

## ECOTOXICOLOGICAL IMPACT OF HERBICIDES BASED ON 2,4 D ON AQUATIC BIOLOGICAL SYSTEMS

Marga Grădilă\*, Mincea Carmen, Elena Hera

Research - Development Institute for Plant Protection, Bucharest

\*correspondence address:

Romania Bucharest 013813 OP 18

Blv. Ion Ionescu de la Brad no. 8

tel. 004-021-2693231, 2693234, fax:004-021-2693239

e-mail: [marga\\_gradila@yahoo.com](mailto:marga_gradila@yahoo.com)

**Abstract:** The aim of this study is to assess the environmental risks of four herbicides based on 2,4 D showing the demonstrating data of the biological systems using aquatic organisms in GLP designed conditions in the eco-toxicological facility of RDIPP Bucharest. In order to assess the four herbicides environmental risk, according to the principles of good laboratory practice, the following stages were ensured: development of the framework structure by ensuring the material base (plant complex to produce de-ionized water, reconstituted and 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 herbicides in terms of acute toxicity against *Daphnia magna* and *Cyprinus carpio*, revealed that two out of them (2,4 - D (2,4 - dichlorophenoxy) acetic acid and herbicide mixture of 2,4 - D and chlorsulfuron ) showed environmental efficiency and low toxicity respectively. When 2,4 – D acid is conditioned, form *ester ethylhexyl* showed a higher toxicity, inducing toxicity symptoms in fish: to lack of response to tactile stimuli, excitation and loss of balance. The mixture of 2,4 D + bromoxynil showed a moderate toxicity to fish and daphnia.

**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 the human health risks assessment is grounded. In 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 of the data related to the environmental risks assessment of herbicides based on 2,4 D 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 guide No.203/17.07.1992 – Acute toxicity test on fishes;
- OECD guide No.202/17.07.1992 –Acute immobilizing test on daphnia;
- GD No. 490/ 16.05.2002;
- GLP general and specific procedures (elaborated on Testing Facility);
- four herbicides based on 2,4 - D (two different forms of conditioning and two mixtures);
- biological material: *Cyprinus carpio*) and *Daphnia magna*.

Static method was used for the determination of the acute lethal toxicity of the four herbicides based on 2,4 D in the two test species, the testing period being 96 hours for fish, and 48 hours for

*Daphnia*. The testing solutions were not renewed during the test, but the temperature, pH, and dissolved oxygen concentration were monitored daily. The main estimated indicators in the aquatic organisms' ecotoxicological tests are shown in Table 1.

**Table 1**

**Aquatic organisms' main estimated indicators in eco-toxicological tests**

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

\***Immobility** – The daphnia are declared immobile, slightly touched and unable movement in 15 seconds after stirring the testing container

## RESULTS AND DISCUSSIONS

According to the Good Laboratory Practice principles, the performance of environmental risk assessment tests of four herbicides based on 2,4 D 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 for the first stage, 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, was settled.

Four herbicides based on 2,4 D: 2,4 - D [(2,4 - *dichlorophenoxy*) *acetic acid* ], 2,4 - D-ethylhexyl [2 - *ethylhexyl* (2,4 - *dichlorophenoxy*) *acetate*], 2,4 - D + bromoxynil and 2,4 - D + chlorosulfuron was tested (Table 2). The herbicide 2,4 - D (2,4 - *dichlorophenoxy*) *acetic acid* did not induced toxicity effects in fish, LC<sub>50</sub>% (96 h) = 18.88 mg a.s./l and NOEC (96 h) = 2,48 mg a.s./l. When 2,4 D acid is conditioned, form ester ethylhexyl [2 - *ethylhexyl* (2,4 - *dichlorophenoxy*) *acetate*], the toxicity increased very much, the average lethal concentration was 0.11 mg a.s./l and the concentration without effect was 0.04 mg a.s./l. The sub lethal concentration of 2,4 - D-ethylhexyl induces toxicity symptoms on fishes: lack of response to tactile stimuli, excitation and loss of balance. Also 2,4 - D-ethylhexyl creates problems in terms of visibility as much foam were formed in the aquariums, the test solution becomes cloudy and symptoms of toxicity in fish can not be observed.

Of the two herbicide mixtures of 2,4 - D, the most toxic to fish was found to be mixed 2,4 - D *acid* + *bromoxynil*. An average lethal concentration was 0.36 mg a.s./l and concentration without effect NOEC <0.1 mg a.s./l (Table 2). At high concentrations, this mixture can cause symptoms of asphyxiation, loss of balance and eliminating massive intestinal contents.

The mixture of *acid* 2,4 - D + *clorsulfuron* didn't induce toxicity effects in fish LC<sub>50</sub>% (96 h) = 14.98 mg a.s./l and NOEC (96 h) = 3,75 mg a.s./l

Table 2

## Herbicides based on 2,4 D toxicity in fish

Herbicide	LC <sub>50%</sub> mg a.s./l	NOEC mg a.s./l	Modification of some behavioral and anatomical indicators
2,4 - D 80% (2,4 - dichlorophenoxy) acetic acid	18,88	2,48	- balance loss at higher concentrations
2,4 - D-ethylhexyl 500 g/l 2 - ethylhexyl (2,4 - dichlorophenoxy) acetate	0,11	0,04	- lack of response to tactile stimuli - excitation and loss of balance
2,4 - D + bromoxynil 280 g/l + 280 g/l	0,36	0,1	- asphyxiation, loss of balance - masive intestinal contents at higher concentrations
2,4 - D + chlorosulfuron 59% + 1%	14,98	3,75	- balance loss at higher concentrations

**b) Environmental risk assessment in daphnia**

The environmental risk assessment for four herbicides based on 2,4 D in daphnia, according to the specific procedures of the biological reconstituted system developed in the laboratory in the first stage includes the following:

- selection of testing specie
- breeding the testing specie
- reproduction conditions insurance
- new born separation
- testing the sensitivity of testing specie

Compared with fish, daphnids were more sensitive to toxic action of herbicides based on 2,4 - D. The herbicide 2,4 - D (2,4 - dichlorophenoxy) acetic acid does not induced toxicity effects in daphnia, the mean inhibitory concentration was 10,66 mg a.s./l and the concentration without effect was 1.68 mg a.s./l. When 2,4 D acid is conditioned, form ester ethylhexyl [2 - ethylhexyl (2,4 - dichlorophenoxy) acetate]. The toxicity increased very much, EC<sub>50%</sub> (48 h) = 0.58 mg a.s /l and NOEC (48 h ) = 0.02 mg a.s. /l because the test solution became cloudy and daphnids was not easily observed. However, it was noted that daphnids float to the surface of the test vessel.

The mixture of 2,4 - D acid + bromoxynil induced a moderate toxicity effect in daphnia, the mean inhibitory concentration was 0.20 mg a.s./l and the concentration without effect was 0.1 mg a.s./l. The toxicity symptoms induced by the sub lethal concentrations of 2,4 D + bromoxynil in daphnia, developed symptoms of excited motion, loss of balance and at higher concentrations, developing a massive daphnia immobilization (Table 3).

The mixture of acid 2,4 - D + clorsulfuron does not show signs of acute toxicity to daphnia, EC<sub>50%</sub> (48 h) = 10,88 mg a.s /l and NOEC (48 h ) = 1,28 mg a.s. /l.

Table 3

## Herbicides based on 2,4 D toxicity in Daphnia

Herbicide	EC <sub>50%</sub> mg a.s./l	NOEC mg a.s./l	Modification of some behavioral and anatomical indicators
2,4 - D 80% (2,4 - dichlorophenoxy) acetic acid	10,66	1,68	-
2,4 - D-ethylhexyl 500 g/l 2 - ethylhexyl (2,4 - dichlorophenoxy) acetate	0,58	0,02	- daphnids float to the surface of the test vessel
2,4 - D + bromoxynil 280 g/l + 280 g/l	0,20	0,1	- exciting movement, balance loss - disorder swimming movements;
2,4 - D + chlorosulfuron 59% + 1%	10,88	1,28	-

**CONCLUSIONS**

- The herbicides based on 2,4 - D showed ecological efficiency, low toxicity effect in fish and daphnia respectively;
- A pesticide manufacturing technology influences its toxicity, 2,4 - D-ethylhexyl is more toxic to the two test species compared with 2,4 - D acid;
- The herbicide 2,4 - D-ethylhexyl form as much foam preventing visibility and presence of a natural aquatic ecosystem therefore preventing oxygenation process, development and penetration of sunlight, and favored eutrophication of waters;
- The mixture of 2,4 - D + bromoxynil showed a moderate toxicity to fish and daphnia;
- The symptoms of toxicity induced by sublethal concentrations of 2,4 - D + bromoxynil in fish are lack of response to tactile stimuli, excitation and balance loss.
- 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 Good Laboratory Practice Principles  
GOVERNMENT DECISION No 490/2002 - methods for eco-toxicity determination/measurement  
GOVERNMENT DECISION No 266/2006 amending and modifying Government Decision nr 63/2002  
OECD No 1- Good Laboratory Practice Principles (1997 revised)  
OECD Guidelines for the Testing of Chemicals no.203/17.07.1992 – Acute toxicity testing on fishes  
OECD Guidelines for the Testing of Chemicals no.202/17.07.1992 – Acute immobilizing testing on daphnia  
OECD series referring to GLP and conformity monitoring