

ASSESSMENT OF THE GENOTOXICITY POTENTIAL OF THE NEMATICIDE INSECTICIDE CARBOFURAN IN WHEAT CELLS

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ABSTRACT

Carbofuran (Furadan), an insecticide-nematicide, is widely used for seed treatment in Brazil, against pests in crops such as wheat, rice and corn. It was assessed for its cytotoxic and or clastogenic potential in root meristems of *Triticum aestivum* (tested *in vivo*). Seed samples (100 grams each) were individually treated with one, two, four and eight liters of the pure commercial product/100 kg seed and germinated on three substrates: virgin soil, sterilized soil and growth chamber (on paper). For all treatments, no cytotoxic or clastogenic effects were observed in the meristem root cells.

INTRODUCTION

Since many insecticides have a mutagenic action, their retention and accumulation may have a detrimental effect on the natural ecosystem and their residues or sub-products in plants may affect human health (Fujii and Inoue, 1983).

Only a limited amount of data on the mutagenic action of carbamate compounds was available until 1976. They were restricted to bacteria and higher plants, where these compounds can undergo metabolic conversion to powerful mutagenic agents. According to the report of FAO/WHO (1977), oxidizing degrading mechanisms in mammals are probably related to the mixed function oxidase systems responsible for the metabolism of the carbamate insecticide Carbofuran. Similar oxidation

systems in plants are apparently active and the same range of products is formed.

Carbofuran showed no mutagenic action when tested on *Salmonella typhimurium* and *Saccharomyces cerevisiae* with and without the fraction S9 (Gentile *et al.*, 1982; Klopman *et al.*, 1985). Blevins *et al.* (1977) also found that Carbofuran did not produce lesions in human fibroblastic DNA. *In vivo* tests on eucariotes, such as *Zea mays* (Gentile *et al.*, 1982), on mice bone marrow cells (Pilinskaya and Stepanova, 1984) and on the sex-linked lethal recessive gene in *Drosophila* (Lee *et al.*, 1983) also demonstrated no mutagenicity.

However Sathaiah *et al.* (1973) found inhibition of spindle fibre formation by Carbofuran in meristem root cells of *Allium cepa* and Singh *et al.* (1977 and 1979) observed the formation of anaphase loops and chromosome fragments in *Hordeum vulgare* seeds, as well as reduced fertility in pollen grains. Sating-Georges-Gridelet (1982) noted an increase in the frequency of micronucleated red blood cells in mice treated orally with this product. Pilinskaya and Stepanova (1984) showed a statistically significant increase in the incidence of metaphase aberrations in human lymphocytes treated *in vitro* with 100 and 300 µg/ml of this insecticide.

Carbofuran (Furadan) is intensively used on wheat (*Triticum aestivum*). According to Ehrenberg (1971), dormant seeds tolerate a wide variation on environmental conditions such as drought and heat. They represent cells

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at the quiescence of the mitotic cycle. When they are imbibed with water, a large fraction of embryonic cells almost immediately go through a DNA synthesis cycle and a subsequent mitosis.

In the present study, the objective was to characterize the action of the insecticide Carbofuran in inducing alterations in the cell cycle of wheat root meristem cells.

MATERIAL AND METHODS

Furadan 350F, the commercial brand of the insecticide Carbofuran manufactured by "FMC do Brasil" and wheat cultivar Tapejara seeds, used in this study, were supplied by the Instituto Agronômico do Paraná (IAPAR - Londrina).

The wheat seeds, obtained from plants which had received no chemical treatment, were treated in 100 gram lots with one, two, four and eight liters of pure commercial Carbofuran per 100 kilograms of seeds. After drying, they were germinated on three different substrates: a) Disposable cups with Methyl Bromide sterilized soil, in a glass house at room temperature; b) Disposable cups with virgin soil, in a green house at room temperature; c) moistened rolled up filter paper, in a climatized growth chamber at 25°C and with 100% humidity. Untreated seeds were germinated on the same three substrates and used as a control.

After the root system of the treated and control groups had formed, one to three young roots (one to two cm in length) were collected from each of 20 seedlings per germination substrate. The roots were placed in individual flasks and fixed in ethanol and acetic acid (3:1) for 24 hours. Roots from soil-germinated seeds were washed in running water before fixation.

The slides were prepared by squashing the roots in 45% acetic acid, using the Feulgen reaction for staining.

The mitotic and phase indices of the treated and control roots were determined by counting the number of cells at interphase and at each phase of mitotic division. Five thousand cells were analyzed for each treatment.

Each of the indices was calculated from the following formula:

$$\text{Mitotic or phase index (\%)} = \frac{\text{number of dividing cells (total or phase)} \times 100}{\text{number of cells analyzed}}$$

The average mitotic and phase indices for the various concentrations of the insecticide and respective controls were calculated after cytological analysis of the roots in each substrate. These data are presented in Tables I, II, III.

The following transformations, $y = \arcsin (IM)^{1/2}$ and $x = \log (1 + \text{dosage})$ were carried out before applying the statistical tests to the data. The first transformation was

Table I - Phase and mitotic indices in *Triticum aestivum* meristem root cells obtained from seedlings treated with different concentrations of Carbofuran and the respective controls germinated in a climatized growth chamber.

Concentration (1/100 kg seeds)	P.I. (%)	Me.I. (%)	A.I. (%)	T.I. (%)	M.I. (%)
0	30.45	30.73	8.66	30.17	7.16
1	49.46	19.13	7.22	24.19	5.54
2	40.42	24.74	12.89	21.95	5.74
4	34.60	28.14	5.19	32.32	7.92
8	41.07	21.00	6.90	31.03	6.38

P.I. = Prophase index; Me.I. = Metaphase index; A.I. = Anaphase index; T.I. = Telophase index; M.I. = Mitotic index.

Table II - Phase and mitotic indices in *Triticum aestivum* meristem root cells obtained from seeds treated with different concentrations of Carbofuran and their respective controls, germinated in sterilized soil.

Concentration (1/100 kg seeds)	P.I. (%)	Me.I. (%)	A.I. (%)	T.I. (%)	M.I. (%)
0	30.71	30.71	13.78	24.80	5.08
1	30.48	36.67	11.90	20.95	8.4
2	27.53	32.73	14.54	25.19	7.7
4	24.54	36.94	12.66	25.86	7.58
8	31.86	29.64	12.74	25.76	7.22

Table III - Phase and mitotic indices in *Triticum aestivum* meristem root cells obtained from seeds treated with different concentrations of Carbofuran and their respective controls, germinated in virgin soil.

Concentration (1/100 kg seeds)	P.I. (%)	Me.I. (%)	A.I. (%)	T.I. (%)	M.I. (%)
0	34.86	17.05	10.94	24.43	7.86
1	28.57	36.88	10.39	24.16	7.7
2	29.61	30.58	12.14	27.67	8.24
4	29.81	43.63	8.40	18.16	7.38
8	41.64	28.33	10.20	19.83	7.0

used to correct the treatment variance heterogeneity and to approximate the data distribution to the normal curve, and the second to make the variances independent from their means.

An analysis of variance and a simple regression analysis for balanced data were used to determine if the differences between the average mitotic indices for the different concentrations were statistically significant.

RESULTS

There were no differences between the mitotic and the average phase indices obtained from the different treatments and the control (Tables I to III).

In all cases, the treated groups behaved similarly. No significant regression of the mitotic indices on Carbofuran doses was observed (under the experimental conditions). The same procedures were carried out for the different phase indices of the cell cycle and the results were not statistically significant.

DISCUSSION

As in animal metabolism, inactive compounds or pro-mutagens can be activated in vegetables to form mutagenic or carcinogenic agents (Wildeman and Nazar, 1982).

Most of the enzymes which take part in the xenobiotic metabolism (Phases I and II) are localized in the endoplasmatic reticula in both organisms (Menn, 1978). Phase III or secondary conjugation enzymes, which do not exist in mammals are found in vegetables (Shimabukuro *et al.*, 1982 "apud" Veleminsky and Gichner, 1988).

According to Plewa *et al.* (1988) the demonstration that plants can activate pro-mutagenic agents increases the interest in the fact that they may activate environmental contaminants and introduce them into the human food chain.

Since the insecticide Carbofuran is a systemic agent, it must be metabolically transformed when in contact with plants. Such metabolism leads to the formation of the derivatives NO-Carbofuran, 3-OH-Carbofuran and 3-K-N-Carbofuran. These compounds are mutagenic *in vitro*: by the tests of Ames and Chinese hamster ovarian cells (Nelson *et al.*, 1981) and they induce chromosome aberrations in human lymphocytes (Pilinskaya and Stepanova, 1984). However, Carbofuran was not genotoxic in *S. typhimurium* and *S. cerevisiae*, even after metabolic activation in plants and rats (Gentile *et al.*, 1982). Plewa and Wagner (1981) also showed that the extract S1, obtained from the activation of Carbofuran in maize, was not able to produce mutagenic effects on the waxy locus of these species *in vitro*.

Carbofuran did not have an antimitotic effect on the meristem root cells of *T. aestivum*, under any of the three germination substrates: virgin or sterilized soil in a glass house and moistened filter paper in a growth chamber.

The virgin soil substrate was included to determine if an interaction between the insecticide and the methyl bromide (used to sterilize the soil) could cause a mutagenic effect. The results indicate that the methyl bromide does not interfere in the cell cycle when used to sterilize soil,

probably because it undergoes volatilization, and that the insecticide nematicide Carbofuran does not have a cytotoxic effect on wheat meristems germinated under these conditions.

Miah and Akhtar (1985) found that in sugar cane roots exposed for 24 hours to Furadan 3G the mitotic index increased with higher doses, being significantly different, however, only at the highest concentration (50 ppm). These results are similar to those of Lee (1976) who demonstrated that derivatives of Carbofuran, such as Plenol Carbofuran and Phenol 3-hydroxy-Carbofuran, are able to interact with indol acetic acid (AIA) to stimulate plant growth.

In agreement with our results, Sathaiah *et al.* (1973) did not find differences between the mitotic stages of the treated and control groups when testing Furadan on roots. They did, however, find irregularly scattered prometaphases and attributed this fact to the inhibition of the spindle fiber formation.

Singh *et al.* (1977, 1979) treated *H. vulgare* seeds with various concentrations of Furadan, and observed no adverse effects on germination. Nevertheless, the insecticide produced a significant decrease in the fertility of the pollen grains, and a significantly greater frequency of aberrant anaphases than the positive control group (EMS). The authors suggest that the appearance of anaphase bridges and chromosome fragments would lead to deficiencies, and occasionally to duplications, which could produce mutations in chlorophyll and other morphological alterations in the offspring.

The present experiment with wheat meristem root cells, showing an absence of atypical mitotic figures and apparently no influence of the treatments on the cell division cycle, lead us to suppose that Furadan was not activated at the meristem level, and therefore, did not act as a cytotoxic agent. Another explanation for these negative results would be the formation of genotoxic subproducts in the meristem cells, with later deactivation by the enzyme system of these cells.

According to the physiological studies of Wildeman and Nazar (1982), there are indications of a high metabolic activity in germinating seeds and young growing plants, particularly in the meristem region. This finding agrees with the hypothesis of deactivation since our work involved a tissue where high metabolic activity occurs naturally. In spite of the different final products of the conjugation reactions in plants and mammals (Veleminsky and Gichner, 1988), the comparison of the two systems shows that, in general, pesticides that induce significant aberrations in plants do so in mouse bone marrow and vice versa (Grover *et al.*, 1988). This is confirmed by the findings of the present work when compared with the negative results obtained by Pilinskaya and Stepanova (1984) and with unpublished data of studies with rat bone marrow carried out by the present authors.

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RESUMO

O inseticida nematocida Carbofuran (Furadan), um composto carbamato, é um produto específico para tratamento de sementes e muito utilizado na agricultura brasileira no combate a pragas de culturas como o trigo, arroz e milho. Este produto foi avaliado quanto a seu potencial citotóxico e/ou clastogênico em meristemas radiculares de *Triticum aestivum* (teste *in vivo*). Cada 100 kg de semente recebeu as seguintes doses: um, dois, quatro e oito litros do produto comercial puro. Estas sementes germinaram em três sistemas: terra virgem, esterilizada e em papel de filtro umedecido e em nenhum deles foram verificados efeitos citotóxicos ou clastogênicos nas células meristemáticas das raízes.

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