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Uptake of Benzo[*a*]pyrene, Carbaryl, DDT and Parathion in Cultured Human Cells: Re-evaluation

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We previously reported that upon incubation of cultured cells with various radioactive pesticides and environmental chemicals, persistent chemicals in the environment were taken up to a greater extent than non-persistent substances by the cells (MURAKAMI and FUKAMI 1978). Since many persistent chemicals have very low water solubilities, the lipophilic character of the chemicals seems to be an essential parameter related to the cellular uptake. It is considered, however, that the nonspecific absorption of compounds to the cell surfaces is included in the extent of cellular uptake of chemicals. Therefore, we have attempted to evaluate the uptake of chemicals in cytoskeleton structures prepared by treatment of cultured cells with ethanol or the non-ionic detergent, Triton X-100. In the present study three insecticides, carbaryl, DDT and parathion, and a carcinogen, benzo[a]pyrene were selected as test compounds.

MATERIALS and METHODS

Cell culture : Human embryonic lung diploid cells (HEL 299) from the American Type Culture Collection (CCL 137) (Rockville, Maryland) were used. The cells were grown as monolayer cultures as described by MURAKAMI and FUKAMI (1976). Antibiotics, penicillin (50 units/ml), streptomycin (100 μg/ml) and Fungizone Squibb (2.5 μg/ml) were contained in the final medium.

Radioactive chemicals : All labeled chemicals were purchased from the Radiochemical Centre, Amersham, England as benzene solutions. Benzo[a]pyrene, ^{14}C-labeled at the 7 and 10 positions, had a specific activity of 60.7 mCi/mmole. Carbaryl, $^{14}CH_3$, had a specific activity of 59.5 mCi/mmole. DDT, universally ^{14}C ring-label, had a specific activity of 29.7 mCi/mmole. Parathion, 1-^{14}C-ethyl labeled, had a specific activity of 19 mCi/mmole.

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Cellular uptake of chemicals : Cells were grown in 75 cm^2 tissue culture flasks containing 10 ml of medium to near-confluence as indicated by microscopic examination. The labeled chemicals were dissolved in ethanol after the benzene was evaporated with nitrogen and were added to the medium to give a final concentration of 1 % ethanol. The concentration of the compounds in the medium was 4 x 10^{-6} M. This dose caused no visible cytotoxicity to the cells.

Fractionation of cells into acid-soluble, ethanol-soluble and acid- and ethanol-insoluble fractions : After a 24 h incubation the medium was removed and the cell monolayers were washed twice with cold 0.9 % NaCl solution (10 ml each). The cell layers were then immersed in 10 ml of cold 4 % $HClO_4$ for 40 min to remove acid-soluble materials, rinsed successively with 80 % and 100 % ethanol (10 ml each). After drying the fixed monolayers were dissolved in 2 ml of 85 % formic acid for 20 min. Each fraction was counted in 15 ml of Aquasol-2 (New England Nuclear).

Preparation of ghost monolayers : After incubation for 4 and 48 h with the chemicals, ghost monolayers were prepared by a modification of the technique described by OSBORN and WEBER (1977). The cell monolayers were washed twice with 10 ml each of cold buffer A (0.01 M Tris-HCl adjusted to pH 7.8, 0.14 M NaCl and 0.005 M $MgCl_2$), and treated with 10 ml of buffer A containing 0.5 % Triton X-100 (Sigma) for 10 min. After treatment with the detergent the monolayers were gently washed twice with 10 ml each of cold buffer A. The fragile ghost monolayers were digested for 15 min with 2 ml of 0.5 N NaOH at room temperature. Total labeled compound uptake was determined by counting a 0.5 ml aliquot of the NaOH digest in 10 ml of a mixture of 5 volumes of toluene with dimethyl POPOP (0.01 g/L) and PPO (6 g/L) and 1 volume of Triton X-100 (MENEGHINI 1974). Total protein per culture was determined by assaying 0.5 ml of the NaOH digest according to the method of LOWRY et al. (1951) as modified by OYAMA and EAGLE (1956) for tissue cultures.

RESULTS

The distribution of labeled compounds in the medium and washings and in various fractions is shown in Table 1. DDT was easily removed from the cells by treatment with ethanol, and very small amount of the

TABLE 1

Radioactivity in the Medium and Washings and in Various Fractions of HEL Cells

Cells in 75 cm^2 culture flasks (10 ml of medium) were treated with 40 nmoles of ^{14}C-labeled chemicals for 24 hours.

	Benzo[a]pyrene		Carbaryl		DDT		Parathion	
	nmoles	%	nmoles	%	nmoles	%	nmoles	%
Medium	23.3	55.3	27.6	87.9	31.1	68.4	33.1	79.6
Washings	1.2	2.9	1.1	3.5	1.7	3.7	3.6	8.7
$HClO_4$-soluble	0.5	1.2	0.2	0.6	0.2	0.4	0.9	2.2
EtOH-soluble	16.7	39.7	0.4	1.3	12.4	27.3	3.6	8.7
$HClO_4$- and EtOH-insoluble	0.4	1.0	2.1	6.7	0.07	0.2	0.4	1.0
Total	42.1	100.1	31.4	100.0	45.47	100.0	41.6	100.2
Recovery (%)	105.3		78.5		113.7		104.0	

TABLE 2

Ghost Monolayer Associated Chemicals (pmoles/mg protein)

Time of treatment (hours)	Benzo[a]pyrene	Carbaryl	DDT	Parathion
4	115	75	29	179
48	169	147	56	440

Cells in 75 cm^2 culture flasks were treated with 4 x 10^{-6} M of various labeled compounds. After the time stated the ghost monolayers were prepared and the radioactivity and protein content in the monolayers were determined.

insecticide remained in the acid- and ethanol-insoluble fraction. The insoluble fraction contained thirty times as much carbaryl and about six times as much parathion as DDT. Approximately 40 % of benzo[a]pyrene that was added in the medium was extracted with the organic solvent, but the carcinogen was found appreciably in the insoluble residue.

The association of the various chemicals to the ghost monolayers after treatment of the cells with the detergent, Triton X-100, is given in Table 2. The amount of association always increased with time of cultivation, and the extent of association of DDT to the ghost was considerably smaller than those of other three chemicals tested.

DISCUSSION

In our previous paper (MURAKAMI and FUKAMI 1978), we found that the extent of cellular uptake of DDT was much greater than parathion. Carbaryl was taken up moderately by the cells, but the extent was considerably lower than that of DDT. The present results indicate, however, that although a large amount of DDT is taken up by the cells, only a small fraction of the compound accumulates in the acid- and ethanol-insoluble fraction or the detergent-resistant cytoskeleton. This suggests that DDT associates mainly in lipid-rich (surface) membrane structures by a nonspecific absorption process. On the contrary, carbaryl and parathion were taken up to a smaller extent than DDT, but the chemicals remained appreciably in the insoluble fraction or the Triton X-100 resistant ghost. The finding may suggest that

carbaryl and parathion are more closely associated with cellular components such as proteins.

It is of interest that a considerable amount of carbaryl is found in the acid- and ethanol-insoluble residue, and it will require further examination. This finding may suggest that the naphthalene ring in the carbaryl molecule is involved in the binding potential of this compound into cellular components. It would obviously be of value to study incorporation and binding of chemicals to cellular constituents in cultured cell systems and the findings obtained should be relevant to the process of toxic action of chemicals in general.

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