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Incorporation of Labeled Pesticides and Environmental Chemicals into Nuclear Fraction of Cultured Human Cells

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During the course of studies on the interaction between xenobiotics and cultured human cells, we found that the rate of cellular uptake of persistent chemicals was greater than non-persistent compounds (MURAKAMI and FUKAMI 1978). The present report describes incorporation data on ten pesticides and environmental chemicals into nuclear fraction of cultured human cells, and compares with the rate of uptake of the chemicals at the cellular level.

MATERIALS and METHODS

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 and 1979). Radioactive compounds used in this work are listed in Table 1.

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 and were added to the medium to give a final concentration of 1 % ethanol. The concentration of the compounds in the medium was 4×10^{-6} M. This dose caused no visible cytotoxicity to the cells.

After incubation for 15 h at 37^{o} C with the chemicals, nuclear fraction was prepared by the technique described by LIEBERMAN and POIRIER (1973). The radioactive medium was removed, and the cell monolayers were washed twice with 0.9 % NaCl solution. The cells were then scraped off by a rubber policeman and pelleted by centrifugation (1,000 rpm, 5 min). Nuclei were prepared by lysing the cells in a 0.32 M sucrose solution containing 0.001 M potassium phosphate buffer (pH 7.5), 0.0015 M $CaCl_2$ and 1 % Triton X-100 (Sigma). After collection by centrifugation (2,000 rpm, 10 min), the nuclei were washed by centrifugations (2,000 rpm, 10

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TABLE 1
Radioactive Compounds Used in This Work

Common name	Chemical name	Specific activity mCi/mmole	Source[1]
Aldrin[2]	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene	76	RCC
Benzo[a]pyrene[3]		60.7	RCC
Carbaryl	1-Naphthyl N-methyl-^{14}C-carbamate	59.5	RCC
Chlordimeform	N'-(2-Methyl-^{14}C-4-chlorophenyl)-N,N-dimethylformamidine	34.5	Schering
2,4-D	2,4-Dichlorophenoxy-1-^{14}C-acetic acid	55	RCC
DDT	Dichlorodi-U-^{14}C-phenyltrichloroethane	29.7	RCC
Dieldrin[2]	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene	85	RCC
HCB	Hexachlorobenzene-^{14}C (U)	35.32	NEN
Parathion	O,O-Di-1-^{14}C-ethyl O-p-nitrophenyl phosphorothionate	19	RCC
PCB[4]	Polychlorinated biphenyl-^{14}C(U)	31.3	NEN

1 Abbreviations : RCC, Radiochemical Centre, Amersham, England; NEN, New England Nuclear, Boston, Massachusetts.
2 Carbon 1,2,3,4 and 10 positions are labeled with ^{14}C.
3 Carbon 7 and 10 positions are labeled with ^{14}C.
4 Isomeric mixture containing approximately 54 % chlorine.

min) once in the same solution without Triton X-100 and once in 0.01 M Tris-HCl buffer, pH 7.5. The nuclear fraction was digested for 15 min with 1.5 ml of 0.5 N NaOH at room temperature. Total labeled compound incorporation into the fraction was determined by counting a 0.5 ml aliquot of the NaOH digest in 15 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). Protein content of the fraction was determined by assaying 0.5 ml of the NaOH digest according to the method of LOWRY et al. (1951).

RESULTS and DISCUSSION

Table 2 summarizes data on the incorporation of

TABLE 2

Incorporation of ^{14}C-Labeled Chemicals into Nuclear Fraction of Cultured Human Embryonic Lung Cells (Results are expressed as means ± the standard deviation of the mean.)

Chemical	pmole/mg of nuclear protein
2,4-D	not detected
HCB	3.5 ± 1.3
Chlordimeform	4.4 ± 2.1
Parathion	4.8 ± 3.0
PCB	36.4 ± 2.7
Dieldrin	49.1 ± 3.0
Aldrin	54.9 ± 9.4
Carbaryl	96.1 ± 29.0
DDT	123.1 ± 12.6
Benzo[a]pyrene	318.8 ± 46.8

^{14}C-labeled chemicals into the nuclear fraction obtained when HEL cells were exposed to the radioactive chemicals. The incorporation rate of a carcinogen, benzo-[a]pyrene was greatest among chemicals tested. DDT, aldrin, dieldrin, and PCB which are generally considered to be persistent chemicals were incorporated markedly into the fraction. Parathion, chlordimeform, and 2,4-D which are classed as non-persistent pesticides, were little or not incorporated into the nuclear fraction. However, the rate of nuclear incorporation of HCB, generally classified as a persistent chemical, was very low like non-persistent pesticides. It is noteworthy that carbaryl was incorporated markedly into the nuclear fraction. This finding coincides with the results reported previously that a considerable amount of the pesticide was taken up by the intact cells (MURAKAMI and FUKAMI 1978) and that carbaryl was found appreciably in the perchloric acid- and ethanol-insoluble fractions of the HEL cells (MURAKAMI and FUKAMI 1979). Recently MILLER et al. (1979) reported covalent binding of carbaryl to amino acid residues of rat liver microsomal protein in vitro. Relatively strong affinity between the insecticide and cells or cellular constituents described in our previous reports (MURAKAMI and FUKAMI 1978 and 1979) and this paper may be due to the covalent binding ability of carbaryl with cellular components described by MILLER et al. (1979). It will require further examination.

In other experiments, the cells were fed with me-

dium containing 4 x 10^{-6} M ^{14}C-labeled dieldrin, aldrin, DDT, and parathion for 24 h. DNA was extracted according to the methods of DIAMOND et al. (1967) and KUROKI and HEIDELBERGER (1971). The amount of bound insecticides was determined by counting an aliquot of DNA fraction. We could not find any binding of the insecticides tested to the cellular DNA (Unpublished results). Similarly, WRIGHT et al. (1977) reported that the interaction between dieldrin and liver DNA was extremely unlikely. Perhaps incorporation of labeled chemicals into the nuclear fraction may be due to the binding, whether it is strong or weak, to the nuclear proteins.

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