Subjecting untrained rats to swimming for 4 hours resulted in about a 3-fold increase in plasma LDH which is in the same range as the serum LDH increases reported by other investigators(1-3) with 6 to 16 hours of exercise in rotating cages. The increased levels apparently resulted from escape of LDH from heart, skeletal muscle, liver, and possibly red cells and platelets. This is further substantiated by the demonstration of histopathological changes in this study and others, and by the finding of elevations of other enzymes in the blood by other investigators(1-5).

Summary. Elevated and variable serum levels of LDH in control rats were found to result from the commonly used method of collecting the blood by cardiac puncture and from the preparation of serum for analysis. Evidence is presented that LDH from heart, red blood cells, and platelets are contributing factors to this discrepancy. When blood was collected from the inferior vena cava and plasma was immediately prepared in plastic tubes for analysis instead of serum, the extraneous factors were largely eliminated. These modifications were employed for the determination of LDH and isoenzymes in untrained rats subjected to 4 hours of swimming, and in untrained controls. The data indicated a 3-fold increase of LDH activity in the plasma of exercised rats. LDH-isoenzyme determination and histological examination indicated that probable sources of the increased LDH activity were the heart, liver, and skeletal muscle.

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Responses of Cell Cultures to Insecticides. IV. Relative Toxicity of Several Organophosphates in Mouse Cell Cultures.* (32261)

JANIS GABLIKS, MYRNA BANTUG-JURILLA, AND LEO FRIEDMAN
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge

The proven toxic effect of organophosphorus insecticides in animals is primarily due to the inhibition of acetylcholinesterase in the central nervous system. The toxicity of malathion and possibly of other organophosphorus compounds is dependent on at least 2 metabolic reactions which occur in the liver microsomes(1,2,3,4,5): the oxidative activation of malathion to malaoxon, a more potent anticholinesterase than the original compound(1), and the hydrolysis of malathion and malaoxon by carboxyesterase, which leads to the detoxification of both compounds(6,7,8). It is this detoxification reaction of malathion which is responsible for its remarkably low mammalian toxicity.

In contrast to this low mammalian toxicity, malathion is relatively toxic to established human-derived Chang liver and HeLa strain cells(9) as well as to primary cell cultures derived from chick embryos(10). Since the activities of esterases(11), of liver glutamate dehydrogenase(12), and of trypsin(13) are affected by organophosphorus compounds, cytotoxicity in cell cultures may also be induced by an effect on other enzyme systems. Because of the mouse's high resistance to malathion, we chose to investigate the suscepti-

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TABLE I. Comparative Cytotoxicity of Insecticides to Cell Cultures.*

Insecticidal compounds	Mouse liver, NCTC #1469			Mouse skin, L-929		
	TD_{50}	ID_{50}	ID_{10}	TD_{50}	ID_{50}	ID_{10}
	(μg/ml)			(μg/ml)		
Malathion	1000	1804	1030	100	106	52
Cygon®	100	310	235	300	370	120
DI-Syston®	5	11	6	50	44	27
Dipterex®	12	2	1	50	56	30

* 48-hr test.

bility of mouse cells in order to compare their susceptibility with the known susceptibility of cell cultures of human origin. This report shows that, in comparison with other organophosphates, mouse liver cells are highly resistant to malathion but are susceptible to its derivative, malaoxon.

Materials and methods. The methods were essentially the same as those described previously(9). Toxicity tests were performed on cell monolayer tube cultures of the established cell lines: mouse liver, NCTC #1469 strain, epithelial-like cells; mouse skin, L-929 strain, fibroblastic cells derived from subcutaneous tissue; and human liver, Chang strain, epithelial-like cells. The mouse liver cultures were grown in Medium 199(14) supplemented with 20% horse serum. The mouse skin and human Chang liver cells were grown in Eagle's medium(15) supplemented with 10% calf serum. In all tests cells were suspended in the appropriate growth medium containing penicillin and streptomycin; 1-ml volumes were planted in culture tubes and incubated in a stationary position at 37°C for 2 or 3 days until a confluent cell monolayer developed.

Insecticides were incorporated into the growth medium at different concentration levels in triplicate. The cultures were incubated up to 72 hours and the toxic effect was evaluated in 2 ways. First, microscopic examination was made to determine morphological alterations and cell destruction. The lowest concentration of an insecticide which induced morphological alteration in 50% of the cell monolayer during the incubation period of 48 hours was expressed as the toxic dose 50% level (TD_{50}).

The second method determined the growth of the culture by measuring the protein content, using the method of Oyama and Eagle (16). Concentrations of an insecticide which inhibited the growth by 10% and 50% during the 48-hour incubation period were expressed as the inhibitory dose 10% and 50% (ID_{10} and ID_{50}). These were calculated by the method of Litchfield and Wilcoxon(17) from the results of 3 or 4 cultures per concentration.

Insecticidal compounds. The insecticides investigated in this study were 0,0-Dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate (malathion†), 0,0-Dimethyl S-(N-methylcarbamoylemethyl) phosphorodithioate (Cygon®†), 0,0-Diethyl S-(2-ethylthio) ethyl phosphorodithioate (DI-Syston®‡), and 0,0-Dimethyl (1-hydroxy-2,2,2-trichloroethyl) phosphonate (Dipterex®‡).

Results and discussion. Comparative cytotoxicity. The cytotoxic effect was evidenced by progressive inhibition of cell growth followed by cytopathic changes in cell morphology and destruction. The magnitude of cell destruction was proportional to the test concentration and the length of incubation. These effects are similar to the cytotoxic effect of the compounds on human Chang liver and HeLa cell cultures(9).

The comparative toxicity, morphological cytopathogenicity (TD_{50}), and growth inhibitory levels (ID_{10} and ID_{50}) are summarized in Table I. In mouse liver cells the compounds exhibited a wide range of toxicity: malathion was the least toxic (ID_{50} = 1804 μg/ml); followed by Cygon, which was approximately 6 times as toxic as malathion; DI-Syston was 160 times as toxic; and Dipterex was 902 times as toxic. In mouse skin cells, however, Cygon (ID_{50} = 370 μg/ml) was the least toxic compound, fol-

† American Cyanamid Co., Princeton, N. J.
‡ Chemagro Corp., Kansas City, Mo.

TABLE II. Comparative Cytotoxicity of Malaoxon to Mouse and Human Liver Cells at 24, 48, and 72 Hours of Incubation.

Cell culture	Growth inhibitory ID_{50} levels (μg/ml) 24 hr	48 hr	72 hr
Mouse liver, NCTC #1469	200	160	5
Human liver, Chang strain	15	20	50

lowed by malathion, Dipterex, and DI-Syston (ID_{50} = 44 μg/ml).

In all the tests conducted in triplicate, the standard deviations of the toxic dose levels did not exceed 15% of the mean value. The results of several repeated titration tests showed good reproducibility.

The low toxicity of malathion in the mouse liver cells correlates well with its low toxicity in the mouse; however, with the other compounds there is no correlation of the relative toxicities in either cell line or *in vivo*.

Acute toxicity in the mouse in terms of the oral LD_{50} show that malathion (LD_{50} = 720 to 3200 mg), the least toxic of these 4 organophosphorus insecticides, is exceeded in toxicity by Dipterex (LD_{50} = 500 mg), Cygon (LD_{50} = 60 mg), and DI-Syston (LD_{50} = 3 to 10 mg)(18).

If the cytotoxic ID_{50} levels of malathion in mouse liver cells (1804 μg) are compared with those in human Chang liver (15 μg) and in HeLa (13 μg) cells as reported from an identical test by Gabliks and Friedman(9) and in chick embryo (approximately 10 μg) as determined by Wilson and Walker(10), it is evident that human liver, HeLa, and chick cells are at least 120 times as susceptible as mouse liver cells.

In an attempt to explain the low toxicity of malathion for mouse liver cells and its marked toxicity for human liver cells, we considered 2 of several possibilities: either malathion might be rapidly inactivated by mouse liver cells or the cells do not form the toxic derivative, malaoxon. The latter possibility was tested by using malaoxon in mouse liver and in human liver cells. The results are summarized in Table II.

In mouse liver cells the ID_{50} level of malaoxon is about 11 times that of malathion at 48 hours; it is about 360 times that of malathion at 72 hours. In Chang liver cells, however, the toxicity of malaoxon was comparable to that of malathion. It is evident that the toxicity of malaoxon increased with increased time of incubation in mouse liver cells whereas it decreased with increased time of incubation in human liver cells. These results suggest that the marked resistance of mouse liver cells is due to their inability to oxidize malathion to malaoxon; human liver cells appear able to transform malathion to malaoxon and are accordingly highly susceptible. Since malathion and malaoxon are also hydrolyzed by aliesterases in the rat and since these aliesterases as studied by Main and Braid(8) are absent in human serum, the metabolism of malathion in man merits further investigation.

The metabolism of malathion in cultivated cells is unknown. As expected, we were unable to detect acetylcholinesterase in Chang liver cells(19). The toxicity of malathion, malaoxon, and the other organophosphorus insecticides to cells *in vitro* may be due to their interference with the activity of other enzyme systems. It has been reported that B type esterases(11) and glutamate dehydrogenase (12) are also inhibited by organophosphorus insecticides.

Growth stimulation of cells. Some of the organophosphorus compounds at concentrations below the acute toxicity levels stimulated growth of the mouse liver cells. The increases in growth during 48 hours of incubation are illustrated in Fig. 1. Cygon at a concentration of 50 μg/ml of medium increased growth to 190% of the control value; at 100 μg growth was 140%. Higher concentration (300 μg/ml) resulted in growth inhibition and cell destruction. Similarly, DI-Syston at 1.0 μg/ml and malathion at 50 μg/ml increased the growth to 130% and 160% respectively. Dipterex did not stimulate the growth of mouse liver cells nor did any of the compounds stimulate the mouse skin L-strain cells. The growth stimulatory effect of DI-Syston has also been observed in DI-Syston chronically treated human HeLa cells (20).

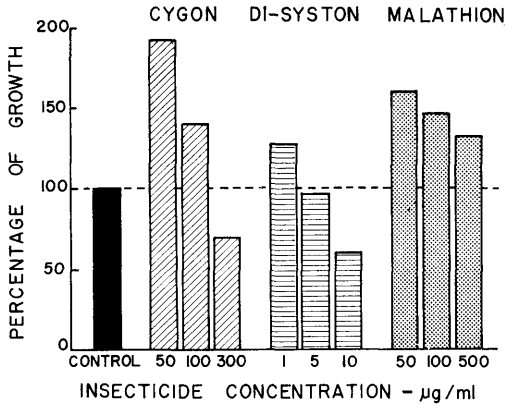

FIG. 1. Growth stimulation of mouse liver NCTC #1469 strain cells by Cygon, DI-Syston, and malathion. Growth was measured as the increase of total cell protein per culture and, for comparison, was expressed as percentages of control values.

Our observations indicate that, at the cellular level, organophosphorus compounds and their metabolites may have effects other than the well-established acute inhibition of acetylcholinesterases. For detection of such effects in various organ systems of different species, the cell and tissue culture technique offers some advantages over *in vivo* studies.

Summary. Mouse liver NCTC #1469 and mouse skin L-929 strain cells vary markedly in their susceptibility to organophosphorus insecticidal compounds. In mouse liver cells, the 50% growth inhibitory level of cytotoxicity was measured as 1804 µg/ml of medium for malathion, 310 µg/ml for Cygon®, 11 µg/ml for DI-Syston®, and 2 µg/ml for Dipterex®. The low toxicity of malathion to mouse liver cells correlates well with its low toxicity to the mouse but contrasts with its high toxicity to human liver Chang strain cells. Mouse liver cells were more susceptible to malaoxon than to malathion, but human liver cells were almost equally susceptible to malaoxon and malathion. Differences in cell susceptibility are largely due to the rates of malathion biotransformation to malaoxon, a more toxic and more potent acetylcholinesterase inhibitor. When tested at concentrations below acute toxicity levels, malathion, Cygon, and DI-Syston stimulated mouse liver cell growth.

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