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Mutagenicity of PCBs and Their Pyrosynthetic Derivatives in Cell-Mediated Assay

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The mutagenicity of PCBs, Aroclor 1242 and Clophen A60 was studied in a mammalian cell assay using V79 Chinese hamster cells as target cells. The method was used as a direct assay without metabolizing cells and as a cell-mediated assay with 5000 R-irradiated rat fibroblasts as metabolizing cells. A wipe soot sample of a PCB fire was studied in the same assay, and the results showed no mutagenicity of the pure PCBs. However, the wipe sample showed weak mutagenic activity which disappeared when metabolizing cells were used. The chemical analysis of the wipe sample showed tri-, tetra- and pentachlorodibenzofurans in addition to PCBs.

Gene Mutation in Mammalian Cells

Various cell types—those of mesenchymal origin as well as tumor origin—are used frequently in mammalian mutagenesis tests. A few systems of epithelial origin are also available. The mesenchymal cells most commonly employed are established aneuploid cells such as Chinese hamster ovary (CHO) and V79, which we use as target cells. Other cell lines include mouse embryo or human skin fibroblasts.

In many instances, the phenotypic change in these cells is the result of changes in enzymes of metabolic systems, and in some cells the mutations in the structural gene and production of abnormal protein have been characterized. The most widely used procedure for selection of mutants is based on 8-azaguanine or 6-thioguanine resistance, which is caused by a reduction in activity of hypoxanthine guanine phosphoribosyl transferase due to a mutation in the HGPRT locus in the X-chromosome. Another selection procedure deals with ouabain resistance that results from a mutation affecting a membrane-associated sodium potassium ATPase.

In the present cell-mediated mutagenesis assay method developed by Huberman (*1*) and Huberman and Sachs (*2*), target cells, V79 with the appropriate markers for mutagenesis, are co-cultivated with lethally irradiated embryo cells or hepatocytes that can metabolize the chemical carcinogens. During cocultivation, the reactive metabolites appear to be transmitted from the cells that metabolize the carcinogen and induce mutations in the cells with the appropriate markers.

Use of this assay makes it possible to demonstrate a relationship between the carcinogenic potency of polycyclic hydrocarbons, nitrosoamines and some other compounds and their ability to indue mutations in mammalian cells.

Direct Mutagenesis Assay in Chinese Hamster V79 Cells

Two hundred target cells are seeded at 200 cells per 60-mm Petri dish to determine cloning efficiencies and at 10^5 cells/per 60-mm Petri dish for selection of ouabain-resistant mutants. The test compound is added after 3 hr. For selection of mutants, a final concentration of 1 mM ouabain is added 2 days after treatment with the test compound. After they are stained with Giemsa, colonies are counted at 6 to 8 days for cloning efficiency and at 16 to 18 days for ouabain resistance.

Cell-Mediated Mutagenesis Assay with Fibroblasts or Hepatocytes

Chinese hamster V79 cells are seeded at 3×10^5 cells in 4 mL of medium on a monolayer irradiated with 5000 R of 2×10^6 polycyclic hydrocarbon-metabolizing golden hamster embryo cells prepared one week earlier. The test compound is added 4 hr later in 1 mL of medium.

In the cocultivation of V79 with primary rat hepa-

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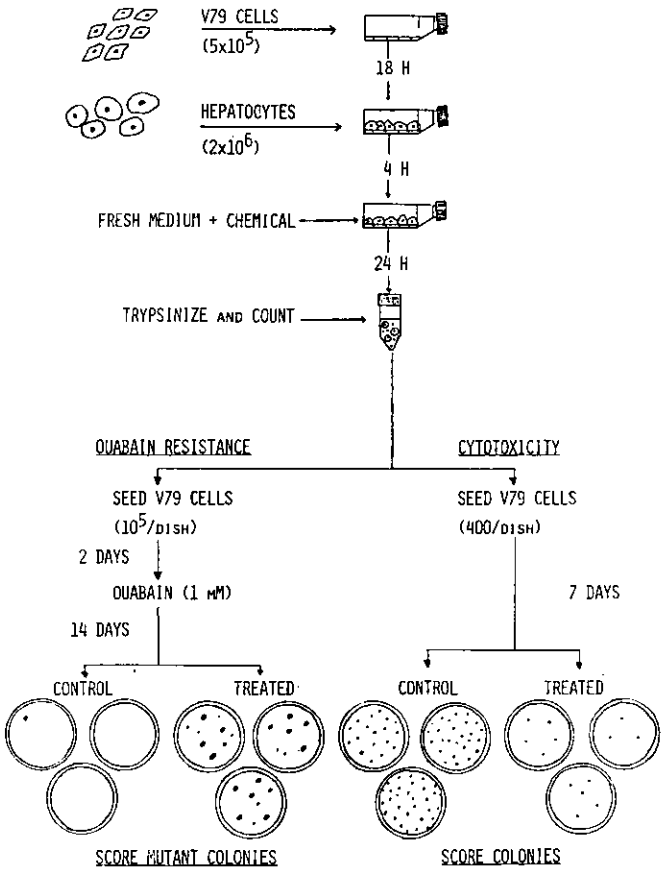

FIGURE 1. Cell-mediated mutagenesis assay.

tocytes, 25-cm^2 T-flasks are seeded with 5×10^5 cells in Dulbecco's modified Eagle medium containing 10% fetal calf serum. after 18 hr, this medium is replaced by complete Leibovitz-15 medium containing 2 10 viable hepatocytes. After 4 hr, at which time the hepatocytes are attached, the medium is replaced by complete L-15 containing 25 mM Hepes buffer and the test compound.

Two days after the treatment the fibroblast cultures and 18 hr later, the hepatocyte cultures are dissociated with a trypsin–EDTA solution and seeded on 60-mm Petri dishes at 200 cells per dish for cloning efficiency and at 10^5 cells per dish for selection of ouabain-resistant mutants. Ouabain-resistant colonies are stained with Giemsa and their number is counted 14 to 16 days after cell seeding.

An expression time of 7 to 8 days is necessary for the selection of 6-thioguanine-resistant mutants. Therefore, after dissociating the hepatocytes and V79 cells or fibroblasts and V79 cells, 10^5 V79 cells are seeded in 100-mm Petri dishes (3 dishes per dose). The cells are dissociated after 6 days and reseeded at 200 cells in 4 mL medium per 60-mm Petri dish (16 dishes per dose) for selection of 6-thioguanine mutants. 6-Thioguanine is added 2 days later in 1 mL of medium at a final concentration of 30 μM. The 6-thioguanine-resistant colonies are stained with Giemsa, and the number is determined 9 days after seeding.

The mutation frequency for ouabain or 6-thioguanine resistance is calculated per 10^6 survivors, based on the cloning efficiency and number of cells seeded for mutant selection. The final results are averages from three to four separate experiments.

Analysis of the Wipe Samples

The wipe test samples of the soot had been extracted in toluene, and three samples were analyzed. The solvent was carefully evaporated by pure nitrogen and the residue was weighed. Before the mutagenesis test a toxicity test was conducted by using cell densities of 20,000 and 200 cells per 60-mm dish. Final concentrations of 1, 3 and 10 μg/mL medium were chosen for the mutagenesis assay. Two PCBs—Aroclor 1242 and Clophen A60—were chosen as references, and the final concentrations 50, 100, 150 μg/mL, respectively, were chosen after the toxicity test.

The results of the toxicity test are present in Table 1, and the results of the mutagenesis test are shown in Table 2.

The highest concentration of pure PCBs used in the final cell-mediated assay was not necessarily the highest possible due to the fact that PCB was interfering with the tissue culture dish material. This is a fact that must be seriously taken into consideration in possible future experiment. A similar problem caused difficulties in some PCB fish toxicity experiments (*3*).

The results showed no ouabain-resistant mutants in

Table 1. Toxicity experiment with V79 cells and PCB as test substances.

	Cloning efficiency, %[a]						
PCB dose, μg/mL	Aroclor 1221	Aroclor 1232	Aroclor 1242	Clophen A30	Clophen A60	Wipe sample without metabolizing cells	Wipe sample with metabolizing cells
50	53	54	47	48	45		
100	43	52	49	54	46		
150	50	37	0	0	42		
1						78	57
3						66	55
10				..		59	
Control	52	52	52	52	52	66	67

[a]After seeding at 200 cells/dish.

Table 2. Induction of 6-thioguanine-resistant mutants in the cell-mediated assay by a wipe test from PCB fire, Aroclor 1242 and Clophen A60.

Concentration of solid material in acetone, μg/mL	Number of 6-thioguanine resistant mutants per 10^6 survivors	
	Without metabolizing cells	With rat fibroblasts
Control	103	0
Wipe sample		
1	287	5
3	386	5
10	340	6
Aroclor 1242		
50	0	
100	0	
150	0	
Clophen A60		
50	0	
100	0	
150	0	

any of the experiments, but 6-thioguanine mutants were obtained from the wipe sample without using metabolizing cells and also when rate fibroblasts were used as metabolizing cells. Because PCBs showed no activity in the direct method, they were not cultivated in cell-mediated assay.

A conclusion can be made that on the basis of the results obtained the wipe samples showed mutagenic activity whereas the pure PCBs showed none.

Chemical analysis of the sample showed the appearance of tri-, tetra-and pentachlordibenzofurans in addition to PCB, which was the main component of the soot sample.

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