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A COMPARATIVE STUDY OF THE CYTOGENETIC EFFECTS OF THE INSECTICIDES HEPTACHLOR, MALATHION, AND METHYL PARATHION IN *Vicia faba*

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ABSTRACT

In order to determine the potential cytogenetic effects of Heptachlor, Malathion and Methyl Parathion, meristems of broad bean root tips were treated with several concentrations of these insecticides for different periods of treatment and recovery.

Heptachlor and Malathion induced chromosomal alterations in anaphase cells as fragments and bridges, chromosomes with inactivated centromeres and isochromosomes, and as micronuclei in interphase cells. Multipolar anaphases also appeared when the mitotic spindle was damaged. Heptachlor induced pycnosis but Malathion did not.

Chromosomal aberrations caused by Methyl Parathion were observed in cells only during metaphase, since a strong c-mitotic effect was induced mostly in the form of fragments. Longer recovery periods (42 and 44 h) revealed tetraploid and pycnotic cells.

Only in the case of Malation was there an increase in chromosome alterations with higher concentrations. However, no such dose response relationship was observed for micronuclei frequency.

RESUMEN

Con el fin de establecer el daño genético que ocasionan el Heptacloro, el Malatión y el Metil Paratión, se realizaron tratamientos sobre las células meristemáticas de la raíz de haba con diversas concentraciones de estos insecticidas y diferentes tiempos de tratamiento y recuperación.

El Heptacloro y el Malatión provocaron alteraciones en los cromosomas que fueron evaluadas en anafase como fragmentos y puentes, cromosomas con el centrómero inactivado e isocromosomas y en células en interfase como micronúcleos. También se observaron anafases multipolares por daño al huso mitótico. En algunas concentraciones de Heptacloro se presentó el fenómeno de picnosis, pero no con Malatión.

Con el Metil Paratión, el registro de aberraciones se realizó en células en metafase debido a que se produjo un fuerte efecto c-mitótico, encontrándose sólo fragmentos. En los tiempos de recuperación mayores (42 y 44 horas) aparecieron células tetraploides y picnosis.

Unicamente en el caso del Malatión se obtuvo incremento de las alteraciones al aumentar la concentración, excepto para micronúcleos.

INTRODUCTION

It has been widely reconognized that chemical pollutants (i.e. pesticides), represent a genetic hazard for mankind important enough to justify studies on their cytogenetic effects. Many of these chemicals used for insect, weed, and disease control, can de considered environmental mutagens (Grant 1973). Among the most commonly used insecticides, which are also of economic importance, are the organophosphorus and organochlorine insecticides.

A number of organochlorine insecticides have had a great impact on ecology, not only because of their persistence in the environment for extended periods of time, but also for their high accumulation potential in living organisms (Maslansky and Williams 1981, Siddiqui *et al.* 1981). An example of this group of compounds is Heptachlor, which has been used for the extermination of grasshoppers, locusts, soil mosquitoes and still other insects (Negherbon 1959, Barbera 1976).

Most organochlorine compounds cause serious harmful effects to certain tissues of animals (i.e. rodents) fed with crops contaminated with these agents. In mammals, and so in humans, these compounds accumulate in several tissues and are detectable in milk and urine (Randaleff 1970, De la Jara and De la Parra 1977, Sddiqui *et al.* 1981). Human faty tissue and blood have been used to measure traces of organochlorine insecticides (Davies 1973).

Clinically, symptoms of organochlorine poisoning are headache, dizziness, hyperirritability, indisposition, nausea, anorexy, vomiting and sometimes muscle contractions, myoclonic spasms, and convulsions (De la Jara and De la Parra 1977).

The precise mode of attack of these insecticides in insects and mammals is still unknown. They may act as toxins on the central nervous system, and may cause a wide variety of neuromuscular symptoms and altered behaviour. Chronic exposure has caused degenerative changes in almost all the vital organs of mammals, and especially in liver kidneys (Randeleff 1970).

The evaluation of the possible mutagenic effects of Heptachlor has been based on the results of dominant lethal assays and on the records of cytogenetic alterations in rat bone marrow cells (Cerey *et al.* 1973). Data from these studies showed that there was an increased number of resorbed fetuses and chromosomal abnormalities, i.e. translocations and DNA aberrations. The effect of Heptachlor was followed through three generations in order to detect it's effects on reproduction. The rat fetuses showed abnormalities in early and late stages of development due to ingestion of Heptachlor by their mothers (Cerey *et al.* 1973).

Two of the most widely used organophosphorus insecticides are Malathion and Methyl Parathion. Both have been applied to bean, corn, sorghum, and tobacco crops to exterminate, green flies, harvest bugs, and other insects.

These insecticides inhibit the enzimatic activity of cholinesterase, which is responsible in hydrolyzing the acetylcholine generated in axon terminals to choline. When active cholinesterase is not present in axon terminals, there is an accumulation of

acetylcholine which blocks normal transmission of nerve impulses at the synapse, and which can cause loss of muscular coordination, convulsion sand death (Randeleff 1970, Cremlyn 1982).

Acute poisoning by organophosphorus compounds presents symptoms similar to an over-stimulation of the parasympathetic nervous system (Toivonen *et al.* 1959); as a result, this group of compounds has been classified as cholinergic stimulants (Gershon and Shaw 1961, Randeleff 1970).

Malathion, can cause chromosomal damage in rat bone marrow cells, spermatogonia, and spermatocytes (Degraeve *et al.* 1979). When Malathion is administered to rats for two generations, there is a decrease in progeny survival and body weight (Kalow and Marton 1961). Malathion also inhibits cell growth in primary cultures of chicken embryo fibroblasts (Wilson and Walker 1966).

Meinél (1974) has shown that parathion causes serious morphological abnormalities in the cervical region of the spine of developing chicken embryos. Lethal effects of Parathion on the offspring of treated female rats has been demostrated (Lobdell and Johnston 1966). Parathion supresses rat fetal development, ossification, and produces cleft palates and fetal death in mice (Tanimura *et al.* 1967).

Both Malathion and Methyl Parathion cause schizophrenic depression and severe memory damage in humans (Gershon and Shaw 1961) and human death (Van Bao *et al.* 1974, Diggory *et al.* 1977, De Berr *et al.* 1980). There are few studies on the cytogenetic effect of these insecticides on humans. A Malathion poisoned individual yielded a larger number of chromosomal aberrations (Yoder *et al.* 1973, Van Bao *et al.* 1974). Sister chromatid exchanges, and the delay of the cell cycle were induced in human cell cultures (Nicholas *et al.* 1979, Chen *et al.* 1981).

Mice skin exposed to Malathion induces micronuclei formation in bone marrow cells (Dulout *et al.* 1982), but on the other hand, no clastogenic effects were observed in peripheral blood cultures from men occupationally exposed to Methyl Parathion (de Cassia Stocco *et al.* 1982).

As it is not possible to use human subjects for this kind of cytogenetic study, we can gain an idea of the cytogenetic hazards to humans by observing the cytogenetic effects on mammals, plants, or microorganisms. In order to detect chromosomal damage induced by insecticides, *Vicia faba* is a highly suitable experimental material. It has advantages such as large sized chromosomes few in number ($2n = 12$), as well as a short cell cycle (Evans and Scott 1964).

Since Heptachlor, Malathion and Methyl Parathion are among the most commonly used insecticides, it is important to evaluate their cytogenetic effects, as well as their concentration response rates. This can be readily achieved by using the chromosomes in the meristematic cells of *Vicia faba* roots.

MATERIALS AND METHODS

Seedlings of broad beans (*Vicia faba* var. major; serial no. C-69-9) with primary root lengths of 4 cm were exposed to different concentrations of: water mixtures of Heptachlor (heptachlortetrahydro-4,7-methanoindene, active ingredient purity 150 g/Kg, Fito-Terra) 50, 75, 100, 125 and 200 ppm; Malathion (diethyl, mercapto succinate, S-ester with 0,0-dimethyl phosphorodithioate, 50 E, Agricultura Nacional, S. A., whose active ingredient purity was 515 g L 50, 75, 100, 125, 200 and 300 ppm; and Methyl Parathion (0,0-dimethyl-0-P-nitrophenyl phosphorothioate,

Bayer, whose active ingredient purity was 500 g L 30, 60, 75, 90 and 120 ppm. Broad been root tips were treated for 1, 2 and 3 h without recovery, and 4 hours with 2, 14, 18, 42 and 44 h of recovery, in a continuos flow, tap water bath of constant temperature (19°C), and aeration. Since Heptachlor is not completely water soluble, it's solutions were constantly stirred to mantain concentration homogeneity during treatment.

Control samples were kept under the same experimental conditions as treated samples, except that distilled water was used in place of solutions of the insecticides. Concentrations of the three insecticides were considerably lower than those recommended for pest control by manufacturers.

All slides were prepared using the Villalobos-Pietrini technique (1965) with some modifications (Gómez-Arroyo 1980), and made permanent using the dry ice technique (Conger and Fairchild 1953).

Cell analysis during anaphase was used to identify chromosome aberration as well as alterations in the centromere and in mitotic spindle behavior. The slides of roots treated with Heptachlor, and with Malathion, and those of the control samples, were examined in order to identify chromosomal alterations in anaphase cells, whereas slides of roots treated with Methyl Parathion were examined for chromosomal alterations in metaphase cells. Two thousand interphase cells from each treatment and recovery period were examined randomly. Pycnotic cells were found in some slides. The data presented here corresponds to one experiment and it's replicate.

RESULTS

Chromosome aberrations caused by Heptachlor and Malathion in *Vicia faba* root meristems were characterized during anaphase by the presence of chromosome, chromatid and subchromatid types of aberrations, the occurence of chromosomes with inactivated centromeres, isochromosomes, as well as multipolar anaphases. Micronuclei were observed in interphase cells.

In short treatment periods, Hepatachlor and Malathion produced subchromatid and chromatid aberrations (fragments and single bridges) within the first few hours. When broad bean roots were treated for 4 h with 14 h of recovery time, chromosome aberrations such as double fragments, and double bridges were noticed. Malathion concentrations of 200 and 300 ppm inhibited mitosis.

Centromeric damage was evident in all treatments and recovery periods, and yielded cromosomes with inactivated centromeres and isochromosomes. Multipolar anaphases due to the alterations of mitotic spindles, were observed in most of the treatment and recovery periods with Heptachlor, but only during the first hours of treatment with Malathion.

With some exceptions, micronuclei were observed within the first hours of treatment using all three insecticides in this study. Pycnosis was induced after 4 hours of treatment with Heptachlor at concentrations of 100, 125 and 200 ppm, and recovery periods of 18, 42 and 44 h.

The average of alterations caused by 50 to 250 ppm Heptachlor is shown in Table I. No increase in alteration frequencies was noted with concetrations higher than 50 ppm.

The alteration values obtained with different concentrations of Malathion are

TABLE I. ALTERATIONS (—CONTROL) INDUCED BY HEPTACHLOR ON *Vicia faba* CHROMOSOMES

Concentration (ppm)	*Total anaphases*	*Agnormal anaphases %*	*Total aberrations %*	*Chromosomes with inactivated centromeres %*	*Isochromosomes %*	*Multipolar anaphases %*	*Mieronuclei %*	*Pycnosis*
50	3923	3.91	1.62	1.30	1.69	0.20	0.18	—
75	7726	3.04	0.54	0.40	2.24	0.64	0.34	—
100	6942	1.77	1.41	0.53	0.89	0.10	0.28	+
125	4594	2.62	1.51	0.87	1.04	0.03	0.48	+
250	3162	3.84	1.35	1.45	1.13	1.00	0.36	+

listed in Table II. Root treatment with higher concetrations of Malathion decreased the frequency of abnormal anaphases and total aberrations, except in treatments where the concentration was 125 ppm. Aberrations increased positively with the dosage.

TABLE II. ALTERATIONS (—CONTROL) INDUCED BY MALATHION ON *Vicia faba* CHROMOSOMES

Concentration (ppm)	*Total anaphases*	*Abnormal anaphases %*	*Total aberrations %*	*Chromosomes with inactivated centromeres %*	*Isochromosomes %*	*Multipolar anaphases %*	*Micronuclei %*
50	13215	1.25	0.78	0.50	0.46	0.03	0.56
75	10575	1.79	1.38	0.60	0.74	0.09	0.53
100	11630	5.36	5.05	1.81	1.90	0.52	1.11
125	9698	5.16	2.46	2.09	1.54	1.67	0.74
200	2779	19.74	11.20	4.46	3.90	11.47	1.21
300	1679	26.72	11.48	4.16	4.04	17.97	0.93

Methyl Parathion induced chromosome breakage in meristematic cells of *Vicia faba* roots when concentrations of 30, 45, 60, 75, 90, and 120 ppm were used for different recovery periods. The estimation of such aberrations was done in cells during metaphase due to the presence of a c-mitotic effect. Chromosome aberrations caused by Methyl Parathion were observed within the first hours of treatment.

Data averages are shown in Table III. Notice that a high number of single fragments were found and that, even though the higher abnormality frequencies were caused by the higher concentrations (90 and 120 ppm), there is no dose-

effect response. After 4 h of treatment, with 42 and 44 h recovery periods, and using concentrations of 60, 75, 90 and 120 ppm, tetraploid cells were observed.

On the other hand, nuclear destruction (pycnosis) was observed in all concentrations after 4 h of treatment with 42 and 44 h recovery periods.

TABLE III. ALTERATIONS (—CONTROL) INDUCED BY METHYL PARATHION ON *Vicia faba* CHROMOSOMES

Concentrations (ppm)	*Total metaphases*	*Abnormal metaphases %*	*Total aberrations (Single fragments) %*	*Tetraploid cells*	*Micronuclei %*	*Pycnosis*
30	559	3.19	2.75	—	0.25	+
45	995	1.11	2.62	—	0.37	+
60	1278	1.98	2.77	+	0.11	+
75	1169	1.06	1.47	+	0.25	+
90	776	4.47	5.68	+	0.12	+
120	1361	2.39	3.60	+	0.22	+

DISCUSSION

Heptachlor, Malathion and Methyl Parathion induced aberrations in *Vicia faba* root meristem chromosomes during the first hours (1, 2, and 3 h) of treatment. According to Kihlman (1966), these kinds of agents belong to chemicals which produce non-delayed effects.

Chromosome and chromatid types of aberrations were observed with all three insecticides. Heptachlor and Malathion produced subchromatid aberrations within the first hours of treatment as well. These insecticides are to be considered S-independent agents, because they produce different types of aberrations and have a non-delayed effect. This means that chromosomal abnormalities caused by these agents did not requiere DNA synthesis, and that the type of aberration induced depends on the stage of the cell cycle initially treated.

Since Heptachlor induced chromosome and chromatid types of aberrations, it's behavior is similar to other organochlorine insecticides, such as: aldrin, which produced the same types of aberrations in human lymphocyte cultures (Georgian 1975), and endrin, which promoted the appearance of single and double fragments, and single anaphase bridges (Wuu and Grant 1966). These results are similar to those obtained for the Heptachlor treatment of *Vicia faba* roots in this study.

Malathion concentrations ranging from 50 to 400 μg/ml caused cytotoxic effects but not chromosomal breakage in Chinese hamster cell cultures (Huang 1973), and concentrations ranging from 10 to 30 μg/ml produced chromosomal damage in human lymphocyte cultures (Walter *et al.* 1980). Malathion used in non-toxic concentrations (2.5-40 μg/ml) increased sister chromatid exchange frequencies in human lymphocyte cultures (Nicholas *et al.* 1979).

In this study, Malathion and Heptachlor had a similar effect in regard to time of ocurrence and type of aberration caused, since both appeared early, and caused both single and double chromosomal fragments. The c-mitotic effetc of Methyl Parathion in this study was in accordance with that also observed by de-Kergammeaux *et al.* (1983) in *Vicia faba* root tips. This c-mitotic effect results in metaphase accumulation, because, avoiding anaphases, only metaphase cells were observed, some of them with single and double chromosome fragments. These same kinds of fragments were described by Wuu and Grant (1966) in barley anaphase cells treated with Malahtion, and by Georgian (1975) in metaphase stages of human lymphocyte cultures treated with Phosphamidon. This evidence indicates that they belong to the group of S-independent agents.

Dichlorvos and Vamidothion, organophosphorus insecticides, produce only chromatid type aberrations (Tezuka *et al.* 1969). Maybe the alkylating properties of this kind of insecticide (Preussmann *et al.* 1969, Bedford and Robinson 1972) induced S-dependent aberrations in which chromosome duplication was needed without regard to the stage of the cell cycle when treated.

In this study, Malathion and Methyl Parathion were S-independent. The results do not support S-dependency. This behavior was not expected, and probably means that these insecticides did not have a direct effect on DNA, but rather interact with lysosome membranes, in agreement with the observations made by Allison and Paton (1965) in mammalian cells in which the liberation of hydrolytic enzymes caused lysosomic breakage and chromosome fragmentation. Gray (1970) reported that DDT caused the breakdown of lysosomes, and hence, according to Clark (1974), it is possible that the weakly mutagenic effect of DDT observed in mice (Swiss albino), and *D. melanogaster,* are mediated by lysosomes.

When lysosomes are damaged, liberated ezymes also capable of promoting chromosomal abnormalities, can mask the direct effect of these insecticides on DNA.

Malathion and Heptachlor also produced centromeric abnormalities yielding chromosomes with inactivated centromeres (Gómez-Arroyo and Villalobos-Pietrini 1983), and yielding isochromosomes which originated from transverse breaks at the centromere (Ramanna and Natarajan 1966, Nicoloff and Gecheff 1976); the apprearance within the first hours of treatment of these types of abnormalities was directly related to the concentration of insecticides applied, so that they had a direct and inmediate effect on the centromere. Independent of how these abnormalities were caused, these chromosomes were not included in normal anaphase kinetics, and micronuclei and aneuploid cells were produced. Wuu and Grant (1966) observed that Endrin an organochlorine insecticide, produced the same type of centromeric alterations.

Heptachlor induced damage to the mitotic spindle causing multipolar anaphases. Giménez-Martín and López-Sáez (1960, 1961) working with Lindane, and Wuu and Grant (1966) working with Endrin, obtained similar results when these two insecticides were applied to root meristems of *Allium cepa* and *Hordeum vulgare.*

The induction of more than one spindle produced cells with different chromosomal numbers possibly leading to polynucleated cells and aneuploidy, conditions which are lethal to daughter cells when widely spread (Brachet 1975).

Malathion and Methyl Parathion acted on the mitotic spindle, the former inducing multipolar anaphases and the later producing an effect similar to colchicine to inhibit spindle development during metaphase. These results agreed with data obtained by Veleminsky and Gichner (1963) in their study of the organophos-

phorus insecticide Systox, which inhibited the formation of the mitotic spindle, and that of Georgian (1975) where Phosphamidon altered spindle proteins in human lymphocyte cultures.

The induction of c-metaphases by Methyl Parathion promoted the appearance of tetraploid cells in the following cycle of development. This is similar to the effect of Dichlorvos and Vamidothion in Chinese hamster cell cultures (Tezuka *et al.* 1980).

Another valuable end-point in revealing cytogenetic effects caused by Heptachlor, Malathion and Methyl Parathion, is micronuclei frequency. Micronucleic analysis measures the production of acentric fragments, chromosomes with inactive centromeres, and isochromosomes (Evans *et al.* 1959, Schmid 1975). Micronucleic analysis is very useful for a rapid estimation of chromosomal damage caused by both physical and chemical agents.

In this study, Heptachlor, Malathion and Methyl Parathion did not significantly increase micronuclei frenquency. In the case of Heptachlor, our results were in agreement with those obtained by Usha-Rani *et al.* (1980) using other organochlorine pesticides in the bone marrow cells of mice but in disagreement with their findings on organophosphorus insecticides.

A relationship between concentrations and abnormal anaphases, total aberrations and multipolar anaphases, was only found in treatments with Malathion.

The cytotoxic effects of Heptachlor and Methyl Parathion, were revealed by the appearence of pycnosis due to nuclear destruction resulting from 4 h treatments with long recovery periods for all concentrations. This was also described by Cerey *et al.* (1973) as chromatid pulverization in bone marrow cells of mice when Heptachlor was administered orally. Pycnosis was also induced by Endrin (Njagi and Gopalan 1981).

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