Research Article

Investigation of Genotoxic Effect of Herbicid Randap 480 ec at Goldfish (*Carassius auratus*).

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ABSTRACT

The genotoxic effects of herbicid Randap 480 EC were evaluated in goldfish Carassius auratus using the micronucleus test and Nucleoplasmic bridge. The frequencies of micronuclei in fish erythrocytes cells of sampling points were elevated when compared to the controls. Micronucleus test in fish erythrocytes were useful to determine genotoxic effects of herbicids in the fish. The micronucleus assays have been used increasingly to evaluate genotoxicity of many compounds in polluted aquatic ecossystems. In order to investigate the frequencies of micronuclei and to assess the sensitivity of species, the results were compared with samples taken at the reference site and maintained in the laboratory, and fish treated with herbicid Randap 480 EC . The micronucleus assay was efficient in demonstrating field pollution and reproducing results in the labotatory. Our results show significant increase of number of micronuclei in erythrocytes of goldfish.

Keywords: Micronucleus assay, genotoxicity, herbicid Randap.

INTRODUCTION

Herbicide Randap is a glyphosate-based herbicide and despite its wide use around the world there are few studies comparing the effects of the active ingredient with the formulated product. In this context the purpose of this study was to compare the genotoxicity of the active ingredient glyphosate with the formulated product RT in order to clarify whether the active ingredient and the surfactant of the RT formula may exert toxic effects on the DNA molecule in juveniles of fish *Prochilodus lineatus* [11]. Genotoxic studies in fish are frequently performed in erythrocytes, due to the ease of sampling and their adaptability to the most common methodologies [1, 8].

MATERIAL AND METHODS

We used the species of fish goldfish (*Carassius auratus*). The fish 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 fungicide for 96 hours.

Slides were stained with Giemsa. The frequency of micronuclei and nuclear abnormalities were estimated by counting 1000 cells in extensions. 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.

Concentrations of herbicid Randap 480 EC , it was in first aquarium 2 ml herbicid Randap 480 EC / 40 liter water, in second aquarium 1.5 ml herbicid Randap 480 EC / 40 liter water, in third aquarium 1 ml herbicid Randap 480 EC / 40 liter water, in fourth aquarium 0.5 ml herbicid Randap 480 EC / 40 liter water. Fifth aquarium uses as control, without herbicid Randap 480 EC, 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 dilution of herbicid Randap 480 EC.

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 72 hours. After fixation, the slides were stained in aqueous Giemsa (diluted in destiled water ratio 1:5) for 50 minute. For each fish prepare 2 slides.

RESULTS AND DISCUSSION

The frequencies of MN in peripheral blood erythrocytes after exposure to the herbicid Randap 480 EC treated for 96 hours, are presented in table 1, Fig. 1.

At first aquaria we registered the 45 micronuclei (MN) and 11 Nucleoplasmic bridge (NB), which is higher compared with other aquaria and with control group.

At second aquaria we registered 39 MN and 8 Nucleoplasmic bridge, while the third has 32 MN and fourth aquaria has 22 MN, at 1000 erythrocyte.

The average numbers of MN at all groups treated with herbicide are 34, 75 MN and 7 Nucleoplasmic bridges, statistically are significantly higher compared with control group. At control group we determine 4 MN / 1000 erythrocytes, and 2 Nucleoplasmic bridges.

Table 1: Average number (per aquarium) of micronuclei (MN) in 1000 erythrocytes of peripheral blood of fish goldfish (*Carassius auratus*) after 96 hours treatment in different concentration of herbicid Randap 480 EC.

Aquarium/ treated for 72 hours	Average number of MNA and Nucleoplasmic bridge /1000 erythrocytes per aquarium		Significancy -P
	MN	Nucleoplasmic bridge	
Aquarium 1(2 ml herbicid /40 l water): Aquarium controll	45	11	S , P = <0.001
Aquarium 2 (1.5 ml herbicid /40 l water): Aquarium controll	39	8	S , P = <0.001
Aquarium 3(1 ml herbicid /40 l water) : Aquarium controll	32	6	S, P = <0.001
Aquarium 4 (0.5 ml herbicid /40 l water) : Aquarium controll	22	3	S , P = <0.001
Aquarium controll	4	2	
Average number of MN and Nucleoplasmic bridge ,at treaed fish , without control group	139: 4 = 34.75	28 : 4 = 7	

Legend: s – Significancy, Ns – Not Significancy

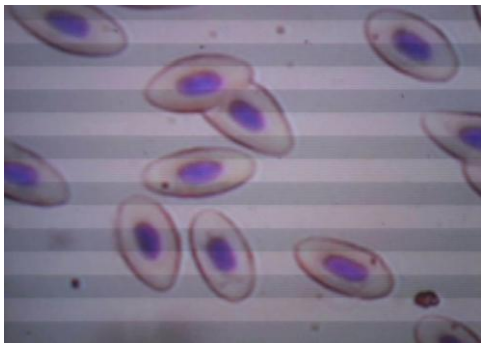

Figure 1: Erythrocytes with micronucleus

The present study brings together information based on in vivo systems to evaluate herbicide randap induced genotoxicity in goldenfish *Carassius auratus*.

The effects of genotoxicity of herbicide are reported to be severalfolds more than control group. Our results are in accordance with investigation by some other authors [2, 3, 4, 10], which showed that the micronucleus with erythrocytes of fishes seems to be efficient to detect the genotoxicity of chemicals.

The increasing use of pesticides in contemporary agriculture is considered a major problem worldwide. Although the application of these agrochemicals is concentrated in terrestrial areas, they can reach the aquatic environment by drift, runoff, drainage and leaching (Cerejeira, 2003), raising a number of environmental concerns especially in systems of shallow waters. Among pesticides, organophosphates constitute the predominant class [9].

CONCLUSIONS

The present findings clearly demonstrate the genotoxic properties of Randap expressed as DNA damage (measured by the micronuclei and nucleplasmic bridge) in erythrocytes of fish *C. auratus* exposed to different concentrations of this herbicide. This result is indicative of a risk to fish populations resulting from the occurrence of this agrochemical in natural water bodies.

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