

ENVIRONMENTAL RISK ASSESSMENT OF SOME FUNGICIDE MIXTURES ACCORDING TO GLP REQUIREMENTS

Marga Gradila*, Mincea Carmen, Elena Hera

Research - Development Institute for Plant Protection, Bucharest

^{1*} correspondence address:

Research-Development Institute for Plant Protection

Bd. Ion Ionescu de la Brad nr. 8, CP 013813, S 1,

Bucharest, ROMANIA

Tel.: 004-021-2693231 (32, 34)

Fax.: 004-021-2693239

e-mail: marga_gradila@yahoo.com

Abstract: The aim of this study is the assessment of environmental risks of four fungicide mixtures showing the demonstrating data of d biological systems using aquatic organisms in GLP conditions designed in the eco-toxicological facility of RDIPP Bucharest.

In order to assess the four fungicides mixtures environmental risk according to the principles of good laboratory practice, we have gone through the following stages: development of the framework structure by ensuring the material base (plant complex to produce de-ionized water, reconstituted and d thermostatic water), water supply for tests, achievement of the control system and software for inspection of the environmental conditions in the acclimation and test rooms, and development of the specific operating procedures and testing.

The acquired data established that the biological systems model of the testing facility agree with the OECD directories concerning the GLP compliance. Following the examination of the fungicides mixtures in terms of acute toxicity against *Daphnia magna* and *Cyprinus carpio* revealed that three out of them (myclobutanil with thiram, captan and myclobutanil, and carbendazim with propiconazole) showed the environmental efficiency, irrespectively low toxicity. The mixture of carbendazim and thiram showed a higher toxicity, inducing toxicity symptoms in fish: inactivity, sudden sinking, decoloration, and weakness, spiral swimming, loss of balance, spasms, slow and heavy breathing.

Key words: eco-toxicology, reconstituted biological system, *Cyprinus carpio*, *Daphnia magna*

INTRODUCTION

The government and general industry are concerned in the quality of the non medical/clinical security studies on which it si grounded the human health risks assessment. With this respect the Organization of Co-operation and Economic Development (OECD) established the criteria to perform these studies. The non medical/clinical security tests involving environmental and human health risks, according to the GLP principles contain laboratory greenhouses and field tests, as well as eco-toxicological tests.

The study shows some the data related to the environmental risks assessment of four fungicide mixtures according to the GLP principles, using the biological pattern systems designed for the testing facility in RDIPP Bucharest.

MATERIAL AND METHOD

The reference material for eco-toxicological test execution is:

- OECD Guideline No.203/ 1992.07.17 – Acute toxicity test in fish;
- OECD Guideline No.202/1992.07.17 –Acute immobilizing test in daphnia;
- GD No. 490/ 16.05.2002;
- GLP general and specific procedures (elaborated in the Testing Facility);

four fungicide mixtures (two based on carbendazim, and another two based on miclobutanil);

- biological material: *Cyprinus carpio*, and *Daphnia magna*.

For the determination of the acute lethal toxicity of the four fungicide mixtures in the two test species it has been used the static method, the testing period being of 96 hours for fish, and 48 hours for

Daphnia. The testing solutions were not renewed during the test, but daily they have been monitored the temperature, pH, and dissolved oxygen concentration. The main estimated indicators in the aquatic organisms' eco-toxicological tests are shown in table 1.

RESULTS

According to the Good Laboratory Practice principles the performance of environmental risk assessment tests of four fungicide mixtures in fish and daphnia comprise the following steps: the accomplishment of a frame structure in order to ensure the equipment, the production of necessary water for the tests, the achievement of the control system and soft designed for checking out the acclimation and testing rooms environmental conditions, and the elaboration of the specific operating procedures.

a) Environmental risk assessment in fish

For the environmental risk assessment in fish, in the first stage it has been settled the reconstituted biological system according to the designed specific procedures in the framework of the laboratory, choosing and purchasing the test species, followed by a period for fish acclimation before testing;

They has been tested four fungicide mixtures: carbendazim + propiconazole, carbendazim + thiram, myclobutanil + thiram and myclobutanil + captan, that are extensively used in the agricultural practice of our country. (Table 2)

The carbendazim and propiconazole mixture showed a very low toxicity effect in fish, LC 50% (96 h) = 1.11 mg a.s./l, and NOEC (96 h) = 0.18 mg a.s./l). When carbendazim has been formulated together with thiram, the mixture toxicity increased very much, the average lethal concentration was of 0.023 mg a.s./l, and the concentration without effect was of 0.0031 mg a.s./l. The variation in time of the induced mortality in fish by the fungicide mixture of carbendazim + thiram it is shown in the Figure 1 (Marga Gradila, 2005).

The sub lethal concentration of carbendazim + thiram induced toxic symptoms of inactivity, sudden sinking, discoloring, weakness, spiral swimming, balance loss, spasms, slow and heavy breathing.

Miclobutanil and thiram mixture developed a moderate toxicity for fish, the lethal average concentration LC 50% (96 h) being of 0.85 mg a.s./l. At concentrations higher than 0.1 mg a.s./l, fish clearly exhibit asphyxiation symptoms.

Miclobutanil and captan mixture didn't induce toxicity effects in fish LC 50 (96 h) = 0.95 mg a.s./l. Anyway at concentrations higher than 1 mg a.s./l the fish suffer, become disturbed, exhibit spasms, and also it has been noticed a massive intestinal matter exclusion.

Environmental risk assessment in daphnia

The environmental risk assessment for fungicide mixture in daphnia, according to the specific procedures of the biological reconstituted system developed in the laboratory, firstly the following:

- selection of testing species;
- breeding the testing species;
- providing of the breeding conditions;
- new born segregation;
- sensitivity testing of the tested species;

Carbendazim and propiconazole mixture induced a moderate toxicity effect in daphnia EC50% (48 h) = 0.31 mg a.s./l, and NOEC (48 h) = 0.04 mg a.s./l. When carbendazim was made up together with thiram, as in the case of fish, the toxicity effect increased very much, the mean inhibitory concentration being of 0.012 mg a.s./l, and the no effect concentration of 0.0031mg a.s./l developing a massive daphnia immobilization. The toxicity symptoms induced by the sub lethal concentrations of carbendazim and thiram in daphnia developed symptoms of agitation and balance loss. (Table 3)

Myclobutanil and thiram mixture developed a moderate toxicity effect in daphnia, the average inhibitory concentration = 0.75 mg a.s./l.

The other two fungicide mixtures based on myclobutanil, irrespectively myclobutanil + thiram and myclobutanil + captan, developed a moderate toxicity in daphnia, but at concentrations higher than 0.1 mg s.a./l the daphnia suffer and almost instantly die.

Table 1

Main estimated indicators in eco-toxicological tests using aquatic organisms

Indicator	Specification
LC ₅₀ % Average Lethal Concentration	Calculated concentration of the testing substance inducing 50% mortality of the fish population at the end of the testing period.
NOEC	Highest concentration showing no observable significant toxic effect in the testing organisms.
Mean Inhibitory Concentration (EC _{50%})	Calculated concentration of testing substance inducing 50% immobility* in testing daphnia.
Morphological and Behavioral deviations	Respiratory disturbances, touching stimuli lack of reaction, balance loss, sudden sinking, color loss or skin pigmentation, etc.

*Immobility – are declared immobile the daphnia slightly touched and unable moving in 15 seconds after stirring contains of testing container.

Table 2

Fungicide mixtures toxicity in fish

Fungicide mixture	LC _{50%} mg a.s./l	NOEC mg a.s./l	Modification of some behavioral and anatomical indicators
carbendazim 300 g/l + propiconazole 75 g/l	1.11	0.18	Balance loss
carbendazim 200 g/l + thiram 400 g/l	0.023	0.0031	Inactivity, sudden sinking, decoloration, weakness, spiral swimming, balance loss, spasms, slow and heavy respiration
myclobutanil 5% + thiram 60%	0.85	0.60	Asphyxiation, balance loss
myclobutanil 3.5%+ captan 46.5%	0.95	0.70	Excited movement, spasms and massive intestinal contain rejection.

Table 3

Fungicide mixtures toxicity in daphnia

Fungicide mixture	EC _{50%} mg a.s./l	NOEC mg a.s./l	Alteration of some behavioral and anatomical indicators
carbendazim 300 g/l + propiconazole 75 g/l	0.31	0.04	-
carbendazim 200 g/l + thiram 400 g/l	0.012	0.0031	Exciting movement, balance loss disorder swimming movements
myclobutanil 5% + thiram 60%	0.75	0.50	-
myclobutanil 3,5%+ captan 46,5%	0.85	0.60	-

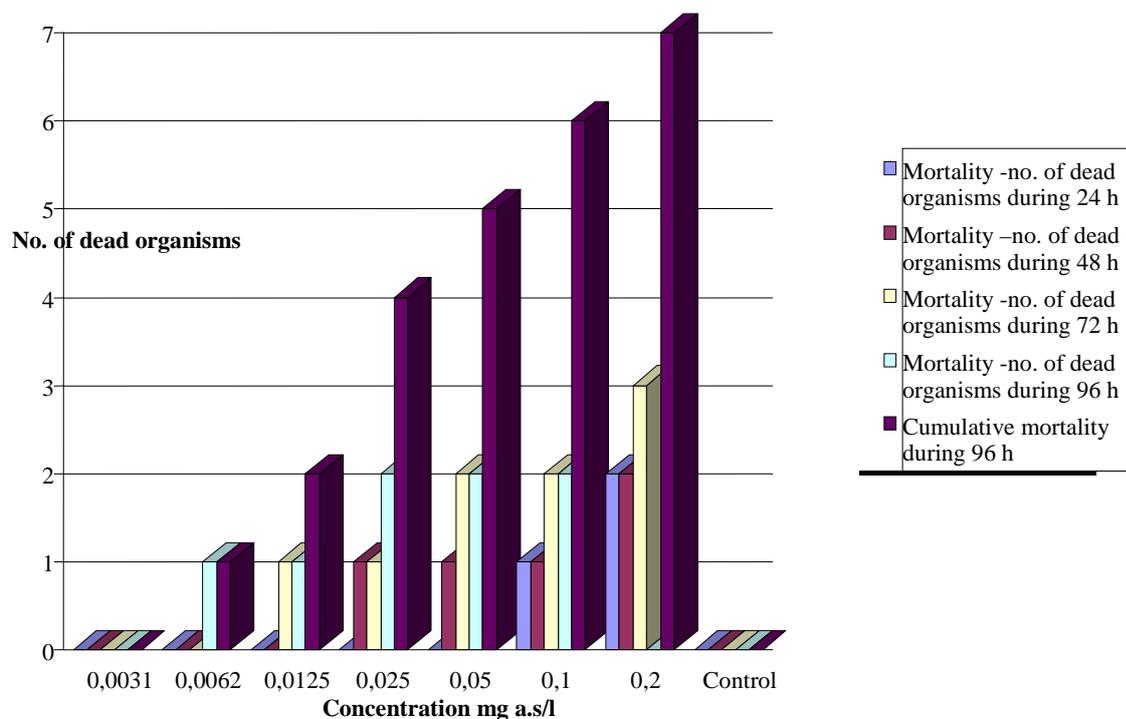


Fig.1. Mortality time variation induced by lethal concentration of solution of carbendazim 200 g/l + thiram 400 g in carp *Ciprinus carpio*

CONCLUSIONS

The fungicide mixtures (*myclobutanil and thiram*, *myclobutanil and captan*, *carbendazim and propiconazole*) showed an ecological efficiency, low toxicity effect in fish and daphnia respectively. *Carbendazim and thiram* mixture induced a high toxicity effect in fish and daphnia, the main recorded symptom being the mortality.

Here you are the toxicity symptoms induced by sublethal concentrations of *carbendazim + thiram*: immobility, sudden sinking, decoloration, weakness, spiral swimming, balance loss, spasms, slow and heavy respiration

The semeiology of the fish kept in pesticide lethal solutions is atypical, and it is similar to the mentioned references in the specialty literature. The progressive pesticide toxicity induction starts with an excited phase, partially balance loss, and the final phase when the balance is completely lost. The death occurs at different periods according to the concentrations of the pesticide solution

The biological systems pattern for fish and daphnia performed in the testing facility, proved their efficacy and validity for the environmental risks assessment of the plant protection products in compliance with the *Good Laboratory Practice Principles*.

REFERENCES

- GOVERNMENT DECISION No. 63/2002 approving the Good Laboratory Practice Principles
 GOVERNMENT DECISION No. 490/2002 - methods for eco-toxicity assessment/measurement;
 GOVERNMENT DECISION No. 266/2006 amending and altering the Government Decision No. 63/2002
 MARGA GRADILA (2005), PHD Thesis "Research concerning the influence of some pesticides in fish from the *Cyprinidae* Family", 173-180
 OECD No. 1- Good Laboratory Practice Principles (1997 revised)
 OECD Guidelines for the Testing of Chemicals no.203/17.07.1992 – Acute toxicity testing in fish;
 OECD Guidelines for the Testing of Chemicals no.202/17.07.1992 – Acute immobilizing testing in daphnia;
 OECD series referring to GLP and monitoring of conformity