Effect on Growth But Not on Chromosomes of the Mammalian Cells After Treatment with Three Organophosphorus Insecticides (36952)

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Due to a widespread concern over environmental contamination by the chlorinated hydrocarbon insecticides, the usage of such once popular compounds has been dwindling rapidly in recent years. Unlike the persistence of the chlorinated hydrocarbons, the organophosphorus insecticides are more susceptible to enzymatic attack and possibly leave little or no residual effect. Recent estimations show that the use of organophosphorus insecticides are drastically increasing. The potential hazards as the result of wide use of such insecticides is not clear.

In an overall project of testing mutagenic and carcinogenic potential of various pesticides in common use, we have studied the effect on cell growth and chromosomes after treatment with three organophosphorus insecticides. Our results indicate that all the compounds are toxic to the cells of human hematopoietic cell lines but cause no observable chromosome damage in either human cells *in vitro* or mouse cells *in vivo* under our experimental conditions.

Materials and Methods. The three organophosphorus insecticides tested were malathion, DI-syston and methyl parathion. Their chemical names, purity and sources are, respectively, *O,O*-dimethyl phosphorodithioate diethyl mercaptosuccinate, 95%, American Cyanamid Co., Princeton, NJ, *O,O*-diethyl *S*-(2-ethylthio) ethyl phosphorodithioate, 96.8%, Chemagro Corp., Kansas City, MO, and *O,O*-dimethyl *O-p*-nitrophenyl phosphorothioate, 98%, Velsicol Chemical Corp., Chicago, IL. The compounds were first dissolved in dimethyl sulfoxide (DMSO) in a concentration of either 10 or 100 mg/ml of DMSO just before use. As determined in a separate experiment, a DMSO concentration below 1% in the medium of human hematopoietic cultures had no observable effect on chromosomes or cell growth. In the present study, only 0.5% or less of DMSO plus a test compound in a culture medium was used.

The human hematopoietic cell lines, B411-4, RPMI-1788 and RPMI-7191 from the Cell Laboratory of Roswell Park Memorial Institute were used in this study. The three lines were all derived from the blood of three normal male individuals, line B411-4 was initiated in Nov., 1968, RPMI-1788 in Oct., 1968 and RPMI-7191 in Jan., 1971. Chromosome studies during the period of the present study showed that B411-4 and RPMI-7191 had normal male diploid constitutions while RPMI-1788 had a pseudodiploid karyotype.

Cultures in rapid growth were treated with a pesticide at several concentrations. At various times after treatment, portions of cells were withdrawn for cell viability counts (trypan blue exclusion) and chromosome study.

Colcemid at a concentration of 0.04 μg/ml was added to a culture generally 2 hr before harvesting for chromosome preparation. Cells were then treated with 1% sodium citrate and fixed in acetic acid–methanol (1:3) fixative. Flame dried slides were stained with Giemsa. Metaphase figures were studied under an oil objective. Clear chromosome lesions such as gaps, breaks, exchanges, dicentrics, pulverization, *etc.*, were recorded.

In each experiment, one culture with nothing added and one culture with only an equivalent amount of solvent DMSO added served as negative controls. Also, cultures were treated with a known chromosome breaking agent—methyl methanesulfonate (MMS)—at several concentrations to serve as a positive control.

The possible effect of methyl parathion

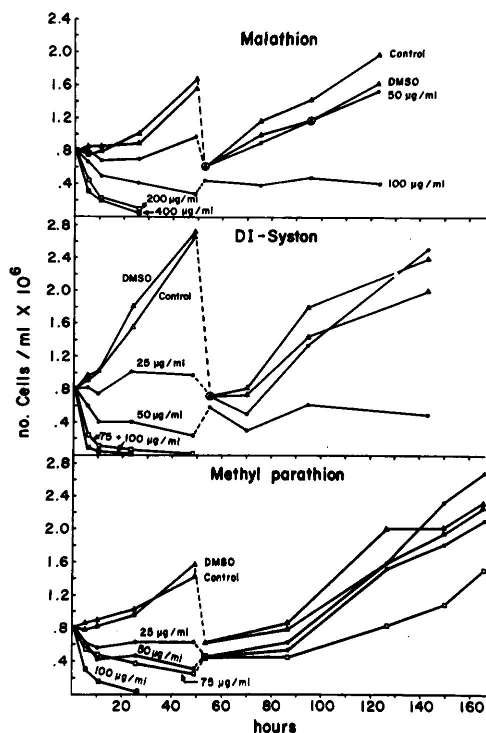

FIG. 1. The effect of various concentrations of the three insecticides on growth of human hematopoietic cells from line B411-4. (- - -) the cultures were centrifuged, washed and fed with insecticide-free medium at adjusted cell concentrations.

on chromosomes of cells *in vivo* was studied. ICR male mice at 2–3 mo of age were injected intraperitoneally with 5, 10, 20, 50 or 100 mg of the compound/kg of body weight. Two animals were used for each dose. Mice with nothing injected or injected with an equivalent amount of DMSO were used as controls. Colchicine solution (0.04%) was injected in the animals at 0.01 ml/g of body weight 22 hr after injection of the pesticide. All animals were killed 24 hr after initial injection. Bone marrow tissue from two femurs was taken out for chromosome study (3).

Results. Each of the three organophosphorus insecticides inhibited cell growth, generally in proportion to the concentration. Figure 1 shows the rate of inhibition after exposure of cells from line B411-4 to the three insecticides. A similar effect on cell growth was observed when the other cell lines were treated with the compounds. In the cultures treated with a relatively low dose of an insecticide, 25 µg/ml of DI-syston or methyl parathion, 50 µg/ml of malathion (Fig. 1), cell growth was observed but at a much lower rate compared with the controls or cultures treated with DMSO.

After about 50 hr of treatment by a pesticide, cultures were centrifuged, washed once with fresh medium, and fed with pesticide-free medium at an appropriate cell concentration (Fig. 1). In the cultures treated with methyl parathion, a rapid normal growth resumed after the removal of the compound, except for the culture treated with 100 µg/ml methyl parathion which died off within 1 day. Resumption of normal growth was also observed in cultures treated with malathion at a concentration of 50 µg/ml and DI-syston at 25 µg/ml. At higher doses cultures either died off within 2 days of treatment or no noticeable resumption of cell growth occurred.

In each sample harvested at different time intervals following a treatment with a pesticide, 100 metaphases were selected for chro-

TABLE I. Incidences of Metaphases with Chromosome Aberrations in the Cultures of Line B411-4 Treated with Malathion.[a]

Time (hr)	Concn (µg/ml)	Metaphases (%) with single gap or break
6	0 (control)	4
	0 (DMSO)	3
	50	2
	100	5
	200	No mitosis
	400	No mitosis
12	0 (control)	3
	0 (DMSO)	1
	50	3
	100	4
24	0 (control)	3
	0 (DMSO)	2
	50	5
	100	2
50	0 (control)	2
	0 (DMSO)	2
	50	0
	100	5[b]

[a] A total of 100 metaphases were examined in each treatment at all time intervals.

[b] One of the metaphases had multiple aberrations.

TABLE II. Chromosome Aberrations Induced by Treatment of Methyl Methanesulfonate in Cell Line B411-4.[a]

Time (hr)	Concn (μg/ml)	Cells (%) with gaps or breaks	Cells (%) with pulverization
6	10	3	1.0
	20	4	2.3
	40	18	14.5
	80	No mitosis	—
12	10	8	1.4
	20	11	31.2
	40	25	74.0
24	10	8	2.0
	20	18	3.1
	40	35	—
48	10	15	75.8
	20	No mitosis	—
	40	No mitosis	—

[a] The percentage of cells with 1–2 breaks in controls and DMSO treated cultures was 1–5% at all time intervals; percentage of cells with pulverized chromosomes was 0–1%. To determine percentage of cells with gaps or breaks, a total of 100 metaphases were studied except in the culture treated with 40 μg/ml for 24 hr, in which only 25 metaphases could be scored. For percentage of cells with pulverization, a total of 300–400 randomly selected metaphases were examined.

mosome aberration study. In some samples however, due to the severe growth inhibition by the treated compound, only a few metaphases could be scored. The result of chromosome studies in cultures treated with any one of the three compounds at any concentration did not show an increase in the incidence of metaphases with chromosome aberrations as compared with controls or cultures treated with DMSO. Table I shows an example of no increase in chromosome damage after cultures of B411-4 were treated with malathion. Similar results were obtained in experiments which involved either B411-4 treated with the other two compounds or other cell lines treated with one of the three compounds.

In the positive controls, cultures of line B411-4 were treated with 10, 20, 40 and 80 μg of MMS/ml of medium. Inhibition of cell growth and induction of chromosome aberrations were observed in all doses (Table II). Cells in cultures treated with 80 μg/ml of MMS died off within 2 days of treatment. In the slides prepared at 6 hr after treatment with 80 μg/ml, no mitosis was found. One day after treatment with 40 μg/ml, mitosis was extremely rare. An initial cell growth was observed in the cultures treated with 10 and 20 μg/ml. However, growth in these cultures decreased drastically by the second day after treatment and all cells were dead within 3 days for the culture treated with 20 μg/ml and 4 days for the culture treated with 10 μg/ml.

The cells with chromosome aberrations induced by MMS can be easily grouped into two categories. In the first, most of the cells had a single chromosome or chromatid gap or break. There were only a few cells with two or more aberrations per cell (Fig. 2a). In the second group, the cells had pulverized chromosomes (Fig. 2b). In such cells, chromosomes were completely fragmented and beyond recognition. The incidence of cells with chromosome aberrations is correlated with the dose and time except in the sample of the culture treated with 20 μg/ml for 24 hr which had fewer cells with chromosome pulverization than the sample harvested at 12 hr after treatment.

ICR male mice injected with methyl parathion at doses of 50 and 100 mg/kg of body weight died within 1 hr of injection. Mice injected with 5, 10, 20 mg/kg all lived 24 hr after injection. One hundred metaphases from bone marrow preparation from each animal were studied. The incidence of metaphases with a single aberration was either 1% or zero in both experimental and control animals.

Discussion. The results obtained show that the three organophosphorus insecticides studied are inhibitory to the growth of human hematopoietic cell lines at the lowest concentrations tested. DI-syston and methyl parathion seemed to have a similar magnitude of inhibition while malathion had the least (Fig. 1). Gabliks and Friedman (2) reported the effect of several organophosphorus insecticides, including malathion and DI-syston on cultured cells of Chang liver, HeLa strain, mouse liver and mouse skin fibroblasts. They found that all the compounds tested were toxic to the cells. The

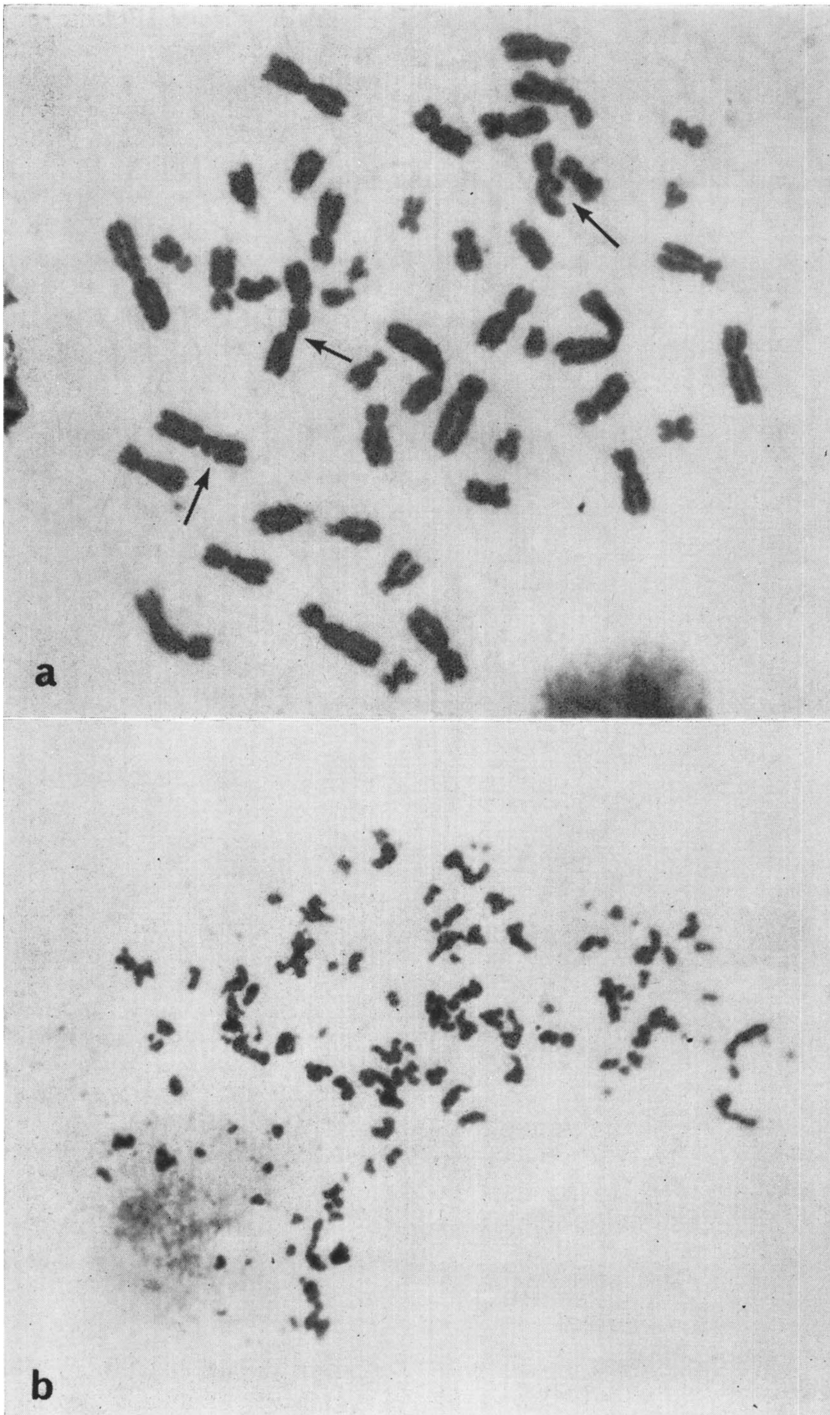

FIG. 2. Chromosome damage observed in the positive control, induced by treatment with methyl methanesulfonate in line B411-4: (a) a cell with chromatid aberrations in three chromosomes (arrows, 20 μg/ml, 12 hr); (b) chromosome pulverization (10 μg/ml, 48 hr).

magnitude of effect, however, varied with the origin of the cells and the compound. For instance, the mouse liver cells were much less susceptible to the treatment with malathion than any other cell cultures. Malathion was less toxic than DI-syston in all cells tested. Wilson and Walker (5) found that growth of cultured cells of chick embryos was inhibited at a dose as low as 10 μg/ml of malathion.

It is widely accepted that the effect of organophosphates in animals is primarily by inhibition of acetylcholine esterase in the nervous system (4). The mode of action of these compounds in cultured cells, however, is virtually unknown. It is unlikely that the effect of organophosphorus insecticides *in vitro* was also due to an inhibitory action on acetylcholine esterase. Nevertheless in the cultured cells these compounds may interfere with other enzyme systems that could be important to cell survival.

The inhibitory effect of the insecticides to the cell growth was not permanent but was readily reversible. The rapid resumption of cell growth following removal of a pesticide and lack of chromosome damage at all time intervals and concentrations indicates that these compounds might only temporarily interfere with some enzyme system(s) with no detrimental effect to genetic material.

It has been pointed out that some organophosphorus insecticides may react with DNA by transalkylation and therefore are possibly mutagenic (1). Failure to observe chromosome damage in the pesticide-treated cultures in the present study, however, does not necessarily indicate that these compounds and their metabolites do not possess mutagenic potential. Further tests of such popular insecticides in other systems should be undertaken.

Injection of methyl parathion into mice at doses of 5, 10, and 20 mg/kg of body weight for 24 hr caused no increase in incidence of cells with chromosome aberrations in the bone marow. Because of the sensitivity of the nervous system to the organophosphates or their metabolites in intact animals, it is difficult to investigate the response of other cells and tissues to such compounds. For instance the doses of 50 and 100 mg/kg of methyl parathion used in this study killed the mice within 1 hr, very likely due to nervous system toxicity. Therefore, one could not study the response of other tissue to the insecticide at these or higher doses.

Summary. Growth of three human hematopoietic cell lines was inhibited by the treatment of any one of the three organophosphorus insecticides used, namely malathion, DI-syston and methyl parathion. Cell growth usually resumed the normal rate after removal of the medium containing the insecticide. Chromosome studies in cultures treated with any one of the insecticides did not show an increase in the incidence of metaphases with chromosome aberrations compared with negative controls. Cultures were treated with a known chromosome breaking agent, methyl methanesulfonate, to serve as a positive control. An inhibition of cell growth and high incidence of chromosome aberrations was observed in these cultures. Methyl parathion was injected into ICR mice at several dose levels and the animals were sacrificed for examination after 24 hr. No increase in incidence of cells with chromosome aberrations was found in the bone marrow.

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