Uptake and Persistence of Pesticides in Cultured Human Cells

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The uptake and persistence of a carcinogen, 3-methylcholanthrene in rat embryo cells have been reported (ZIMMERMAN et al. 1975). However there have been few, if any, reports on the uptake and persistence of pesticides in cultured cells. As a preliminary step to elucidate the mechanism of pesticide toxicity at the cellular level, the incorporation of some pesticides into cultured human embryonic lung cells was studied. In the present study five insecticides were selected as test compounds. They were persistent chlorinated hydrocarbons, DDT, aldrin and dieldrin, an organophosphorus compound, parathion, and a novel broad-spectrum acaricide-insecticide, chlordimeform (also known as chlorphenamidine or Galecron^{TM}).

Materials and Methods

Human embryonic lung diploid cells (HEL 299) (PETERSON et al. 1968) from the American Type Culture Collection (CCL 137) (Rockville, Maryland) were used in these experiments. The HEL cells were grown in Eagle minimal essential medium, supplemented with 10 % fetal bovine serum (Flow Laboratories, Rockville, Maryland), sodium pyruvate (110 mg/l), Bacto peptone (500 mg/l), glycine (7.5 mg/l), serine (10.5 mg/l), penicillin (50 units/ml), streptomycin (100 µg/ml), and buffered with Hepes buffer (5 mM) and $NaHCO_3$ (4.5 mM). Cells were grown as monolayer cultures in tightly stoppered culture bottles at 37° C.

To determine the cellular uptake of pesticides, cells in 5 ml of growth medium were planted in 24 cm^2 culture bottles. After 24 hours of incubation the starter medium was replaced with 5 ml of medium and the cells were allowed to grow to confluence as indicated by microscopic examination. Radioactive pesticides (0.02 µmole) in 0.05 ml of ethanol were added to the cultures (Table 1). This dose of pesticides caused no visible cytotoxicity to the cells. After a 24 hour incubation period this medium was replaced with 5 ml of fresh medium containing no test pesticide. At the time of this medium change, the cellular uptake of pesticide

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TABLE 1

Radioactive Pesticides Used in This Work

Common name	Chemical name	Specific activity mCi/mmole	Source
Chlordimeform	N'-(2-Methyl-^{14}C-4-chlorphenyl)--N,N-dimethylformamidine	34.5	Schering
DDT	1,1,1-Trichloro-2,2-bis(p-chlorophenyl-^{14}C)ethane	6.25 and 5.88	New England Nuclear
Aldrin [a]	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene	79	Radiochemical Centre
Dieldrin [a]	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-dimethano-naphthalene	85	Radiochemical Centre
Parathion	O,O-Diethyl-1-^{14}C -O-p-nitrophenyl-phosphorothioate	15.9	Radiochemical Centre

a Carbon 1,2,3,4 and 10 positions are labeled with ^{14}C.

at zero time was determined from two culture bottles. The cultivation was continued for 9 or 10 days with two additional changes of medium during the incubation period.

Each point (every two or three days) in the cellular persistence curves is an average number taken from two bottles. The medium was removed and the cell monolayers were washed twice with calcium and magnesium free phosphate buffered saline. To the cultures 2 ml of 1 % sodium dodecylsulfate (SDS) was added and mixed gently (HUBERMAN et al. 1971). Ten minutes later, 0.5 ml aliquots of the SDS solution were placed into liquid scintillation counting vials. Ethanol (4 ml) was added, followed by 10 ml of the scintillation medium previously described (MURAKAMI and FUKAMI 1974). The samples were counted for 5 minutes on a Beckman LS-150 Liquid Scintillation System. Counting efficiencies were determined by reading directly from the quenching curve which was plotted against external standard ratio, and counts per minute were converted to disintegrations per minute.

Results and Discussion

Cellular uptake and persistence of chlordimeform, DDT and parathion are illustrated in Fig. 1. At the beginning of the cultivation with the test insecticides,

DDT, a persistent chemical, was incorporated into the HEL cells about 20 to 30 times greater than the non-persistent pesticides, chlordimeform and parathion. During incubation, the amounts of all pesticides taken up by the cells decreased with time while maintaining the initial proportions. In separate experiments, aldrin and dieldrin which are very stable in the environment gave cellular persistence curves identical with that of DDT as shown in Fig. 2. Approximately 10 % of the persistent insecticides added in the medium was initially incorporated into the cells.

Based on the cellular uptake, the compounds tested clearly fall into two groups. One group comprises DDT, aldrin and dieldrin which are generally classed as persistent pesticides. During the initial stage of incubation the incorporation rates of these persistent compounds into the cells were approximately 20 to 70 times greater than chlordimeform and parathion which are classified as non-persistent insecticides.

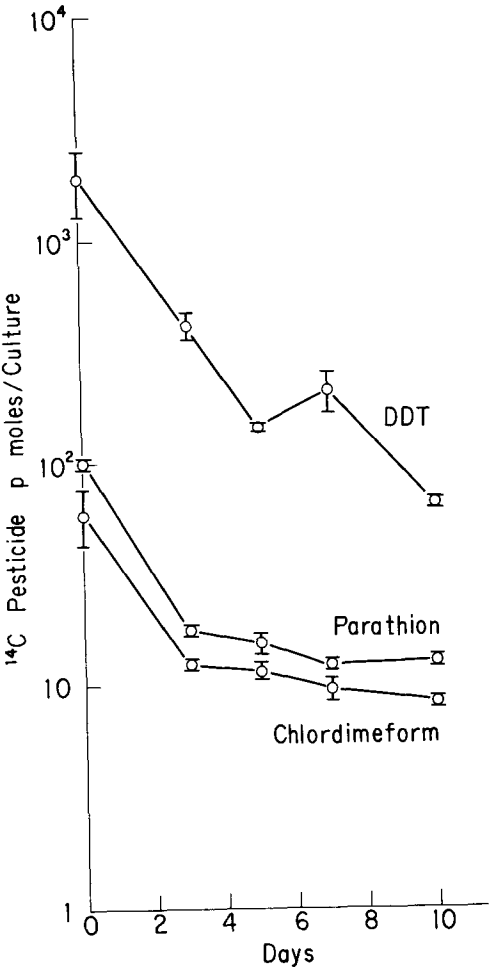

Fig. 1. Uptake and persistence of chlordimeform, DDT and parathion in cultured human embryonic lung cells.

Summary

The uptake and persistence of ^{14}C-labeled pesticides in cultures of human embryonic lung diploid cells were studied. Chlordimeform, DDT, parathion, aldrin and dieldrin were selected as test compounds. Results obtained from these experiments divided the chemicals into two groups. The first group consisted of three chemicals DDT, aldrin and dieldrin which are generally classified as

persistent insecticides while the second group was composed of chlordimeform and parathion, generally considered to be non-persistent insecticides. The rate of initial cellular incorporation of first group was approximately 20 to 70 times greater than the non-persistent insecticides. During the course of an incubation, the amounts of pesticide taken up by the cells decreased gradually.

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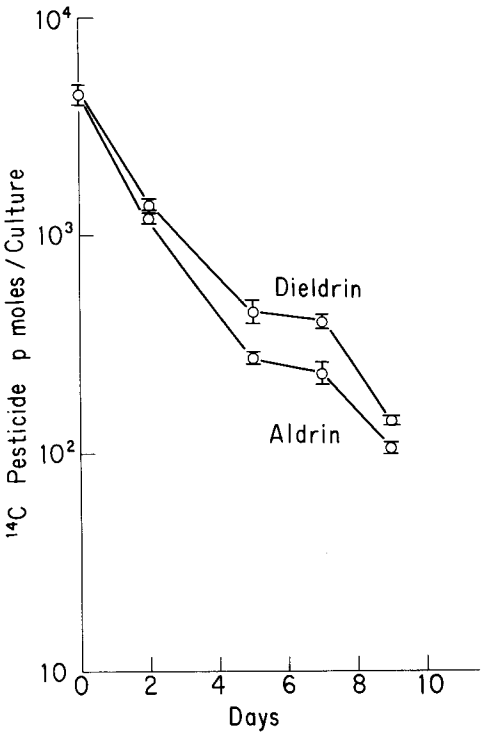

Fig. 2. Uptake and persistence of aldrin and dieldrin in cultured human embryonic lung cells.

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