# THE INDUCTION OF MICRONUCLEUS UNDER THE INFLUENCE OF ACETAMIPIRID INSECTICIDE ON THE GOLDFISH (*CARASSIUS AURATUS*) AFTER THE TIME TREATMENT OF 24 AND 72 HOURS

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**Abstract**

As a result of environmental, pollution by mutagen substances are created micronucleus, which are genetically defects, which may cause mutation and cancerous diseases. Based in this fact, we have attempted to do a research, which will prove that genetically damages are as result of environment pollution. Our research is based in Micronucleus-test method, according to Schmid. This method is used to be observed micronucleus, as mentioned above are created as result of environment pollution in blood of living organism. As we expected, during the research with this method, we have faced in genetically damage as aftermath of our environment pollution for research. For the research we have decided fish (goldfish), whereas environment pollution is created referring to our needs about research. The result is astonishing. As higher is level of pollution the higher will be the number of micronucleus, more precisely number of genetically damages. We have used 50 fish for our research diffused in five aquariums, distinguished by dose and day of treatment with insecticide. As our environmental pollutant for research we used acetamipirid insecticide in goldfish. The research has resulted what has proved by Schmid with Micronucleus-Test method, which proved the presence of genetic damages harmonized with the amount of pollution. Our research opens the way for safe research in environment polluting to see real situation on the ground and the ability to make managerial policies to prevent pollution, moreover to prevent many diseases caused by such pollution.

**Key words**: Micronucleus, acetamipirid, fish, blood, aquarium.

## 1. Introduction

People's organism nowadays is attacked from many chemical agents, and unfortunately their exposure is not just in these groups, which are exhibited in working places but in whole environment, especially in urban places. Research of genes' damage with micronucleus-test method gives us opportunities to determine: scale of pollution in living environment, and mutagen's acting capabilities, which may cause genetically damage.

Considerating that fish are water inhabitants, they have abilities to response toxic contaminants in similar way as the high vertebrates do, likewise these can be also used as indicators for different chemicals which may be mutagenicity [3].

Based in this fact, fish are perfect objects for mutagenicity or cancerous researchs of different components into water environment.

Micronucleus-test (MNT) is developed technic by Schmid [5], using cells as study object, moreover treating them with chemicals as genotoxic. test from toxic agents.

With this method is able to observe chromosome's damage, which may be detected according to genetically researchs, with water contaminants in fish's fringe blood that may be mutagen [2].

## 2. Material and methods

Material that has been used are fish. Their weight has varied from 22gr to 73gr. Fish's number used for research was 50, divided in five aquariums, four of them are used to test acetamipirid insecticide, while in another aquarium are placed fish. In aquariums we have put 40l of drinking water. Acetamipirid Insecticide used in four aquariums, is fixed dose from 0.125 ml/l (5ml insecticid/40 l water), 0.100 ml/l(4ml insecticid/40 l water), 0.075 ml/l (3ml insecticid/40 l water ), and 0.050 ml/l (2ml insecticid/40 l water).

The fish in the first aquarium on the concentration 0.125ml/l, and the fish in the second aquarium on the concentration 0.100ml/l under 24 hours treatment. In the third and fourth aquarium, the fish are placed on the concentrations 0,075 ml/l and 0,050 ml/l for 72 hours.

In fifth aquarium we have placed fish that'll be examinated. In each aquarium are applied pumps, their function is to supply them with air(oxygen).

Fish that have been treated for 24 and 72 hours are taken in another aquarium, not containing insecticid substances, but just drinking water. After 24 hours treatment with insecticid is taken their blood.

The blood was taken from the posterior part of the body from the caudal vein wherefrom each fish

has been taken a drop of blood, aftewards we placed it on glasses of object, than using another glass is done spreading (for each fish are done four preparations of spreading-painting).

Drying of preparations is done for 24 hours, while hitching is done with 96% of ethyl alcohol for 20 min, afterwards all of the preparations have been painted, accordintg to Schmid's method 1975, there also is used Gimza's solution, which is subtilized with distilled water in proportion 1:5 (Gimza paints stone material with darkness colour comparing with citoplazmatik material).

After subtilizing Gimza's solution is done preparations' painting for 55 min., all of the preparations are irrigated with distilled water.

Observation is done with microscop of lights increased for 400 time. In this state is made cells' photography and micronucleus's counting. About 2000 cells of blood has been counted from each fish, as for every single fish are prepared four preparations, each preparation has counted up to 500 erythrocytes.

## 3. Results and discussion

According to acquired results, we have average frequency of the micronucleuses in aquariums on table 1. In first aquarium treated with 0.0125 ml/l with insecticid, presented 18.8 MN. In second aquarium treated with 0.100ml/l presented 16.9 MN. In third aquarium treated with 0.075ml/l presented 14.6 MN. In fourth aquarium treated with 0.050ml/l presented 11.7 MN. While in not treated aquarium are presented 4.2 MN.

Moreover, our results are based in Kligerman's research [4] which confirm that fish who live in polluted water have higher frequencies scale of micronucleuses.

Average weight of fish in all five aquariums is 34.39gr, whereas average length is 13.26 cm.

**Table 1**: Average frequence of micronucleuses, weight and length of fish through aquariums.

| Aquariums | Micronucleuses (MN) | Weight (gr) | Length(cm) |
|---|---|---|---|
| Aq-1 0.125ml/l | 18.8 | 39.7 | 14.1 |
| Aq-2 0.100ml/l | 16.9 | 32.9 | 13.25 |
| Aq-3 0.075ml/l | 14.6 | 31.25 | 12.85 |
| Aq-4 0.050ml/l | 11.7 | 32.3 | 12.7 |
| Aq-5 controla | 4.2 | 35.8 | 13.2 |

**Table 2.** Standard deviation of the micronucleus, average frequency in aquariums with various dilutions and different time treatments.

| Aquariums | Average of MN-es for aquariums | Standard Deviation |
|---|---|---|
| Aq-1 | 18.8 | 3.610 |
| Aq-2 | 16.9 | 3.410 |
| Aq -3 | 14.6 | 3.062 |
| Aq- 4 | 11.7 | 2.751 |
| **A –contr.** | 4.2 | 2.710 |

. We have also presented the standard deviation results of the micronucleus average frequency, at the fish treated in dilutions and various time treatments in table 2.

These statistical analyzes were processed with statistical program SIGMA STAT 3.0, version 2004.

In table 3. we present statistical analysis results of t-test and significance of average frequency of the MN-es into fishes with various dilutions and different time treatments.

Our results are in accordance with the results of other author [1], who found a significant increase in the level of MN frequency in erythrocytes of fishes (*Clarias batrachus*), after treatment for 48, 72 and 96 hours, with 2.4 herbicide dichlorophenoxyacetic, used for destroying bad aquatic plants with wide leaves.

From results of presented in table 3. is seen that the frequency of micronucleus (MN) in erythrocytes of fish treated for 24 and 72 hours, including concentrates 0.125, 0.100, 0.075 and 0.050 ml/l is in higher significant scale($p<0.001$), compared with control fish group.

In the aquarium, under 24 hours treatment where acetamipirid insecticide was 0.125ml/l dilution, MN-

**Table 3**. Statistical analysis (t-test and significance) of the MN's average frequency at the fish treated in different dilutions and time treatments.

| Aquariums | t-test | significance |
|---|---|---|
| Aq-1 / Aq-2 | 1.208 | P=0.243 NS |
| Aq-1 / Aq-3 | 2.803 | P=0.012 S |
| Aq-1 / Aq-4 | 4.943 | P=<0.001 S |
| Aq-1 / Aq-control | 10.149 | P=<0.001 S |
| Aq-2 / Aq-3 | 1.586 | P=0.130 NS |
| Aq-2 / Aq-4 | 3.751 | P=<0.001 S |
| Aq-2 / Aq-control | 9.141 | P=<0.001 S |
| Aq-3 / Aq-4 | 2.228 | P=0.039 NS |
| Aq-3 / Aq-control | 7.965 | P=<0.001 S |
| **Aq-4 / Aq-control** | 6.060 | P=<0.001 S |

NS-It is not significance. S- It is significance

us frequencies were higher (18.8 MN/2000 erythrocytes), but not in significant scale (P=0.012), comparing with MN frequency in fish treated with 0.1ml/l dilution for 24 hours (16.9 MN/2000 erythrocytes).

In the aquarium, under 24 hours treatment where acetamipirid insecticide was 0.125ml/l dilution MN frequency is high while significant scale is (p<0.012), compared to fish's group treated 72 hours in 0.075ml/l dilution

On the other hand in the aquarium, under 24 hours treatment where acetamipirid insecticide was 0.125ml/l dilution MN frequency is also high with significant scale (p<0.001), compared to fish group treated 72 hours under 0.050ml/l dilution.

We have also observed higher MN-es frequencies in significance scale in fish treated for 24 hours under 0.1125ml/l dilution, compared with group control. In the aquarium, where the dilution of acetamipirid insecticide was 0.100ml / l in a 24 hours time treatment, the frequency of MN was higher (16.9 MN/2000 erythrocytes), but not in a significant scale, compared with the frequency of MN-fish treated dilution of 0.075ml / l for 72 hours (14.6 MN/2000 erythrocytes).

In the aquarium, where the dilution of acetamipirid insecticide was 0.100ml/ l in a 24 hours time treatment, the frequency of micronucleus was higher in a significant scale (p<0.001), compared with the frequency of the group of fish treated with dilution of 0.075ml / l for 72 hours.

Higher frequency of micronucleus in significant scale (p<0.001), was ascertained at the fishes treated for 24 hours too in a dilution of 0.100ml/l, compared with control group.

In the aquarium where the dilution of acetamipirid insecticide was 0.075ml/l, in a 24 hours time treatment, the frequency of MN was higher (14.6 MN/2000 red blood cells), but not in a significant scale compared with the frequency of fish treated in the dilution of 0.050ml/l for 72 hours (11.7 MN/2000 erythrocytes)

Higher frequency of micronucleus in significant scale (p<0.001), was also ascertained at the fishes treated for 72 hours, in a dilution of 0.100ml/l, compared with control group.

Similarly higher frequency of micronucleus in significant scale (p<0.001), was also ascertained at the fishes treated for 72 hours, in a dilution of 0.050ml/l, compared with control group.

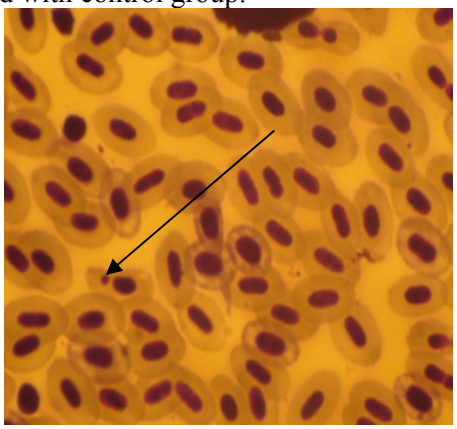

**Figure 1**:.Micronucleuses in the peripheral blood erythrocytes in (fish) *Carassius auratus*, treated for 24 hours in a dilution with acetamipirid insecticide 0.125ml / l.

## 4. Conclusions

Based on the research done on the genotoxic effect of the acetamipirid insecticide in the frequency of micronucleus of erythrocytes in goldfish (Carassius auratus), the following conclusions have been made:

- Higher frequency of micronucleus in a insignificant scale showed in the fish treated 24 hours in 0.125 ml/l dilution compared to fish treated 24 hours in 0.100 ml/l dilution.
- Higher frequency of micronucleus in a insignificant scale showed in the fish treated 72 hours in 0.075 ml/l dilution compared to fish treated 72 hours in 0.050 ml/l dilution.
- Higher frequency of micronucleus in a significant scale showed in the fish treated 24 and 72 hours in 0.125, 0.100, 0.075, 0.050 ml/l dilution compared to fish not treated with insecticide (control).
- Higher frequency of micronucleus in a significant scale showed in the fish treated 24 hours in 0.125 ml/l dilution compared to fish treated 72 hours in 0.075, 0.050 ml/l dilution.
- Higher frequency of micronucleus in a insignificant scale showed in the fish treated 24 hours in 0.100 ml/l dilution compared to fish treated 72 hours in 0.075, 0.050 ml/l dilution.
- Higher frequency of micronucleus in a significant scale showed in the fish treated 72 hours in 0.075 ml/l dilution compared to fish not treated with insecticide (control).
- Higher frequency of micronucleus in a significant scale showed in the fish treated 72 hours in 0.050 ml/l dilution compared to fish not treated with insecticide (control).

## 5. References


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