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INDUCED MICRONUCLEI UNDER THE EFFECT OF FUNGICIDE PROMENOL KOMBI AFTER SEVEN DAYS OF TREATMENT

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Summary

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The objective of this study is to investigate the genotoxic effect of fungicide in erythrocytes of *Carassius auratus* fish. The fish were exposed to a sublethal concentration of fungicide for 7 days. This paper explores the biomonitoring potential of micronucleus test on waterborne pollutants. Fish were treated with four different concentrations of insecticide 5, 4, 3 and 2 mL insecticide/40 L water. The frequency of micronuclei and nuclear abnormalities in erythrocytes, was significantly higher in fish treated with the fungicide promenol kombi compared with control group of fish.

Key words: erythrocytes, fish genotoxic effect, insecticide, micronuclei

INTRODUCTION

Biomarkers are biological responses to environmental chemicals at the individual level or below demonstrating departure from normal status (Walker *et al.*, 2003). Biomarker responses may be at the molecular, cellular or 'whole organism' level.

An important thing to emphasize about biomarkers is that they represent measurements of effects, which can be related to the presence of particular levels of environmental chemicals; they provide a means of interpreting environmental levels of pollutants in biological terms.

MATERIAL AND METHODS

Experimental design

We used one species of fish, *Carassius auratus*. The fish were collected in the lake Vasileva, central part of Kosovo. After the capture, they were placed in aquaria with aerated tap water and taken to the laboratory. After acclimation to reduce the stress of capture and transport, fish were treated with insecticide for 72 hours.

Golden fish (*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 we put ten (10) fish, so the total number of fish was 50. Concentration of fungicide promenol kombi in the first aquarium was 5 mL /40 L water, in the second aquarium 4 mL /40 L water, in the third aquarium 3 mL /40 L water, in the fourth aquarium 1 mL /40 L water. The fifth aquarium was used as control, without fungicide, and contained only drinking water.

Slide preparation and staining

The fish was cut in caudal region and smears of peripheral blood were made on free clean slides. Slides were stained with May-Grunwald-Giensa The frequency of erythroblasts, micronuclei and nuclear abnormalities were estimated by counting 2000 cells in extensions.

For each fish three slides were prepared. Slides were coded for each fish. The smears are air-dried and fixed in absolute ethanol for 25 min. After fixation, the slides were stained in aqueous Giemsa (diluted in destiled water ratio 1:3) for 45 min.

RESULTS

The frequency of micronuclei (MN) is shown in Table 1 and Fig. 1 and 2.

In the first aquarium we detected 54 MN, which is higher compared with other aquaria and with control group. In the second aquarium we determined 46 MN, while the third and fourth aquaria had 30 and 22 MN/1000 erythrocytes respectively.

The average numbers of MN at all groups treated with insecticide were statistically significantly higher compared with control group (P<0.001).

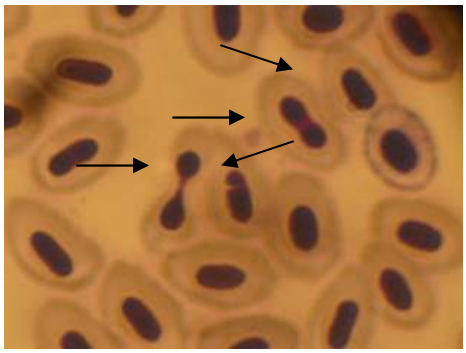

Fig. 1. Nuclear abnormality

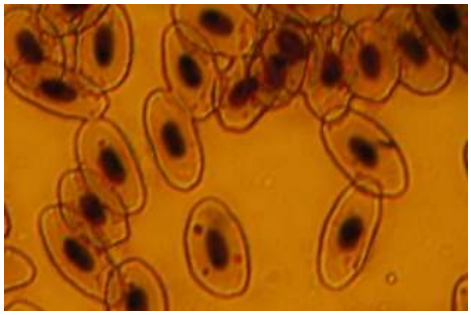

Fig. 2. Micronucleus

Table 1. Average number (per aquarium) of micronuclei (MN) in 1000 erythrocytes of peripheral blood of fish Carasius aureus after 7-day treatment with different concentrations of the fungicide promenol kombi

Aquarium	Average number of MN	Significance
Aquarium 1 (5 mL fungicide /40 L water)	54	P<0.001
Aquarium 2 (4 mL fungicide /40 L water)	46	P<0.001
Aquarium 3 (3 mL fungicide /40 L water)	33	P<0.001
Aquarium 4 (2 mL fungicide /40 L water)	20	P<0.001
Aquarium 5 (control)	5	
Average number of MN in all treated fish, without control group	38.25	

DISCUSSION

Genotoxic evaluation of aquatic environment is a key mechanism for translating the principle of sustainable development into action. Genotoxic pollutants have been associated with gene mutation (mutagenic) and proliferation of tissue (carcinogenic potential). These chemicals are capable of transforming the future generations if unchecked because of its potential to cause genetic hazards.

Our findings are in concordance with the results of other authors, such as Shugart (1988), who investigated the genotoxic and neoplastic effects in both vertebrate and invertebrate organisms following metabolism of certain PAHs. Of the compounds selected for the environmental assessment, such effects were observed under laboratory conditions for B[*a*] P, phenanthrene, and naphthalene (Black *et al.,* 1983; Hose *et al.,* 1984; Hose, 1985; Shugart, 1988). Exposure of fish to PAHs leads to clastogenic effects resulting from DNA damage.

CONCLUSIONS

Based on this investigation we can conclude that the fungicide promenol kombi damaged the chromosome of erythrocytes of fish *Carassius auratus*. The frequency of micronuclei found was significantly higher in treated groups compared with control group.

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