# RESEARCH ON THE LETHAL DOSE OF AZOXYSTROBIN FOR THE SPECIES *Carassius gibelio* (Bloch, 1782)

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**Abstract**. Azoxystrobin is an important factor contributing to the reduction of biodiversity in aquatic ecosystems, especially since it is found in water bodies in Europe, the USA, Germany, Sweden, China in a considerable proportion, between 0.16-3.03 μg/L, and through accumulation it concentrates in the tissues of aquatic organisms, and through the trophic chain in humans. In order to establish the lethal dose of azoxystrobin for the species of Carrassius gibelio, a case study was initiated and carried out, the general objective of which was to identify the concentration of doses of azoxystrobin with direct/indirect effects on the physiology and behavior of the species of *Carrassius gibelio*. The research conducted confirmed the hypotheses launched at the beginning of the study, namely that exposure of Carrassius gibelio species to a dose of 1-2 mg/L azoxystrobin does not induce physiological and behavioral changes in the first 48 hours, respectively that exposure of Carrassius gibelio species to a dose of 2.1-5 mg/L azoxystrobin induces physiological and behavioral changes (the behavior of the specimens was increasingly agitated, they tended to feed more and more often, to hit the walls of the aquarium, to make irregular, chaotic movements, to rise higher to the surface of the water) and that exposure of *Carrassius gibelio* species to a dose of 5.1-7 mg/L azoxystrobin is lethal at 168 hours (in the case of specimens exposed to a dose of 5 mg/L), respectively 48 hours after exposure (in the case of specimens exposed to doses of 6, respectively 7 mg/L).

**Keywords:** azoxystrobin, lethal dose, *Carassus gibelio*, behavioral and physiological changes.

**Rezumat. Cercetări privind doza letală de azoxistrobin pentru specia de *Carassius gibelio* (Bloch, 1782).** Azoxistrobinul reprezintă un factor important care contribuie la reducerea biodiversității ecosistemelor acvatice, mai ales că acesta se găsește în corpurile de apă din Europa, SUA, Germania, Suedia, China într-o proporție considerabilă, între 0,16-3,03 μg/L, iar prin acumulare se concentrează în țesuturile viețuitoarelor acvatice, iar prin lanțul trofic și în cele umane. În vederea stabilirii dozei letale de azoxistrobin pentru specia de *Carassius gibelio* a fost inițiat și realizat un studiu de caz al cărui obiectiv general a fost identificarea concentrației dozelor de azoxistrobin cu efecte directe/indirecte asupra fiziologiei și comportamentului speciei menționate. Cercetarea realizată a confirmat ipotezele lansate la începutul studiului și anume că expunerea speciei de *C. gibelio* la o doză cuprinsă între 1-2 mg/L azoxistrobin nu induce modificări fiziologice și comportamentale în primele 48 de ore, respectiv că expunerea speciilor de *C. gibelio* la o doză cuprinsă între 2,1-5 mg/L azoxistrobin induce modificări fiziologice și comportamentale (comportamentul exemplarelor era din ce în ce mai agitat, acestea aveau tendința de a se hrăni mai mult și mai des, de a se lovi de pereții acvariului, de a efectua mișcări neregulate, haotice, de a se ridica mai mult la suprafața apei) și că expunerea speciei la o doză cuprinsă între 5,1-7 mg/L azoxistrobin este letală la 168 de ore (în cazul exemplarelor expuse la o doză de 5 mg/L), respectiv 48 de ore de la expunere (în cazul exemplarelor expuse la doze de 6, respectiv 7 mg/L).

**Cuvinte cheie:** azoxistrobin, doză letală, *Carassius gibelio*, modificări comportamentale și fiziologice.

## INTRODUCTION

Azoxystrobin is a methacrylate compound structurally related to natural strobilurins, which are compounds derived from some fungal species and act by inhibiting electron transport in pathogenic fungi. This fungicide has several functional groups and complex metabolic pathways with the formation of a large number of metabolites, such as: azoxystrobin acid or R234886) or (E)-2-{2-[6-(2-cyano-phenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylic acid, -(2-cyanophenoxy)-6-hydroxypyrimidine (R401553) and 2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]benzoic acid (R402173).

Of these azoxystrobin metabolites, (E)-2-(2-[6-cyanophenoxy)-pyrimidin-4-yloxyl]-phenyl-3-methoxyacrylic acid has been shown to have high toxicity to aquatic organisms.

The specialized literature (Armstrong, S., Clough, J., 2009; Crupkin A., C., Fulvi A.,B., Iturburu F.,G. et al., 2021; Hoseinifar SH, Yousefi S, Van Doan H. et al., 2020; Murányi, D., Kovács, T., Katona, G., Fekete, J., 2015; Raza GA, Ghaffar A, Hussain R. et al., 2022; Yamin A, Naz S, Hussain R. et al., 2020) revealed that exposure of freshwater fish to azoxystrobin concentrations between 1-5 mg/L, within a period of 96 hours causes the following effects: induction of oxidative stress, inhibition of energy metabolism, destruction of cellular structures, modification of respiratory rhythm, significant decrease in the immune system and fertility, dysregulation of metabolism amino acids and fatty acids, ascorbic acid synthesis, damage to DNA and cell walls of cells.

The crucian carp (*Carassius gibelio*) is the most widespread freshwater fish in Romania. Originally from North Asia, it initially spread to China, then throughout the world.

The usual size of the crucian carp is 10-15 cm, and its weight can vary from 80-150 grams to 1.5-2 kg.

In muddy waters, its scales are black, in waters rich in vegetation it is green, and in clear waters the color of the crucian carp is golden-brown. The shape of the head is similar to that of the carp, but it lacks whiskers, and the shape of the mouth is obtuse, with thin lips. Reproduction occurs in spring when the water reaches temperatures of 8-10

degrees. Spawning lasts until the end of summer. In this species, all specimens over 2-3 years old are females, because males, after this period, suffer from the phenomenon called gynogenesis.

In order to establish the lethal dose of azoxystrobin for the species of *C. gibelio*, a case study was initiated and carried out.

General objective: to identify the concentration of doses of azoxystrobin with direct/indirect effects on the physiology and behavior of the species of *C. gibelio*.

Hypotheses launched:

1. exposure of the species of *C. gibelio* to a dose of azoxystrobin between 1-2 mg/L azoxystrobin does not induce physiological and behavioral changes in the first 48 hours.
2. exposure of the species of *C. gibelio* to a dose of azoxystrobin between 2.1-5 mg/L azoxystrobin induces physiological and behavioral changes and can be lethal after a certain time interval.
3. exposure of the species of *C. gibelio* to a dose of azoxystrobin between 5.1-7 mg/L azoxystrobin is lethal in the first 48 hours.

## MATERIALS AND METHODS

Material used:
Eight specimens of *C.gibelio* were exposed for 8 days to a dose of 1, 2, 3, 4, 5, 6, 7 mg/L azoxystrobin.
Working method used: observation.
Sample used: The research was carried out in November 2024 on a sample consisting of 56 specimens of *C. gibelio*, with a length between 2-8 cm and a weight between 10-80 grams.

## RESULTS AND DISCUSSIONS

Experiment a was carried out privately. Eight specimens of *C. gibelio* were introduced into 10 l of water over which 1 mg/L azoxystrobin was poured. The biological material was observed for eight days, during which no behavioral and physiological changes were reported, which confirms hypothesis 1 of the study launched, namely that exposure of *C. gibelio* species to a dose of 1 mg/L azoxystrobin does not induce physiological and behavioral changes.

Eight specimens of *C. gibelio* were introduced into 10 l of water and exposed to doses of 2, 3, 4, 5, 6 and 7 mg/L azoxystrobin.

The induced effects are presented in table 1.

Table 1. Effects of exposure of *C. gibelio* species to various doses of azoxystrobin.

| Sample | Exposure dose | Exposure time | Observed effects |
|---|---|---|---|
| 8 pieces Length=2-8 cm Weight=10-30 grams | 1 mg/L | 24 hours | No behavioral and physiological changes were noted. |
| | | 48 hours | No behavioral and physiological changes were noted. |
| | | 72 hours | No behavioral and physiological changes were noted. |
| | | 96 hours | No behavioral and physiological changes were noted. |
| | | 120 hours | No behavioral and physiological changes were noted. |
| | | 144 hours | No behavioral and physiological changes were noted. |
| | | 168 hours | No behavioral and physiological changes were noted. |
| | | 190 hours | No behavioral and physiological changes were noted. |
| 8 pieces Length=2-8 cm Weight=10-30 grams | 2 mg/L | 24 hours | No behavioral and physiological changes were noted. |
| | | 48 hours | No behavioral and physiological changes were noted.. |
| | | 72 hours | Slightly agitated behavior. |
| | | 95 hours | Slightly agitated behavior, tendency to cover more of the aquarium surface by making circular movements |
| | | 120 hours | Slightly agitated behavior, rises to the surface of the water more often. |
| | | 144 hours | Slightly agitated behavior, the number of rises to the surface of the water increases more and more. |
| | | 168 hours | Slightly agitated behavior, lifting movements at the water surface are more frequent and irregular. |
| | | 190 hours | Slightly agitated behavior, the number of rises to the water surface is twice as high as in the previous period. |
| 8 pieces Length=2-8 cm Weight=10-30 grams | 3 mg/L | 24 hours | Very agitated behavior. |
| | | 48 hours | Agitation behavior, movement in the aquarium is achieved through hesitant and pendulum movements. |
| | | 72 hours | Agitation. Weight loss of 0.4 g, tendency to feed more often. |
| | | 96 hours | Agitation, the number of rises to the surface of the water increases. |
| | | 120 hours | Agitation. Weight loss of 0.7 g, tendency to feed more often, to move a lot in the aquarium, by describing slightly hesitant movements. |
| | | 144 hours | Agitation. |
| | | 168 hours | Agitation. Weight loss of 0.9 g, tendency to eat more. |
| | | 190 hours | Agitation behavior, tendency to move a lot in the aquarium, describing uncertain and irregular movements, the number of rises to the surface of the water is greater. |

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| 8 pieces Length=2-8 cm Weight=10-30 grams | 4 mg/L | 24 hours | Very agitated behavior. |
| | | 48 hours | Very agitated behavior. Weight loss of 1.2 g. |
| | | 72 hours | Very restless behavior, tendency to move a lot in the aquarium, by describing uncertain and irregular movements. |
| | | 96 hours | Very agitated behavior. Weight loss of 1 g. |
| | | 120 hours | Very agitated behavior, tendency to move a lot in the aquarium, describing uncertain and irregular movements, the number of rises to the surface of the water is greater. |
| | | 144 hours | Very agitated behavior. Weight loss of 1 g. |
| | | 168 hours | Very agitated behavior, tendency to move even more in the aquarium, by describing increasingly uncertain and irregular movements, the number of rises to the water surface being even greater. |
| | | 190 hours | Very agitated behavior. Weight loss of 1 g, tendency to eat more than before, to move more and more chaotically. |
| 8 pieces Length=2-8 cm Weight=10-30 grams | 5 mg/L | 24 hours | Very agitated behavior. |
| | | 48 hours | Very agitated behavior. Weight loss of 1 g, tendency to feed more often and more. |
| | | 72 hours | Increasingly agitated behavior, the number of rises to the surface increases. |
| | | 96 hours | Very agitated behavior. Weight loss of 1.3 g, tendency to feed more than before, to move more and more chaotically, irregularly in the aquarium, the number of rises to the surface is increasing more and more. |
| | | 120 hours | Very agitated behavior. Weight loss of 1.3 g. |
| | | 144 hours | Very agitated behavior, they tend to hit the walls of the aquarium, they move with increasingly weaker, more uncertain and chaotic movements. |
| | | 168 hours | Death |
| | | 190 hours | Death |
| 8 pieces Length=2-8 cm Weight=10-80 grams | 6 mg/L | 24 hours | Very agitated behavior, the number of rises to the water surface is twice as high as in the case of other specimens exposed to other doses of azoxystrobin. |
| | | 48 hours | Death |
| | | 72 hours | Death |
| 8 pieces Length=2-8 cm Weight=10-80 grams | 7 mg/L | 24 hours | Very agitated behavior, the lifting movements to the water surface are chaotic and performed with a very high frequency, covering the entire aquarium. |
| | | 48 hours | Death |

In the case of specimens exposed to a dose of 2 mg/L of azoxystrobin for 24 and 48 hours, the same situation was observed, while the exposure of these specimens to the same dose for an interval between 72 and 190 hours led to the recording of a state of slight agitation in their behavior.

For specimens exposed to a dose of 3 mg/L of fungicide, agitated behavior was observed throughout the monitoring interval and, starting with the third day, a slight weight loss of 0.3 grams/specimen/48 hours.

For specimens exposed to a dose of 4 mg/L fungicide, a very agitated behavior and a slight weight loss of 1 gram/specimen/48 hours were observed throughout the monitoring interval.

In the first 24 hours of exposure of goldfish specimens to a dose of 5 mg/L azoxystrobin, a very agitated behavior was observed, while their exposure to the same dose for a period between 48 and 168 hours also had the effect of decreasing the weight of these specimens by 1-1.3 grams.

Exposure to a dose of 5 mg/L azoxystrobin led to the death of the tested specimens on the eighth day.

For specimens exposed to a dose of 6 mg/L azoxystrobin, a very agitated behavior was observed in the first 24 hours after exposure. At 48 hours after exposure, the specimens began to lose weight by 2 grams, so that at 72 hours after exposure, the death of all specimens was observed.

In the case of specimens exposed to a dose of 7 mg/L azoxystrobin, a very agitated behavior was observed in the first 24 hours after exposure. At 48 hours after exposure, the death of all specimens was observed.

Throughout the testing period, no increase in length was recorded for any specimen, regardless of the dose of fungicide to which they were exposed.

For specimens exposedto a dose of 5 mg/L azoxystrobin, death occurred starting on the seventh day of exposure.

For specimens exposed to a dose og 6 and 7 mg/L azoxystrobin, death occurred from the second day of exposure which confirms the second hypothesis launched by the initiated study, namely that exposure of the species to a dose higher than 5 mg/L azoxystrobin is lethal (MURÁNYI et al., 2015; EDWARDS et al., 2016; CHANG et al., 2017; COOPER et al., 2020; HOSEINIFAR et al., 2020; YAMIN et al., 2020 AKRAM et al., 2021; CRUPKIN et al., 2021).

It was noted that, as the dose of azoxystrobin and the duration of exposure increased, the behavior of the specimens became increasingly agitated, they tended to feed more and more often, to move increasingly chaotically, irregularly in the aquarium, describing pendulum, uncertain movements and showing a tendency to hit the walls of the aquarium (in the case of specimens exposed to a dose of azoxystrobin of 5 mg/L, on the sixth day after exposure), the number of rising to the surface increases more and more as the dose of toxic substance and the duration of exposure increase, and the rising movements to the surface of the water are increasingly hesitant and chaotic, and performed with an increasingly greater frequency, with a tendency to cover the entire aquarium.

## CONCLUSIONS

The research confirmed the hypotheses launched at the beginning of the study, namely that exposure of *C. gibelio* species to a dose of 1-2 mg/L azoxystrobin does not induce physiological and behavioral changes in the first 48 hours.

The research also confirmed the second and third hypotheses launched, namely that exposure of *C. gibelio* species to a dose of 2.1-5 mg/L azoxystrobin induces physiological and behavioral changes, respectively that exposure of *C. gibelio* species to a dose of 5.1-7 mg/L azoxystrobin is lethal at 168 hours (in the case of specimens exposed to a dose of 5 mg/L), respectively 48 hours after exposure (in the case of specimens exposed to doses of 6 and 7 mg/L, respectively).

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