

## Research Article



## Assessment of the Genotoxic Effect of Fungicide Propamex 722 sl at Goldfish (*Carassius auratus*), after Treatment for 96 hours

Ilmije Vllasaku<sup>1</sup>, Hamit Ismaili\*<sup>2</sup>, Kemajl Kurteshi<sup>2</sup>, Agron Krasniqi<sup>3</sup>, Samir Mulaki<sup>3</sup>

<sup>1</sup>State advisor in Ministry of Agriculture, Macedonia

<sup>2</sup>Department of Chemistry, Faculty of Natural Sciences, University of Prishtina, Kosovo

<sup>2</sup>Department of Biology, Faculty of Natural Sciences, University of Prishtina, Kosovo

<sup>4</sup>Master study, Department of Chemistry, Faculty of Natural Sciences, University of Prishtina,

\*Corresponding author's E-mail: [hamit.ismaili@yahoo.com](mailto:hamit.ismaili@yahoo.com)

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### ABSTRACT

The main objective of this investigation was to assess the genotoxic effect of fungicide propamex 722 SL on fish. We used the species of fish goldfish (*Carassius auratus*). Concentration of fungicide propamex 722 sl in first aquarium 2 ml fungicide / 40 liter water, in second aquarium 1.5 ml fungicide / 40 liter water, in third aquarium 1 ml fungicide / 40 liter water, in fourth aquarium 0.5 ml fungicide / 40 liter water. Fifth aquarium uses as control, without fungicide propamex 722 sl, contain only drinking water.

**Keywords:** Micronucleus test, Genotoxicity, Fungicide.

### INTRODUCTION

Pesticides as pollutants in aquatic environment induce several chromosomal abnormalities. Chromosomal studies have received considerable attention in recent years, in part from a growing interest in the evaluation of genotoxicity of environmental toxicants and carcinogens.

Propamex (Propamocarb hydrochloride) 722 SL is systemic fungicide with protective action, used to control soil born and leaf diseases in vegetables and ornamental plants. The product is high effective when applied as soil disinfectant and foliar spray. The product is absorbed by the roots, and transported acropetally to the stems and leaves.

The interest to study the impact of fungicides propamex 722 sl is mainly related to their toxicity. Like all pesticides, they can affect human health and the environment, hence the need for assessing their effects<sup>1</sup>. The genetic and genotoxic effects of fungicide thiophanate-methyl (TM) were assessed by the micronucleus (MN) test, the single-cell gel electrophoresis (SCGE) assay, or comet test, and chromosome analysis. The MN test is a simple and sensitive method to detect both chromosome fragments and whole chromosomes, i.e. both clastogenic and aneugenic activity<sup>2, 9</sup>. Propamex (propamocarb hydrochloride 722 g / l), represent the most extensively used fungicide worldwide, including Kosovo.

### MATERIAL AND METHODS

The fish goldfish (*Carassius auratus*) were collected in the lake Stublina nearby city Gjilan, east part of Kosovo. After the capture, they were placed in aquariums with aerated tap water and taken to the laboratory. After acclimation to reduce the stress of capture and transport, fish were treated in aquarium with herbicide for 96 hours.

Slides were stained with Giemsa. The frequency of micronuclei and nuclear abnormalities were estimated by counting 1000 cells in extensions. At each aquarium put 10 fish.

Fish goldfish (*Carassius auratus*) was chosen for this study because it is very adapt for investigation, also due to proven sensivity to genotoxic chemicals. In each aquarium put ten (10) fish, total number of fish is 50 fish.

Concentration of fungicide propamex 722 SL, it was in first aquarium 2 ml fungicide propamex 722 SL / 40 liter water, in second aquarium 1.5 ml fungicide propamex 722 SL / 40 liter water, in third aquarium 1 ml fungicide propamex 722 SL / 40 liter water, in fourth aquarium 0.5 ml fungicide propamex 722 SL / 40 liter water. Fifth aquarium uses as control, without fungicide propamex 722 SL, contain only drinking water.

### Experimental design

Fish goldfish (*Carassius auratus*) were placed in five different aquaria, each one containing tap water (negative control) and four different aquaria containing different concentration of fungicide propamex 722 SL.

The fish was cut in caudal region and smears of peripheral blood were made on free clean slides.

### Slide preparation and staining

For each fish prepare three slides. Slides were coded, for each fish. The smears are air-dried and fixed in absolute ethanol for 25 minute. Treatment it was 96 hours. After fixation, the slides were stained in aqueous Giemsa (diluted in distilled water ratio 1:5) for 50 minute. For each fish prepare 2 slides.



## RESULTS AND DISCUSSION

The frequencies of MN and Nucleoplasmic bridge in peripheral blood erythrocytes after exposure to the fungicide propamex 722 SL treated for 96 hours, are presented in table 1.

The number of micronucleated (MN) erythrocytes were estimated for each fish in each aquaria. At first aquaria we detect the 61 micronuclei(MN) and 10 nucleoplasmic bridge (NB), which is higher compared with other aquaria and with control group.

**Table 1:** Average number (per aquarium) of micronuclei (MN) and Nucleoplasmic bridge in 1000 erythrocytes of peripheral blood of fish goldfish (*Carassius auratus*) after 96 hours treatment in different concentration of fungicide propamex 722 SL.

Aquarium/ treated for 96 hours	Average number of MNA and Nucleoplasmic bridge /1000 erythrocytes per aquarium		Significancy -P
	MN	Nucleoplasmic bridge	
Aquarium 1(2 ml fungicide /40 l water): Aquarium controll	61	10	S , P = <0.001
Aquarium 2 (1.5 ml fungicide /40 l water) : Aquarium controll	56	7	S , P = <0.001
Aquarium 3 (1 ml fungicide /40 l water) : Aquarium controll	48	6	S, P = <0.001
Aquarium 4 (0.5 ml fungicide /40 l water) : Aquarium controll	23	3	S , P = <0.001
Aquarium controll	2	1	
Average number of MN and Nucleoplasmic bridge, at treated fish, without control group	188 : 4 = 47	26 : 4= 6	

Legend: s – Significancy, Ns – Not Significancy

An advantage of chromosomal studies is that they reveal a measure of sub-lethal effects of xenobiotics in vivo. In epidemiological studies, it has been shown that people with elevated frequencies of chromosomal aberrations (CA) in their peripheral blood lymphocytes have a significantly elevated risk of developing cancer<sup>5</sup>. This work aimed at adding to the contrasting evidence collected on the genotoxicity of pesticides in general<sup>4</sup>, and of TM in particular<sup>7,11</sup>.

The results show higher number of micronucleus in erythrocytes fish. By decreasing the concentration of fungicide decrease and number of micronuclei. At first aquaria the number of micronuclei (61 Micronucleus) and nucleoplasmic bridge (10 Nucleoplasmic Bridge) was higher compared with other aquaria and control group (2 Micronucleus, 1 Nucleoplasmic Bridge).

Several authors<sup>2,6,8</sup>, have reported genotoxic effects in different species of fish using cytogenetic analysis. Exposure of fish to pollutants and toxicants for prolong

At second aquaria we determine 56 MN and 7 NB, while the third has 48 MN and 6 NB and fourth aquaria has 23 MN and 3 NB, at 1000 erythrocyte.

The average numbers of MN and NB at all groups treated with fungicide are 47 MN and 6 NB, statistically are significantly higher compared with control group. At control group we determine 3 MN and 1 NB / 1000 erythrocytes.

period, even at low levels, leads to chromosomal aberrations including gene changes<sup>3</sup>.

The results are also in accordance with other studies in world which showed a high frequency of micronuclei and nuclear abnormalities in fish and other organisms exposed to various pesticides<sup>10,12</sup>.

## CONCLUSIONS

Based in these investigations, we can conclude that fungicide propamex 722 SL has genotoxic effect in erythrocytes of fish.

We demonstrated that exposed the fish to fungicide propamex 722 SL induced genomic damage (measured as MN induction, comet tail length and chromosome aberrations) and that the frequency of these markers and exposure time are significantly correlated.

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