

INDUCTION OF MICRONUCLEI IN PERIPHERAL ERYTHROCYTES OF CYPRINUS CARPIO FISH BY METHYL PARATHION

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ABSTRACT

Methyl parathion (MP) is a commonly used organophosphorus insecticide, commercially available as Folidol 600. This chemical substance was tested to determine micronucleus (MN) frequencies in peripheral erythrocytes of the fish *Cyprinus carpio* treated *in vivo*. The concentrations tested were 1.25, 2.50, 5.00 and 7.50 ppm. Fish were killed after 24 or 48 h of treatment. There was a significant increase in the frequency of micronuclei in the animals treated with 2.50 and 5.00 ppm after 24 h. At higher dosages this effect disappeared probably due to the increased cell lethality. The frequency of MN induction was lower at 48 than at 24 h after treatment. There was a dose-dependent increase in MN frequency which, however, was significant only at the highest concentration (7.50 ppm) compared to control.

RESUMEN

El metil paratión (MP) es un insecticida organofosforado disponible comercialmente como Folidol 600. Esta sustancia fue probada para determinar la frecuencia de micronúcleos (MN) en eritrocitos periféricos de peces *Cyprinus carpio* tratados *in vivo*. Las concentraciones utilizadas fueron 1.25, 2.50, 5.00 y 7.50 ppm. Los peces fueron sacrificados 24 y 48 h después del tratamiento. Se notó un aumento significativo en la frecuencia de micronúcleos en los animales tratados con 2.50 y 5.00 ppm y sacrificados después de 24 h. A concentraciones mayores este efecto desapareció probablemente debido al incremento de la letalidad celular. La frecuencia de inducción de MN fue menor después de 48 h que a 24 h del tratamiento. En la primera hubo una elevación en la frecuencia de MN dependiente de la concentración que, sin embargo, fue significativa cuando se comparó con el testigo solamente en la mayor (7.50 ppm).

INTRODUCTION

Methyl parathion (MP) is a broad-spectrum anticholinesterase organophosphorus insecticide widely used against crop pests. In Brazil it is also being employed at pisciculture sites to select the zooplankton and to eliminate predators. Methyl parathion is commercially available as Folidol 600.

Negative results have been reported in mutagenic assays with MP on *Salmonella* and *Streptomyces* (Carere *et al.* 1978), and positive results in *Salmonella* with and without S9 mix (Waters *et al.* 1980, Rashid and Mumma 1984, Breau *et al.* 1985). The compound is probably mutagenic

in *Escherichia coli*, inducing 5-methyltryptophan (5-MT) resistance (Mohn 1973a, b). MP is mutagenic in somatic and germ line cells of *Drosophila* and induces sex-linked recessive lethals in immature male germ cells (Tripathy *et al.* 1987).

The results from studies on mammals are also conflicting. Negative results were obtained for chromosome aberrations in bone marrow cells and spermatogonia, and for the frequency of pre and postimplantation fetal lethality in a dominant lethal mutation assay in mice (Degraeve and Moutschen 1984). MP caused no observable chromosome damage either in human cells *in vitro* or in mouse cells *in vivo* (Huang 1973). However, an increased fre-

quency of chromosome aberrations was described in mouse bone marrow cells (Kurinnyi 1975) and of sister chromatid exchanges in human lymphoid cells (Sobti *et al.* 1982) and in V79 Chinese hamster ovary cells (Chen *et al.* 1981).

The micronucleus test (MNT) was developed in mammalian polychromatic erythrocytes of the bone marrow (Schmid 1975). Nevertheless, it has also been applied to nucleated piscine erythrocytes in the genotoxicity evaluation of test compounds *in vivo* (Hooftman and de Raat 1982, Hose *et al.* 1984, Manna *et al.* 1985, 1987, Das and Nanda 1986). This assay can detect agents which cause chromosome breaks and agents which affect the mitotic spindle. Thus, micronuclei (MN) are indicators of clastogenic or aneugenic effects.

Since micronucleated piscine erythrocytes have been proved to be sensitive indicators of genetic damage, the purpose of our study was to evaluate the cytogenetic (clastogenic or aneugenic) effects of methyl parathion in *Cyprinus carpio* fish using the MNT.

MATERIALS AND METHODS

Chemical agent

Methyl parathion (0,0-dimethyl-0-p-nitrophenyl phosphorothioate) (CAS N° 298-00-0) is an organophosphorus insecticide commercially available as Folidol 600 (Bayer). The chemical structure of the compound is shown in figure 1.

MP was dissolved in water collected from an IBAMA pisciculture site and diluted to the desired concentrations. *Cyprinus carpio* fish were exposed to methyl parathion contaminated water for 24 and 48 h.

In vivo assay on peripheral erythrocytes of *Cyprinus carpio* for the determination of micronucleus frequencies

Cyprinus carpio fish weighing approximately 1.0 g were purchased from an IBAMA pisciculture site, Uberlândia (MG) Brazil, and kept in laboratory aquaria containing water collected from the same site, in order to maintain zooplankton concentration and avoid chlorinated tap water. The aquaria were aerated frequently and the animals

were allowed to acclimatize for one week prior to treatment. A batch of 56 fish were equally released in four different aquaria (fourteen animals in each aquarium), containing different concentrations (1.25, 2.50, 5.00 or 7.50 ppm) of MP dissolved in the aquarium water. Fish were then killed, following 24 and 48 h of treatment. These time periods were arbitrarily chosen. Fourteen fish kept in the same kind of water, but without MP, and killed at the same time intervals served as controls.

Seven fish were used for each concentration level and for each sampling period, including the controls. Fish were cut in the caudal region, and blood smears were immediately prepared and fixed in absolute methanol for 15 min. On the following day, the material was stained with Giemsa diluted 1:20 in sodium phosphate buffer, pH 6.8, for 10 minutes. A total of 2,000 cells, 500 per slide, from each individual were examined microscopically under 1000 X magnification. Coded and randomized slides were scored using blind review by a single observer. The number of micronuclei (MN) and micronucleated cells (MNC) were expressed per 100 cells.

Statistical analysis

The data were analyzed statistically by the conditional test for the detection of rare events (Pereira 1991), with the level of significance set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The frequencies of MN and MNC observed in *Cyprinus carpio* treated with MP for 24 h were significantly higher in fish treated with 2.50 and 5.00 ppm ($P < 0.008$ and 0.005, respectively) than those in control fish. At a concentration of 7.50 ppm, the increase in frequency was not significant ($P > 0.07$), probably due to the increased cell lethality (Table I). An inconsistent result was found in this experiment. The frequency of MN and MNC observed in fish treated with 1.25 ppm of MP for 24 h was significantly lower than that in controls ($P < 0.005$) (Table I).

High frequencies of MN and MNC were expected to be found in the controls, based on previous observations of high incidence of MN in fish (*Oreochromis mossambicus*) collected from the same site of pisciculture (IBAMA-Uberlândia) due to environmental pollutants (Nepomuceno and Spanó 1992).

On the other hand, the frequencies of MN and MNC present in *Cyprinus carpio* treated with MP for 48 h were significantly higher ($P < 0.005$) only in fish treated with 7.50 ppm (Table II). For 1.25, 2.50 and 5.00 ppm doses, the data on MN and MNC did not differ significantly from the respective controls ($P > 0.4$, 0.08 and 0.06, respectively) (Table II). The lower frequencies of micronuclei obtained after 48 h of treatment compared to those obtained after 24 h maybe due to the toxicity of MP leading to cell death.

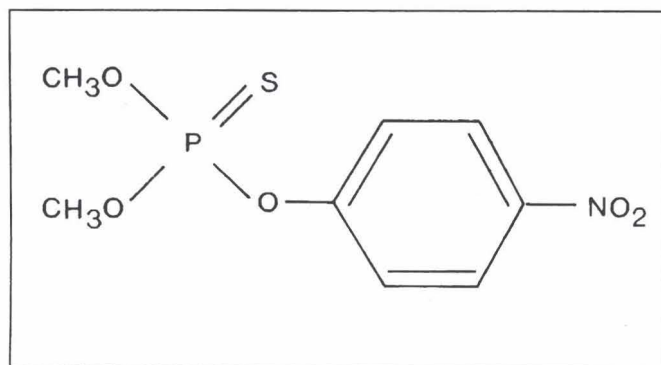


Fig. 1. Structural formula of the methyl parathion

TABLE I. FREQUENCY OF MICRONUCLEATED CELLS (MNC) AND MICRONUCLEI (MN) IN PERIPHERAL ERYTHROCYTES OF *CYPRINUS CARPIO* FISH EXPOSED TO METHYL PARATHION CONTAMINATED WATER FOR 24 HOURS

Dose (ppm)	No. of indiv.	Total cells	Total MN	Total MNC	\bar{X} (%)	\pm	SD
					MN		MNC
0.00	7	14000	72	71	0.51 \pm 0.35		0.50 \pm 0.35
1.25	7	14000	41*	41*	0.29 \pm 0.20		0.29 \pm 0.20
2.50	7	14000	105*	102*	0.75 \pm 0.32		0.72 \pm 0.30
5.00	7	14000	278*	275*	1.98 \pm 2.58		1.96 \pm 2.55
7.50	7	14000	91	91	0.65 \pm 0.57		0.65 \pm 0.57

* Significantly different from the control according to the conditional test for the detection of rare events (Pereira 1991) with the level of significance set at $\alpha = 0.05$.

Most of the earlier studies with organophosphates used bacterial test systems and the reports on mammalian test systems were mostly negative possibly due to the toxicity and the rapid degradation of the test chemicals in *in vivo* and *in vitro* experiments (Wild 1975).

The induction of 5-MT resistance mutations in *E. coli* shows that MP is probably mutagenic. However, under the experimental conditions described, the compound is not significantly mutagenic at doses which do not inactivate the cells, whereas at higher doses an increase in relative mutation frequency occurs with a parallel decrease in survival (Mohn 1973a).

Incubation of human lymphoid cells (LAZ-007) with 20 μ /ml caused a significant increase in SCEs. However, cytotoxic effects of MP were dose related and led to cell death (Sobti *et al.* 1982).

Wild (1975) described the general aspects of the chemistry, toxicity and metabolism of organophosphates. According to Breau *et al.* (1985), the most mutagenic phosphorothioates are those containing strong electron-withdrawing substitutes (as in MP).

Kurinyi (1975) observed chromosome lesions in bone marrow of mice treated with 10 mg/kg MP, considering this compound to be slightly clastogenic.

There is a lack of information about the formation and frequency of micronuclei in the haematopoietic tissues of *Cyprinus carpio* fish. There is no information about the time taken for the completion of the mitotic cycle in blood cells, nor about the time taken for the blood cells to reach the peripheral circulation. Because of this, we cannot assure that micronuclei found had been formed during haematopoiesis at different times (24 or 48 h) of

TABLE II. FREQUENCY OF MICRONUCLEATED CELLS (MNC) AND MICRONUCLEI (MN) IN PERIPHERAL ERYTHROCYTES OF *CYPRINUS CARPIO* FISH EXPOSED TO METHYL PARATHION CONTAMINATED WATER FOR 48 HOURS

Dose (ppm)	No. of indiv.	Total cells	Total MN	Total MNC	\bar{X} (%)	\pm	SD
					MN		MNC
0.00	7	14000	54	54	0.38 \pm 0.34		0.38 \pm 0.34
1.25	7	14000	51	51	0.36 \pm 0.19		0.36 \pm 0.19
2.50	7	14000	40	40	0.28 \pm 0.22		0.28 \pm 0.22
5.00	7	14000	72	72	0.51 \pm 0.28		0.51 \pm 0.28
7.50	7	14000	168*	168*	1.20 \pm 1.85		1.20 \pm 1.85

* Significantly different from the control according to the conditional test for the detection of rare events (Pereira 1991) with the level of significance set at $\alpha = 0.05$.

treatment with MP. However, in spite of these considerations, based on our data and on previous reports, we may conclude that MP has genotoxic effects.

Although organophosphates are not persistent in the environment and do not pose a serious residue problem (Wild 1975), the continued and increased use of these pesticides may lead to unintentional deleterious effects on the environment and on animal and human health.

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