Persistent Pesticides and Environmental Chemicals Are Taken Up to a Greater Extent than Non-persistent Compounds by Cultured Human Cells

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Pesticides and other man-made chemicals have played significant roles in increasing human needs such as food production and industrial uses, but in consequence of these uses some of these substances have entered the biosphere as persistent toxic chemicals. Therefore, the development of relevant test systems to evaluate the persistency of chemicals in the environment is required in order to eliminate the possibility of human exposure to such persistent chemicals. During the course of an examination of the uptake and persistence of pesticides in cultured human cells, we found that the rate of initial cellular incorporation of persistent pesticides, DDT, aldrin, and dieldrin was considerably greater than non-persistent insecticides, parathion and chlordimeform (MURAKAMI and FUKAMI 1976). The present communication presents cellular uptake data on eleven pesticides and environmental chemicals of different classes. The results demonstrate that persistent chemicals are taken up to a greater extent than non-persistent ones by cultured cells. This test method may be useful for evaluating the persistent potential of chemicals which have the possibility of environmental pollution.

Materials and Methods

Human embryonic lung diploid cells (HEL 299) from the American Type Culture Collection (CCL 137) (Rockville, Maryland) were used in these experiments. The cells were grown as monolayer cultures as described previously (MURAKAMI and FUKAMI 1976). Radioactive compounds used in this work are listed in Table 1.

To determine the cellular uptake of chemicals, cells in 5 ml of growth medium were planted in 24 cm^2 culture bottles. After 24 hours of incubation at 37° C the starter medium was replaced with 5 ml of medium and the cells were allowed to grow to near-confluence as indicated by microscopic examination. Radioactive chemicals (0.02 μmol) in 0.05 ml of ethanol were added to the cultures. The chemical concentration in the medium is about 4×10^{-6} M.

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TABLE 1

Radioactive Compounds Used in This Work

Common name	Chemical name	Specific activity mCi/mmol	Source**	Use
DDT	1,1,1-Trichloro-2,2-bis(p-chlorophenyl-^{14}C)ethane	29.7	RCC	Insecticide
Aldrin*	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethano-naphthalene	69	RCC	Insecticide
Dieldrin*	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	Insecticide
Malathion	O,O-Dimethyl-S 1,2-di(ethoxycarbonyl) ethyl-1,2-^{14}C]phosphorodithioate	4.6	RCC	Insecticide
Parathion	O,O-Diethyl-1-^{14}C-O-p-nitrophenyl-phosphorothioate	19	RCC	Insecticide
Carbaryl	1-Naphthyl N-methyl-^{14}C-carbamate	57	RCC	Insecticide
Chlordimeform	N'-(2-Methyl-^{14}C-4-chlorophenyl)-N,N-dimethylformamidine	34.5	Schering	Insecticide
2,4-D	2,4-Dichlorophenoxy-1-^{14}C-acetic acid	54	RCC	Herbicide
2,4,5-T	2,4,5-Trichlorophenoxy-1-^{14}C-acetic acid	54	RCC	Herbicide
HCB	Hexachlorobenzene-^{14}C(U)	23.4	RCC	Seed disinfectant, intermediate for organic synthesis, plasticizer
PCB	2,4,5,2',4',5'-Hexachlorobiphenyl-^{14}C(U)	15.2	RCC	Industrial chemical

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

** Abbreviation : RCC, Radiochemical Centre.

In controls, the same amount of chemicals was simply mixed with 5 ml of medium. Culture bottles were sampled at 4, 24, and 48 hours. At each period, the medium was removed and the cell monolayers were washed twice with cold 0.8 % NaCl solution and digested with 2 ml of 0.5 N NaOH at room temperature (ZIMMERMAN et al. 1975). Fifteen minutes later, 0.5 ml aliquots of the NaOH digest were placed into liquid scintillation counting vials. Radioactivity was measured on a Beckman LS-150 Liquid Scintillation System, after the addition of 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). 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.

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.

The growth of the culture was measured by determination of total cell protein after 48-hour exposure to chemicals. The different compounds were applied at the concentration of 0.02 µmol /5 ml of culture medium. The growth of treated cultures was expressed as the percentage of the control growth (EtOH treated).

Results and Discussion

Table 2 summarizes cellular uptake data obtained when HEL cells were exposed to ^{14}C-labelled chemicals over a period of 48 hours. The incorporation rates of DDT, dieldrin, and aldrin which are generally considered to be persistent pesticides, were greatest among chemicals tested. PCB which is also classed as a persistent chemical, was taken up markedly by the cells, but the rate was slightly lower than the chlorinated insecticides. HCB, another persistent chemical, was also accumulated significantly. Malathion, parathion, chlordimeform, 2,4,5-T, and 2,4-D, generally considered to be non-persistent pesticides, were taken up to a smaller extent than the persistent compounds. A considerable amount of carbaryl was taken up by the cells. This finding may suggest that the naphthalene ring in the carbaryl molecule is involved in the incorporation potential of this compound into the cells.

In a general way, it appears that an inverse relationship exists between the uptake of the test compounds by HEL cells and their solubility in water (Table 2). However, a close correspondence does not exist between them. This result may suggest that in addition to the lipophilic character, some factors such as binding capacity to proteins and other cellular constituents are involved in the uptake of chemicals by cultured cells.

In order to examine the state of the cells during the experiment, the effect of the chemicals on cell growth was investigated. Most compounds were not toxic at the same concentration and over the same experimental period as the uptake experiments. The herbicides, 2,4-D and 2,4,5-T, though less incorporated than the insecticidal and industrial chemicals, appear to be more toxic. It is therefore evident that differences in uptake are not the cause of the different degrees of cytotoxicity of the chemicals.

HAAG et al. (1975) reported that 2.7×10^{-6} M of 2,4-D caused a loss in cell differentiation of cultured chicken muscle cells. Using HeLa cells, LITTERST et al. (1969) and BLEVINS and DUNN (1975) found independently that carbaryl was more toxic than some other insecticides tested. Our findings on the inhibition of cell growth by chemicals are in accord with the results reported in the above papers. The drug-metabolizing system of the HEL cells was investigated by the measurement of the ability of the cell to convert the chemicals from a benzene soluble to an aqueous soluble form, as described by LOCKE et al. (1971), and any compounds tested were not metabolized by the cells.

TABLE 2

Uptake of ^{14}C-Labelled Chemicals into Cultured Human Embryonic Lung Cells
(Results are expressed as means ± the standard error of the mean.)

	Cell-associated chemical (pmol/mg of cell protein)			Water solubility*	Cell growth
	4 h	24 h	48 h	(mg/l)	(Per cent of control)
DDT	3690 ± 243	4060 ± 277	4830 ± 326	0.04	94
Dieldrin	3550 ± 175	3860 ± 122	4380 ± 240	0.1	97
Aldrin	3590 ± 369	3290 ± 224	3640 ± 383	0.01	99
PCB	1040 ± 80.8	1250 ± 40.4	1740 ± 98.0	0.001	111
HCB	993 ± 67.3	916 ± 78.6	778 ± 3.2	---	88
Carbaryl	278 ± 49.0	551 ± 110	945 ± 21.3	40	87
Malathion	323 ± 163	164 ± 111	180 ± 45.3	145	107
Parathion	70.7 ± 32.5	31.1 ± 2.5	22.6 ± 9.6	24	97
Chlordimeform	13.0 ± 2.8	18.7 ± 5.6	32.4 ± 5.7	250	96
2,4,5-T	6.6 ± 1.0	12.0 ± 0.4	9.9 ± 0.8	280	83
2,4-D	2.6 ± 0.3	5.0 ± 1.5	3.7 ± 1.0	730	79

* References for water solubilities quoted : DDT, Dieldrin, Aldrin, Carbaryl, and Parathion (LITTERST et al. 1969); PCB (HAQUE and SCHMEDDING 1975); Malathion (MELNIKOV 1971); Chlordimeform (NOYAKU NO KAGAKU TO ŌYŌ 1972); 2,4,5-T and 2,4-D (BÖHM and MÜLLER 1976).

Conclusion

It can be concluded that persistent chemicals were taken up to a greater extent than non-persistent substances by cultured human cells. Increasing uptake of the chemicals by the cells accorded with decreasing water solubility in general, but a strict correspondence between them could not be obtained. We propose that the method employed in this study be used for detecting a compound with persistent character in the environment. It will be a useful addition to examinations of n-octanol-water partition coefficient and of accumulation in fish and other organisms which have been used for evaluating the persistent potential of chemicals in the environment.

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