and by Vandoren et al. (1984) who used HeLa cells.

I now report the use of a cell culture assay using growing cells as a screen for aquatic toxicity.

MATERIALS AND METHODS

The human established cell line KB and primary African green monkey kidney (AGMK) cells I used were the same as described (Mochida et al. 1983). These cells were cultured in Eagle's minimum essential medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% newborn calf serum (Flow Laboratories, Inc., Stanmore, Australia), penicillin G (100 U/mL), streptomycin sulfate (100 μg/mL) and L-glutamine (292 μg/mL). These 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 chemicals were used : Cd($CdCl_2$), Cu($CuCl_2$) and Zn($ZnSO_4 \cdot 7H_2O$) (Wako Pure Chemical Ind. Co., Osaka, Japan).

Aquatic samples were obtained directly from the Hii river and from ground water in Shimane Prefecture, Japan, between May and September 1984. These water samples are the main source of drinking water for residents in Shimane Prefecture and are not polluted with industrial or agricultural products. Five samples per month were obtained. These samples were sterilized by passage through a 0.22-μm Millipore filter

(Millipore, Massachusetts, U.S.A.).

Table 1. The ID50 values of heavy metals to KB and AGMK cells.

metals	ID50 (μM)[1]	
	KB cells[2]	AGMK cells[3]
Cd	5.5	3.0
Cu	150.0	100.0
Zn	100.0	82.0

1 Concentrations of heavy metals 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 cell.

Toxicity tests were as described previously (Mochida et al. 1983). The metals used were dissolved in distilled water.

To determine the toxicity test of the water, the KB and AGMK cells were suspended in medium at a cell density of 1 X 10^5 cells/mL and 5-mL volumes were seeded into 50-mm new plastic Petri dishes (Nunc, Roskilde, Denmark). One day later the medium was removed. 5-mL of fresh medium made up with water sample was added to the plastic Petri dishes. Test and control cultures were incubated under the same atmospheric condition in a humidified 5% CO_2 - 95% air incubator at 37°C. A control was prepared by the same process, using distilled water. After 72 h of additional incubation, cell viability (numbers determined by the nigrosin exclusion method (Paul 1975)) was determined using a Bürker-Türk cell counter. Results were determined as a percentage inhibition of cell growth according to the following formula :

$$\frac{\text{number of viable cells in treated plates}}{\text{number of viable cells in control plates}} \times 100$$

RESULTS AND DISCUSSION

Model metals (Cd, Cu and Zn) as aquatic pollutants (chemical toxicants) were used to establish that the assay is capable of detecting toxic effects by inhibition of cell growth (%). Cd, Cu and Zn were chosen because their toxicity is fairly well established.
Table 1 shows the ID50 (50% inhibition of cell growth) values obtained with heavy metals (Cd, Cu and Zn). Cd was more toxic than Cu and Zn to KB and AGMK cells.

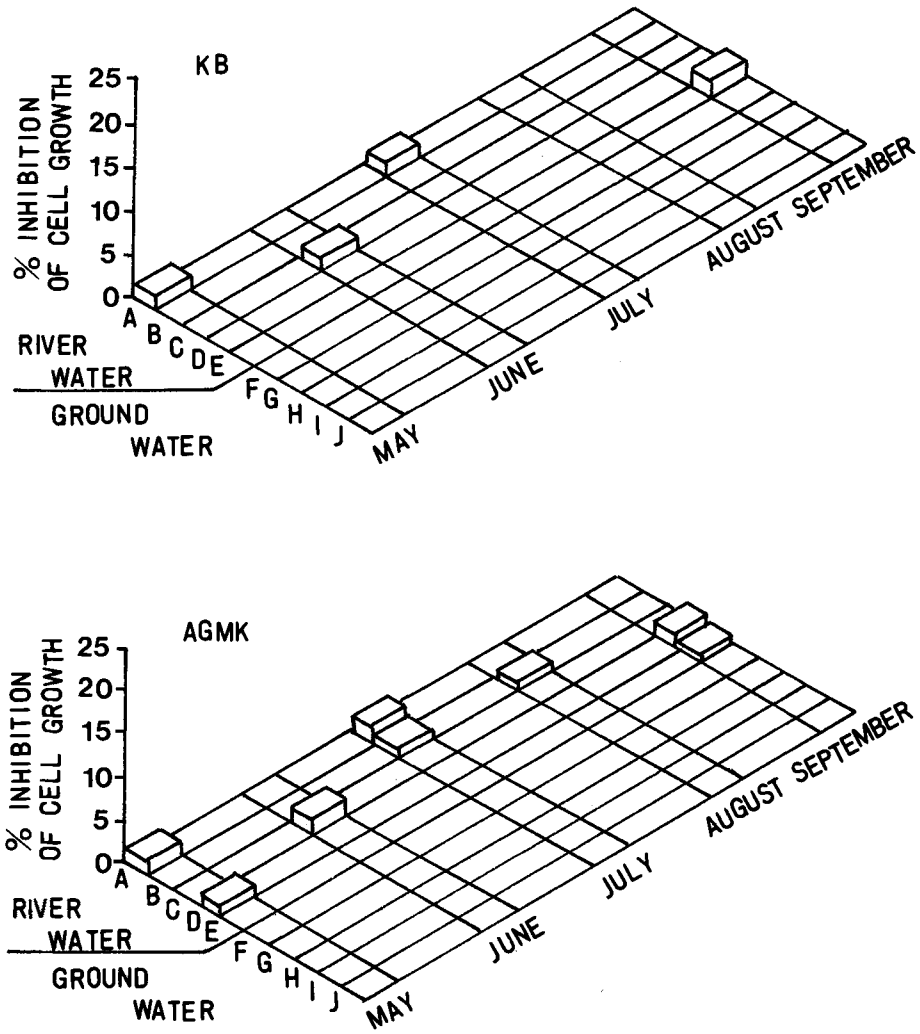

Figure 1. Effects of river (A-E) and ground (F-J) water on KB and AGMK cells.

Kfir and Prozesky (1981) and Richardson et al. (1977) found that cell culture assays were more sensitive to chemicals than were fish and Daphnia bioassay. I found that the cell culture assay used here was more sensitive than the algae bioassay (Rachlin et al. 1983). This cell culture assay is a simple and rapid quantitative system for determination of aquatic toxicity.

Figure 1 shows effects of river and ground water on KB and AGMK cells. Aquatic samples (100% river and ground water) in Shimane Prefecture, Japan, obtained between May and September 1984 were not toxic to KB and AGMK cells.

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