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CYTOLOGICAL EFFECTS OF SOME CARBAMATE INSECTICIDES. II. INDUCTION OF SISTER CHROMATID EXCHANGES IN *Vicia faba* BY LANNATE-90

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ABSTRACT

The carbamate insecticide methomyl-containing technical formulation of lannate-90 was tested for its ability to induce sister chromatid exchanges (SCE). Meristematic cells of *Vicia faba* root tips were used as test system. Treatments of 2 h with several concentrations of this insecticide were applied followed by the differential stain technique including 5-bromodeoxi uridine incorporation and the Feulgen reagent. The results showed a significant increase of SCE frequencies when compared with the control ($P<0.001$) and a concentration-response relationship was observed, thus sustaining that lannate-90 is an indirect acting genotoxic in *Victa faba*.

RESUMEN

El insecticida carbámico lannate-90, una formulación técnica a base de metomilo fue evaluado en su capacidad para inducir intercambios de cromátidas hermanas (ICH). Para este fin fueron usadas las células meristemáticas de la raíz principal de *Vicia faba* como sistema de prueba. Éstas fueron tratadas con diferentes concentraciones del plaguicida por dos horas y a continuación se siguió la técnica de tinción diferencial que incluyó la incorporación de 5-bromodesoxiuridina y del reactivo de Feulgen. Los datos mostraron un incremento significativo en las frecuencias de ICH ($P<0.001$), notándose una relación de concentración efecto. Así, se sostiene que el lannate-90 tiene actividad genotóxica indirecta en *Vicia faba*.

INTRODUCTION

Humans beings are constantly exposed to xenobiotics in drugs, foods, and, most importantly, in the environment. It is well established that several chemicals affect adversely human health. Most cancers are due to exposure to chemical found in tobacco, in food, and in the air and water. Active research is under way to determine the extent of exposure of humans to foreign chemicals (González and Gelboin 1993).

Pesticides constitute a major group of xenobiotics and are among the most widely used chemicals in agriculture (Rao *et al.* 1988). Their use has resulted in increased availability, improved quality, and lower prices of a large number of crops. Without pesticides, agricultural production would drop by 30 % to 50 % (Baker and Wilkinson 1990, Zilberman *et al.* 1991). However, the benefits of the use of pesticide must be considered in the light of increasing concerns regarding environmental degradation, worker safety, and public health (Dennis and Weisenburger 1993).

The widespread and increasing use of agrochemicals may cause unintentional deleterious effects on the environment. The occurrence of their residues in the food chain demand a careful monitoring since could be harmful to present and future generations (Hemavathy and Krishnamurthy 1987a). The possible mutagenic, carcinogenic or teratogenic properties of some of the agrochemicals has been shown.

Methyl carbamates are systemic as well as contact insecticides widely used everywhere. Methomyl is available in different formulations as water-soluble powder or liquid. Lannate-90 is a powder methomyl-based carbamate pesticide used for the control of a great variety of insect pest in diverse field crops. This interfere with the neural transmission blocking the enzyme cholinesterase in insects and other animals (Baron 1991).

To evaluate the damage at genetical level induced by many physical and chemical mutagens of the environment and to know its risk to human health, several test systems using bacteria, cultured mammalian cells, fungi, plants and mammals as target cells or target organisms have been described.

The methomyl genotoxicity has been evaluated by mean of several different assays (reviewed by Bonatti *et al.* 1994). Negative results have been obtained in most test systems, particularly with that detect gene mutations or DNA damage (Guerzoni *et al.* 1976, Simmon *et al.* 1976, 1977, Blevins *et al.* 1977a,b, Gopalan and Njagi 1981, Valencia 1981, Wojciechowski *et al.* 1982, Moriya *et al.* 1983, Sandhu *et al.* 1985, Hemavathy and Krishnamurthy 1987a). On the other hand it has been reported as an efficient numerical and structural chromosome alteration inducer

(Bolognesi *et al.* 1994). It did not show carcinogenic activity either in rats (Kaplan and Sherman 1977) or dogs (Baron 1991). Neither teratogenic or fetotoxic effects were observed in pregnant rats or rabbits (Baron 1991).

The use of higher plants as test systems for screening and monitoring environmental mutagens is widely know. Both genetic and chromosomal alterations can be analyzed, and this fact besides with other advantages, make them worthwhile biological test assays (Gómez-Arroyo and Villalobos-Pietrini 1995).

Valencia-Quintana *et al.* (1993) established positive correlations between cytogenetic damage induced in the meristematic cells of the root tips of *Vicia faba* after treatments with lannate-90 and vydatel-24. The former has proven to be a concentration related inducer of chromatid type aberrations. Another cytogenetic technique to study the effect of low chemical concentrations was the SCE assay system. The induction of SCE has been described as a rapid and sensitive end-point for genotoxicity test. In certain cases it can be induced by concentrations ten fold lower than those that produce chromosomal aberrations (CA) (Perry and Evans 1975, Stetka and Wolff 1976, Latt *et al.* 1981)

Vicia faba has been used in numerous studies to detect genotoxicity through CA (Valencia-Quintana *et al.* 1993, Gómez-Arroyo and Villalobos-Pietrini 1995) and SCE (Kihlman and Andersson 1984, Gómez-Arroyo *et al.* 1988a,b, 1989, 1997)

Due to the exposure to the carbamate pesticides as agricultural contaminants with alkylant capability, it is important to determine the genetic risk of exposed individuals. For this reason, the aim of the present investigation is to evaluate the induction of SCE in meristematic cells of *Vicia faba* as well as to define the genotoxic profile of this insecticide.

MATERIALS AND METHODS

Vicia faba (var. minor) seeds were germinated between two cotton layers wetted with tap water at 20 °C in the dark. The primary roots reaching 2-3 cm were selected and put in a solution containing 100 μM of 5-bromodeoxyuridine (BrdU), 0.1 μM of 5-fluorodeoxyuridine (FdU) and 5 μM of uridine (U) for one replicative cycle (20 h). Then, they were incubated for 2 h with lannate-90 (Du Pont, México), in concentrations of 500, 1000, 1500, 2000 and 2500 ppm of the active ingredient, dissolved in distilled water.

Fresh solutions of BrdU, FdU and U were applied in a second replicate cycle (20 h). Treatments were carried out in the dark at 19 °C. Controls were exposed to distilled water and subjected to the same procedure.

After this treatment, meristems were cut, treated with 0.05 % colchicine for 3 h and stained by Feulgen differential technique described by Tempelaar *et al.* (1982) modified as follows: cuttings were fixed with glacial acetic acid for 1 h, then put into ethanol-acetic acid (3:1) for two days at –20 °C and later in ethanol 70 % for 15 min, and hydrolyzed in 5 N HCl for 80 min at 28 °C. Then they were washed 3 times with distilled water and stained with the Schiff reagent (Feulgen staining) for 10 min in the dark. Cuttings were treated with 2 % pectinase dissolved in 0.01 M citrate buffer (pH 4.7) for 15 min at 28 °C, followed by 45 % acetic acid for 10 min and then they were finally transferred to cold 70 % ethanol for 30 min.

The all «squash» was carried out using 45 % acetic acid and the slides were made permanent by a dry-ice technique (Conger and Fairchild 1953), dehydrated by means of two absolute butanol changes and then mounted in Canada balsam. Slides were scored in code so that it was not known to which group they belonged.

For each concentration tested, 250 subacrocentric chromosomes and 50 metacentric chromosomes were scored in each experiment and its replicate. These chromosome numbers are equivalent to 25 metaphase cells. A terminal SCE was considered as one exchange and an interstitial SCE as two exchanges. An analysis of variance (ANOVA) was used to determine differences between groups. When a significant F value was found ($P<0.05$), the Newman Keuls multiple comparisons test was applied to establish differences at the $P<0.001$ level among control and several concentrations.

RESULTS AND DISCUSSION

Genotoxic effects like gene mutation and CA has been reported for several carbamate pesticides *in vitro* (Baron 1991, Bonatti *et al.* 1994).

In the case of methomyl, not all reports agreed in its genetic activity. Methomyl appears to elicit weak or no response in microbial gene mutation assays and no response in bacterial and *in vivo* mammalian test for DNA damage. However, it was able to induce structural and numerical chromosomal aberrations in plants (Njagi and Gopalan 1981) and animal systems (Debuyst and Van Larebeke 1983, Hemavathy and Krishnamurthy 1987a,b, Bolognesi *et al.* 1994 and references therein, Bonatti *et al.* 1994) (**Table I**).

Positive results about aberration induction were obtained earlier in our laboratory with lannate-90 in *Vicia faba* (Valencia-Quintana *et al.* 1993)

In an extensive number of studies, *Vicia faba* root tips assay has demonstrated to be a highly sensitive assay to determine genetic damage produced by several agents. This plant has proven to be a sensitive and reliable system for the detection of SCE induced by different chemicals such as antibiotics, metals, anilines, pesticides and environmental xenobiotics (Gómez-Arroyo and Villalobos-Pietrini 1995).

When the Newman-Keuls multiple comparisons test ($P< 0.001$) was applied, it was confirmed that the treatments with lannate-90 in *Vicia faba* root tips meristems significantly increased the SCE. By the other side a concentration-response relationship was observed (**Table II**). At the highest concentrations tested, 2000 and 2500 ppm, the value was more than twice of the control.

There are not studies on capacity of lannate-90 to induce SCE in plant systems. Parathion methyl (folidol), dimethoate

TABLE I. CHROMOSOMAL ALTERATION INDUCED BY METHOMIL

Test organism	Genetic effect	Compound (purity)	Results	References
Saccharomyces cerevisiae	mitotic recombination	methomyl (99%)	–	Simmon *et al.* 1976, 1977
Vicia faba	CA	lannate 20 (methomyl 20%)	+	Njagi and Gopalan 1981
Vicia faba	CA	lannate-90 (methomyl 90%)	+	Valencia-Quintana *et al.* 1993
Vicia faba	SCE	lannate-90 (methomyl 90%)	+	This paper
Drosophila melanogaster	chromosomal translocation	lannate 20 (methomyl 20%)	–	Hemavathy and Krishnamurthy 1987a
Human peripheral blood *in vitro*	SCE	methomyl (not specified)	+	Debuyst and Larebeke 1983
Human peripheral blood *in vitro*	SCE CA micronuclei	methomyl (99%)	– + +	Bonatti *et al.* 1994
Human peripheral blood *in vitro*	SCE CA micronuclei	lannate 25 (methomyl 25 %)	– + +	Bonatti *et al.* 1994
Mouse sperm germ cells (*in vivo*)	CA	lannate 20 (methomyl 20%)	+	Hemavathy and Krishnamurthy 1987b
Mouse bone marrow (*in vivo*)	micronuclei	methomyl (99%) lannate 25 (methomyl 25%)	+ +	Bolognesi *et al.* 1994

(rogor), oxidemeton methyl (metasistox), azinphos methyl (gusation) and phoxim (bay 77488), like methomyl, induced SCE, and concentrations as low as 0.5 ppm (methyl parathion) produced tissue damage and decreased the mitotic index. Besides, NF-133, bensultap thiocyclam, dimehypo, monocrotophos, omethoate (Xing and Zhang 1990, Zhang *et al.* 1991) and propoxur (Gómez-Arroyo *et al.* 1995), also induced SCE in this plant.

The results mentioned above as well as those obtained in this work demonstrate that SCE in *Vicia faba* is an adequate assay for the detection of agents thar could imply a potential genetic hazard. Its easy handling and its relative low cost, makes it a valuable biological test assay for genetic damage (Gómez-Arroyo and Villalobos-Pietrini 1995).

Kihlman and Andersson (1984) obtained 29 SCE per metaphase in *Vicia faba* control roots, when cells remained through two cycles in BrdU, which agrees well with 28.4 SCE per metaphase scored in this work.

In relation to the molecular mechanism involved in the genetic damage, in general, the carbamate pesticides act as alkylating agents with S-dependent and delayed effects. As it has been demonstrated, lannate-90 in *Vicia faba* is a S-dependent agent since it produces only chromatid type aberrations in treatments after of 4 h and 18 and 44 h of recovery time (Valencia-Quintana *et al.* 1993). Considering that SCE is a S-dependent event (Wolff *et al.* 1974, Painter 1982), lannate-90 should be an efficient SCE-inducer.

TABLE II. SISTER CHROMATID EXCHANGES INDUCED BY LANNATE-90 IN *Vicia faba*[a]

Treatment	SCE/metaphase
	X ± SE
Control	28.40 ± 1.13
500	39.92 ± 1.65*
750	46.92 ± 1.88*
1000	48.45 ± 1.93*
1500	49.48 ± 2.16*
2000	56.33 ± 2.64*
2500	63.44 ± 2.37*

[a] n = 50 metaphases in two experiments; * Significant differences among control and each concentration were obtained by analysis of variance, F = 31.136, P value < 0.0001, and therefore the Student-Newman-Keuls multiple comparisons test was applied with P < 0.001.

This work showed that lannate-90 is an efficient inductor of SCE and is in agreement with Debuyst and Larebeke (1983) who described that methomyl caused an increase in SCE but only in presence of S9 mixture. On the opposite, Bonatti *et al.* (1994) found that SCE did not increased significantly. The differences could be due to the different type of formulation used in each case. In the present study, lannate-90 as active agent was tested. In the second, methomyl was applied without specified purity and in the later work lannate-25 was used. Second, it is important to consider that in the latter work, *in vitro*, the metabolic activation was not used.

The two different results obtained with the three insecticide preparations suggest that metabolic activation was needed to make the damage evident. In the case of *Vicia faba*, an assay *in vivo*, it has been demonstrated that plants have the capacity to metabolize pesticides and other xenobiotics (Sandermann 1982).

On the other hand, Bonatti *et al.* (1994) suggested that the genotoxicity profile of methomyl is similar to the effects described for the mouse skin tumor promoter phorbol-12-myristate acetate (PMA), that, although not mutagenic, could induce CA and DNA damage acting indirectly, i.e. forming active oxygen species. PMA was indeed found to induce structural CA, but was a weak inducer of SCE (Emerit and Cerutti 1981).

Methomyl was not mutagenic in two bacterial systems, however, it induced structural and numerical CA in plants, animals, and human lymphocytes *in vitro* and, at least in its technical formulation, caused DNA damage of the oxidative type (Bonatti *et al.* 1994). This results suggest an indirect mutagenic mode of

action of this carbamate pesticide.

The induction of OH^8dG by methomyl hinted that DNA oxidative damage could account for the formation of single strand breaks (Bolognesi *et al.* 1994). Therefore an indirect mode of action of this carbamate pesticide, through the formation of hydroxyl radicals, is considered to be a relevant factor for DNA damage and potential genotoxic effects, evidenced by DNA strand breaks and oxidized bases (Fraga *et al.* 1990).

Moreover, the possibility of oxidative damage as a causal factor for the genotoxicity of methomyl has some support in the experimental evidence of enzyme-inhibiting activities of different methyl carbamate pesticides (Bolognesi *et al.* 1994). Hemavathy and Krishnamurthy (1987a) propose a similar mechanism with regard to the methomyl that may be operating under our study conditions and that inhibition of some enzymes could have occurred leading to oxidative damage of DNA and to genetic events. Enzymes involved in the defense against harmful oxygen species, such as superoxide dismutase, catalase, and glutathione transferase, are inhibited by this pesticides (Rannug and Rannug 1984).

The high SCE frequency induced in *Vicia faba* by different concentrations of lannate-90 means that this biological material is extremely sensitive and useful for the detection of damage caused by chemical agents on DNA. From this data, and other positive results obtained by Bolognesi *et al.* (1994) and Bonatti *et al.* (1994) and our earlier work (Valencia-Quintana *et al.* 1993), the potential genetic hazard of lannate-90 must be considered as an actual problem, that should be handled carefully.

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