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CYTOLOGICAL EFFECTS OF SOME CARBAMATE INSECTICIDES. I. METHOMYL AND OXAMYL IN *Vicia faba*

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ABSTRACT

The cytological effects of two carbamate insecticides, methomyl and oxamyl, were analysed in the meristematic cells of the root tips of *Vicia faba*. The former induced chromosomal alterations which were analyzed in anaphase cells as chromatid aberrations (single fragments and bridges), chromosomes with inactivated centromeres, isochromosomes and disturbances in the mitotic spindle as multipolar anaphases, as well as micronuclei scored in interphase cells. Only the chromatid aberration frequencies augmented with the increment of concentration of methomyl. In relation to its action upon cell division, it was observed that methomyl had an inhibitory effect demonstrated by the low mitotic index. On the other hand, oxamyl at the concentrations tested did not induce clastogenic alterations neither produce any effect on the mitotic index.

RESUMEN

Los efectos citológicos de dos insecticidas carbámicos, metomilo y oxamilo, fueron analizados en las células meristemáticas de la raíz principal de *Vicia faba*. El primero indujo alteraciones cromosómicas que fueron identificadas en células en anafase como aberraciones cromatídicas (fragmentos y puentes sencillos), cromosomas con el centrómero inactivado, isocromosomas y disturbios en el huso mitótico como anafases multipolares, también se observaron micronúcleos en células interfásicas. Unicamente las frecuencias de aberraciones cromatídicas aumentaron con el incremento de las concentraciones de metomilo. Con respecto a su acción sobre la división celular, se notó que este insecticida causó inhibición, demostrada por el bajo índice mitótico. Por otro lado, el oxamilo a las concentraciones analizadas no provocó alteraciones clastogénicas ni afectó el índice mitótico.

INTRODUCTION

The methyl carbamate pesticides have been available for use since 1957. These compounds are an important group of environmental chemicals used as insecticides, fungicides or herbicides. Several of these agrochemicals have been shown as mutagens in different test systems and no general conclusion can be drawn from the data available, as the results vary according to the test and the compound employed (Moutschen-Dahmen *et al.* 1984).

Methomyl is a carbamate insecticide extensively used in Mexico for the control of insects pests in crops of tobacco, cotton and varieties of fruits and vegetables. The genotoxicity of this compound has been described in some reports (Blevins *et al.* 1977, Valencia 1982, Hemavathy and Krishnamurthy 1987a, b) but unfortunately the results from these studies are not conclusive.

Oxamyl is another carbamate pesticide which is applied to similar plant species and which controls insects, nematodes and acarids. No reports were available in the literature about the potential clastogenic or other cytological effects of this chemical.

Considering the paucity of information about the cytogenetic effects of methomyl and the lack of reports in the case of oxamyl, the present studies were undertaken to determine the cytogenetic activity of these insecticides on the root tip meristematic cells of *Vicia faba* which is recommended as one of the most suitable bioassays in the GeneTox Programme for the most pressing cases.

MATERIAL AND METHODS

Treatments were made to 3-4 cm primary roots of *Vicia faba* (var. minor) seedlings. Concentrations of 500, 1000, 2000 and 3000 ppm of methomyl (Lannate 90) and oxamyl (Vydate L-24), both purchased from Du Pont, whose purity of the active ingredients was 90% and 24% respectively, were applied for 4 hours with 18 and 44 hours of recovery, in a continuous flow of tap water at 19°C and aeration. All the steps were carried out in the dark. For a comparative analysis, concentrations and exposure times were selected from preliminary experiments with methomyl and, the same concentrations were used for oxamyl. In a preliminary treatment with methomyl, a group of root tips were exposed for 3 hours without recovery time and no cytological effects were detected.

For microscopic observation the root tips were stained with Schiff's reagent. Slides were made permanent with liquid air to freeze the tissue (Meneses *et al.* 1991), followed by two changes of butanol and mounted in Canada balsam. The slides were handled by code in order to avoid knowing to which group (control vs treated) they belonged. The data presented in this paper correspond to one experiment and its replicate.

Chromosomal analyses were made in anaphase cells in order to identify chromosome alterations (bridges and fragments) as well as alterations in the centromere (chromosomes with inactivated centromeres and isochromosomes) and mitotic spindle disturbances, through the appearance of multipolar anaphases.

As micronuclei in interphase provide a simple method to detect the presence of chromosomal damage (Gómez-Arroyo *et al.* 1986), analyses of micronuclei were made in 2000 interphase cells.

It has been described that chromosomal alterations can lead directly to cell death (Schwartz 1989). It was therefore decided to calculate the mitotic index and to consider it as an indicator of such damage. This index was calculated following the analysis of 2000 cells per concentration.

RESULTS AND DISCUSSION

In meristematic root-tip cells of *Vicia faba* with methomyl, no chromosome type aberrations were found; only chromatid type aberrations (fragments and bridges) were detected. The increment of the frequencies were concentration related (Table I). In this study, fragments and bridges were induced by methomyl in all concentrations tested. Similar types of abnormalities were also observed in *Vicia faba* and *Gossypium barbadense* with the carbamate insecticide rogor (Amer and Farah 1974) and in *Vicia faba* after treatment with organophosphorus insecticides (Amer and Farah 1983, 1985).

The frequency of aberrations on chromosomes of *Vicia faba* decreased with the recovery time (Table I). Similar effects have been observed with the alkylating agents N-methyl-N'nitro-N-nitrosoguanidine (Schwartz 1989, Kaina *et al.* 1990) and with methyl methanesulfonate (Schwartz 1989). Presumably this behavior is due to the removal of adducts and/or repair of that alkylation damage during recovery which leads to DNA strand breaks and alkali-labile lesions. On the other hand, according to Beranek (1990), the differences observed with methomyl during different times of recovery, may possibly be the reflection of different sensitivity of the cells treated in the G_1-phase (18 hours of recovery time) and in the G_2-phase (44 hours of recovery time) which is in accordance with the alkylating activity of this insecticide.

Alterations in the centromere with methomyl included its inactivation, as well as its transverse breakage resulting in isochromosomes. Disturbances in the mitotic spindle, appeared as multipolar anaphases. None of these effects showed a consistent response in relation to the concentrations applied (Table I).

In addition to the above mentioned abnormalities, another valuable end-point revealing cytogenetic effects, was the micronuclei test. However, the findings in this study did not show a relationship between micronuclei production and the concentrations used. On the contrary, an asymptotic distribution was observed, making this assay a qualitative monitor to determine damage caused by this insecticide on *Vicia faba* root tip cells (Table I). These results agreed with those obtained by Gómez-Arroyo *et al.* (1985) with heptachlor, malathion and methyl parathion in the same assay system.

Methomyl also inhibited cell division in *Vicia faba* root meristems (Table II). A statistically significant and concentration-dependent decrease of the mitotic index (MI) in all cases suggested a mitodepressive action of this compound. Such an effect was also observed after treatment with other different pesticides in the same plant system (Abdel-Rahem and Ragab 1989, Adam *et al.* 1990).

Carbamates are known to inhibit proteins by binding to them (Rannug and Rannug 1984). It is possible that the same mechanism could be occurring with methomyl causing inhibition of some en-

TABLE I. FREQUENCIES OF ALTERATIONS (-CONTROL) INDUCED IN THE CHROMOSOMES OF *Vicia faba* AFTER 4 HOURS OF EXPOSURE TO DIFFERENT CONCENTRATIONS OF METHOMYL

Concentration (ppm)	Total anaphases	Abnormal anaphases %	Total chromatid aberrations %	Single fragments %	Single bridges* %	Chromosomes with inactivated centromeres %	Isochromosomes %	Multipolar anaphases %	Micronuclei** %
18 hours of recovery time									
500	11456	1.50	1.34	0.36	0.90	0.52	0.03	0.12	0.40
1000	7682	3.01	2.19	1.24	0.95	1.59	0.00	0.27	1.35
2000	4297	4.32	4.20	2.63	1.57	0.25	0.21	0.38	0.00
3000	1963	6.07	6.85	2.01	4.84	0.44	0.05	0.38	0.85
44 hours of recovery time									
500	9398	1.12	0.49	0.40	0.15	0.09	0.00	0.21	0.40
1000	5516	1.82	1.78	0.44	1.43	0.00	0.05	0.06	0.15
2000	3346	3.16	3.05	1.78	1.32	0.14	0.00	0.18	0.30
3000	1446	3.61	5.16	1.14	4.07	0.00	0.00	0.00	0.20

* With and without fragments

** 2000 cells analyzed in interphase for each treatment

Note: 19881 anaphases were observed in the control following 18 hours recovery and 18185 in the 44 hours recovery period

zymes leading to DNA damage, and therefore, to clastogenic and physiological events.

On the other hand, oxamyl in this study did not induce clastogenic alterations or any effect on the MI. This may be due to the fact that the concentrations herein employed were insufficient to cause cytological damage or that the chemical itself may be nonclastogenic.

The differential mutagenic action of these chemicals may be related to their physicochemical properties. Metabolic transformations will influence the types and the relative frequencies of cellular reaction products and hence their biological effects (Lawley 1976).

Elespuru *et al.* (1974), established that differences in the mutagenic potency of three N-Methyl-N-nitroso compounds including the nitroso derivative of carbaryl, a carbamate pesticide, may be due to formation of different reactive intermediates within the cell or to differential uptake of the compounds, governed by the structure of the rest of the molecule. Besides lipid solubility is a factor influencing biological activity. The methyl carbamate group of carbendazim is suspected to play an important role in spindle inhibitory action (Seiler 1976) and other alterations (Grover *et al.* 1988, Pandita 1988).

Since both methomyl and oxamyl are methyl carbamate compounds and the only difference in their molecules are the radicals CH_3- in the former and $(CH_3)_2NCO$ in the latter, it may be suspected

TABLE II. MITOTIC INDEX OF *Vicia faba* ROOT TIPS EXPOSED DURING 4 HOURS TO DIFFERENT CONCENTRATIONS OF CARBAMATE INSECTICIDES

Methomyl			Oxamyl		
Concentration (ppm)	MI %	z value	Concentration (ppm)	MI %	z value
		18 hours of recovery time			
0	20.05		0	9.95	
500	15.45	3.81*	500	9.15	0.86 ns
1000	9.60	9.30*	1000	9.70	0.27 ns
2000	6.75	12.35*	2000	10.55	-0.63 ns
3000	4.85	14.57*	3000	10.45	-0.52 ns
		44 hours of recovery time			
0	19.00		0	12.00	
500	12.10	6.02*	500	11.75	0.24 ns
1000	7.75	10.49*	1000	12.65	-0.63 ns
2000	6.10	12.31*	2000	12.20	-0.19 ns
3000	3.75	15.19*	3000	12.35	-0.34 ns

z (0.001) = ± 3.291
* inhibitory effect

z (0.10) = ± 1.282
ns not significant

that in some way these radicals are differentially envolved in the activity of these insecticides; methomyl is a clastogen for the meristematic cells of *Vicia faba,* whereas oxamyl is not. This conclusion confirms that the close structural similarity of these two chemicals does not always lead to a similar biological activity, although it might be expected to.

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