

CHRONIC ASPECTS OF IMIDACLOPRID ON THE FISHES FROM *CYPRINIDAE* FAMILY

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Abstract: This paper presents the results of a short-term chronic toxicity test at saplings carp (*Cyprinus carpio*), saplings crucian (*Carassius auratus*) and Chinese carp saplings (*Ctenopharyngodon idella*) bred a period of 35 days in solutions sublethal of imidacloprid. The purpose of these research was to determine the maximum concentration of imidacloprid for cyprinidae family and assess the safety concentration of this pesticide. The test results showed that *Cyprinus carpio* is most sensitive to sublethal concentrations of imidacloprid. Maximum permissible value of imidacloprid in water for *Cyprinus carpio* represent the safety concentration that can protect all aquatic organisms in the ecosystem studied.

Key words: eco-toxicology, *Cyprinus carpio*, *Carassius auratus*, *Ctenopharyngodon idella*, „Safety concentration”

INTRODUCTION

Research conducted in recent years in the chronic eco-toxicology at fish are designed to protect all aquatic organisms in the ecosystem studied. Therefore, research on sublethal concentrations of pesticides have intensified. The end result of these research represent accumulation of data about values maximum allowable in water (Maximum Acceptable Toxic Concentration - MATC) and "Safety Concentrations" for pesticides.

This paper presents data on the chronic toxicity of imidacloprid on the fish from *cyprinidae* family. The research was conducted in the Laboratory of Eco-toxicology of R.D.I.P.P Bucharest.

MATERIAL AND METHOD

For ecotoxicological tests were used following materials:

- imidacloprid (4 g /l);
- biological material: *Cyprinus carpio*, *Carassius auratus*, *Ctenopharyngodon idella*

The saplings of *Cyprinidae* had ages between 3 to 4 weeks and an average weight of 5 g and have been purchased from Research Station Nucet, Dâmbovița.

The reference material for eco-toxicological test performance was:

- OECD guide No.203/17.07.1992 – Acute toxicity test on fishes;
- OECD guide N0.305/14.06.1996 – Bioconcentration, fish test.
- HG no. 490 16/05/2002;
- GLP general and specific procedures (elaborated by RDIPP).

RESULTS AND DISCUSSIONS

The first chronic toxicology studies on fish were conducted on complete vital cycles (Mount and Stephan, 1967). These studies have demonstrated greater sensitivity of some stages of development. Possibility of conducting research for sensitive stages has led to more rapid methods and less expensive to evaluate chronic toxicity. The results obtained showed that short-term tests conducted on embryonic stages, juveniles and young saplings stages, lead to estimates of concentrations maximum of pesticides comparable to those obtained by testing on complete vital cycles.

The maximum concentration of pesticide (MATC) is the concentration which not affects at a prolonged exposure (chronic) the survival, reproduction and growth of various species of fish and the „Safety Concentration" defines the maximum concentration of pesticide which not affect at a prolonged exposure the most sensitive fish species in aquatic ecosystems.

The lowest value of „MATC” corresponding to the most sensitive of fish species is the safety concentration that protects all species of the aquatic system studied.

Since chronic toxicity bioassays on fish are preceded by the acute toxicity tests, initial was evaluated the acute toxicity for determining the median lethal concentrations of imidacloprid at the three species of *Cyprinidae*.

a) Acute toxicity of imidacloprid on the fish from *Cyprinidae* family

For the determination of the acute lethal toxicity of imidacloprid at *Cyprinidae* it was used the static method, the testing period being of 96 hours. The main indicators estimated in the fish ecotoxicological tests (Table 1). Cumulative mortality was recorded at 24, 48, 72 and 96 hours and median lethal concentration was calculated statistically. The temperature, pH and dissolved oxygen concentration had been monitored daily. Also were observed morphological and behavioral changes.

Table 1

Aquatic organisms' main indicators estimated in eco-toxicological tests	
Indicator	Specification
LC ₅₀ % Mean Lethal Concentration	Calculated concentration of testing substance inducing 50% mortality of the fish population at the end of the testing period.
NOEC Concentration no effect	Highest concentration showing no observable significant toxic effect on testing organisms.
M.A.T.C. Maximum permissible concentration in water	The maximum concentration of pesticide that prolonged exposure (chronic) did not affect survival, reproduction and growth of various species of fish
Factor a Safety Concentration	Maximum concentration that does not affect the long exposure most sensitive species in the ecosystem
Modification of functional indices	Indices of growth, mortality, food consumption and efficiency of feed utilization for growth

The fungicide imidacloprid had a acute toxicity moderate at saplings of *Cyprinidae*, but at higher concentrations (depending on species) were observed: inactivity, diving sudden, weakness, spiral swimming, lack of balance, breathing slow and cumbersome, spasms and mortality.

The saplings of crucian proved more resistant to the toxic action of imidacloprid, the lethal median concentration LC₅₀ (96 h) was 24.8 mg a.s./l (Table 2) compared with saplings of carp at that the LC₅₀ (96 h) it was 6.68 mg a.s./l. (Table 4). This resistance could be explained by the fact that this species has a ecological plasticity high and a great adaptability, being present in all lakes and ponds in our country.

Results of the acute toxicity of imidacloprid to saplings of *Ctenopharyngodon idella* are shown in Table 3; the value of median lethal concentration was of 13.2 mg a.s./l, close to value obtained for *Cyprinus carpio*. At the lots of control have not registered mortality and symptoms of acute toxicity.

From analysis of results obtained can be observed that saplings of carp were the most sensitive to the toxic action of imidacloprid.

Table 2

The acute toxicity of imidacloprid on <i>Carassius auratus</i>						
Concentration ml c. p./l	No.fish tested	Cumulative mortality at:				Modification of some behavioral and anatomical indicators
		24 h	48 h	72 h	96 h	
1.25	7	0	0	0	0	-
2.5	7	0	0	0	1	-
5	7	0	0	1	2	
10	7	1	1	1	4	balance loss
20	7	2	2	1	6	lack of response to tactile stimuli, excitation and loss of balance
40		3	3	1	7	mortality
Martor	7	0	0	0	0	-

NOEC = 1.25 ml c.p./l = 5 mg a.s./l

LC₅₀ (96 h) = 6.2 ml c.p./l = 24.8 mg a.s./l

R = 0.99184

Table 3

The acute toxicity of imidacloprid on *Ctenopharyngodon idella*

Concentration ml c. p./l	No.fish tested	Cumulative mortality at:				Modification of some behavioral and anatomical indicators
		24 h	48 h	72 h	96 h	
0.625	7	0	0	0	0	-
1.25	7	0	0	1	1	-
2.5	7	0	1	1	2	
5	7	1	1	1	4	inactivity
10	7	1	1	2	5	lack of balance, breathing slow and cumbersome
20	7	2	3	2	7	mortality
Martor	7	0	0	0	0	-

NOEC = 0.625 ml c.p./l = 2.5 mg a.s./l LC₅₀ (96 h) = 3.3 ml c.p./l = 13.2 mg a.s./l R = 0.99884

Table 4

The acute toxicity of imidacloprid on *Cyprinus carpio*

Concentration ml c. p./l	No.fish tested	Cumulative mortality at:				Modification of some behavioral and anatomical indicators
		24 h	48 h	72 h	96 h	
0.312	7	0	0	0	0	-
0.625	7	0	0	0	1	-
1.25	7	0	0	0	2	sudden immersion swimming spiral
2.5	7	0	1	1	2	inactivity
5	7	2	2	2	-	spasms, lack of balance, breathing slow and cumbersome
10	7	4	2	1	-	mortality
Martor	7	0	0	0	0	

NOEC = 0.312 ml c.p./l = 1.24 mg a.s./l LC₅₀ (96 h) = 1.67 c.p./l = 6.68 mg a.s./l R = 0.99652

b) Chronic toxicity of imidacloprid on the fish from *cyprinidae* family

Based on the results of acute toxicity, were conducted chronic sub-lethal tests, to assess the influence of imidacloprid on mortality, growth and efficiency of feed utilization (Table 5).

In chronic toxicity tests the fish were fed with pelleted foods, and administered daily ration was of 2% by weight of the batch. At the lots of control have not registered effects on growth, mortality and efficiency of feed utilization at species tested (Table 6, 7 and 8).

From the data analysis it is observed that concentration of 5.0 mg a.s./l of imidacloprid does not alter the growth, survival and efficiency of feed utilization and represents the limit that no toxic effects on saplings of crucian and the maximum amount allowable in water (MATC) of imidacloprid, for *Carassius auratus*. Application factor (a) which signify the ratio of the MATC (5.0 mg s.a./l) and LC₅₀ (24.8 mg s.a./l) is equal to 0.20 and represent safety concentration for *Carassius auratus*.

The concentration of 5.0 mg a.s./l imidacloprid had changed the values of growth and efficiency of feed utilization in saplings of carp compared to the lot control. Thus, the increase was 51.8% compared with the control and efficiency of feed utilization of 53.3%, while at control was 103.7% (almost double the concentration of 1.25 mg a.s./l imidacloprid).

At sapling of chinese carp the growth was 97.9 % at the concentration of 2.5 mg a.s./l imidacloprid compared with the control and only 60.6 at concentration of 10.0 mg a.s./l imidacloprid.

Because the *Cyprinus carpio* was the most susceptible at action toxic of imidacloprid, we estimate that the concentration of imidacloprid at 1.25 mg a.s./l represent the limit which does not affect at the long-term exposure the fish species most sensitive of aquatic ecosystem studied respectively the ecosystem of the farm Nucet, Dambovița.

Above this maximum concentration of imidacloprid was recorded mortality and efficiency of feed utilization decreases greatly, the more resistant was *Carassius auratus* at which the toxic effects occur at concentrations above 10.0 mg s.a./l imidacloprid.

Therefore, the application factor = 0.2 determined at *Carassius auratus*, can not be regarded as a safety concentration. In contrast, the application factor = 0.18 determined for *Cyprinus carpio* represent

safety concentration which can protect all species studied of aquatic ecosystem (fish, daphnids, algae etc.) against toxic actions of imidacloprid.

Table 5

Indices of growth, mortality, and efficiency of feed utilization		
Indicator	Formula	Significant symbols
Growth (C)	$C = \frac{\overline{Gf} - \overline{Gi}}{\Delta t}$ (Ricker, 1970)	\overline{Gf} - final average weight; \overline{Gi} - average initial weight; Δt - experimental period (days)
Mortality (M)	$M = \frac{Ni - Nf}{\Delta t}$ (Ricker, 1970)	Ni și Nf = initial and final number of fish, Δt - experimental period (days)
Efficiency of feed utilization (E)	$E = \frac{\Delta G}{R \cdot \Delta t} \cdot 100$ (Ivlev, 1960)	ΔG = fish growth calculated from the difference between \overline{Gf} and \overline{Gi} R = amount of food consumed during the experimental period

Table 6

Growth, mortality and efficiency of feed utilization at <i>Carassius auratus</i>										
Variant	t°C	Δt (days)	Ni	\overline{Gi}	Nf	\overline{Gf}	C	C% control	M	E
Control	25	35	10	28	10	589.4	16.0	100	0	102.2
imidacloprid 5.0 mg a.s./l	25	35	10	28	10	546.2	14.8	92.5	0	94.3
imidacloprid 20.0 mg a.s./l	25	35	10	28	8	370.3	9.8	61.2	0.05	62.3

MATC = 5.0 mg a.s./l

a = 0.20

Table 7

Growth, mortality and efficiency of feed utilization at <i>Ctenopharyngodon idella</i>										
Variant	t°C	Δt (days)	Ni	\overline{Gi}	Nf	\overline{Gf}	C	C% control	M	E
Control	25	35	10	30	10	710.6	19.4	100	0	108.0
imidacloprid 2.5 mg a.s./l	25	35	10	30	10	698.2	19.0	97.9	0	106.6
imidacloprid 10.0 mg a.s./l	25	35	10	30	4	411.8	10.9	56.1	0.17	60.6

MATC = 2.5 mg a.s./l s.a./l

a = 0.18

Table 8

Growth, mortality and efficiency of feed utilization at <i>Cyprinus carpio</i>										
Variant	t°C	Δt (days)	Ni	\overline{Gi}	Nf	\overline{Gf}	C	C% control	M	E
Control	25	35	10	30	10	680.2	18.5	100	0	103.2
imidacloprid 1.25 mg a.s./l	25	35	10	30	10	620.6	16.8	89.7	0	93.7
imidacloprid 5.0 mg a.s./l	25	35	10	30	3	366.2	9.6	51.8	0.20	53.3

MATC = 1.25 mg a.s./l

a = 0.18

CONCLUSIONS

- The fungicide imidacloprid had a acute toxicity moderate on the fish from *cyprinidae* family, but at higher concentrations (depending on species) were observed: inactivity, diving sudden, weakness, spiral swimming, lack of balance, breathing slow and cumbersome, spasms and mortality;
- Based on the research it was observed that symptoms of poisoning at fish bred in solutions lethale of imidacloprid is atypical similar to that described in the literature;
- Installation phases progressive with pesticide poisoning are: the phase of excitation, phase of partial loss of equilibrium and the final phase of total loss of balance. Death occurs at different time intervals depending on the concentrations of pesticides;
- Test results of chronic toxicity showed the species *Cyprinus carpio* is the most sensitive to sublethal concentrations of imidacloprid;

- Maximum allowed imidacloprid in water for *Cyprinus carpio*, represent the safety concentration that can protect all aquatic organisms in the ecosystem studied from the toxic action of this fungicide.

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