

## BIOLOGICAL SYSTEMS MODEL ACCOMPLISHMENT ON GLP COMPLIANCE

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### Abstract

The aim of this study is to display the designed stages of two biological systems pattern including aquatic organisms as fish and *Daphnia*. The accomplishment of these two biological systems pattern was possible thanks to the Ministry of the Agriculture and Rural Development by an endowment PHARE project of the equipment for the Eco-toxicology Laboratory. In order to accomplish the proposed biological systems according to the Good Laboratory Practice, the following stages have been run: the design of the structure frame providing the material base, (the complex water supply installation with equipment for deionization, reconstituted and thermostated reconstituted), the supply of the necessary water for tests, achievement of the control system and checking soft for the surveillance of the environment conditions from the acclimatization rooms, as well as the specific elaboration of the specific operating procedures.

**KEY WORDS:** eco-toxicology, biologic system, reconstituted water, specific procedure.

### INTRODUCTION

The Good Laboratory Practice Principles (GLP) has been elaborated by the Organization for Economic and Co-operation and Development (OECD) in order to promote the data tests validity and quality used in order to establish the security of the chemical substances and products. GLP is a managerial concept covering the organizational process, and the conditions the laboratory studies are planned, achieved, monitored, registered and reported.

Aiming the chemicals assessment and their use regarding the environment and human protection, these principles need to be followed in the testing facilities where they are carried out studies that are submitted for the approval of the national authorities.

The aim of this study is two biological systems pattern to establish in the RDIPP Eco-toxicological Facilities, one with fish and the other with *Daphnia* according to GLP.

### MATERIAL AND METHOD

Materials: OECD GUIDELINES, “Organization of Economic Co-operation and Development”, and GD No. 490/2002.05.16. Section C, Annex No. 3.

Methods: Practical application of the regulations mentioned in the previous materials

### ACCOMPLISHMENTS AND DISCUSSIONS

The accomplishment of these two biological pattern systems was possible thanks to the Ministry of the Agriculture and Rural Development, supported through a PHARE project for endowment of the Eco-toxicology Laboratory. The proposed biological systems, in compliance with Good Laboratory Practice pursued the following stages:

#### 1) Testing facility organization

Testing facility includes locations, staff, and operational units needed for the studies development. In the testing facility were settled the fish and *Daphnia* testing system units that offer an

sufficient separation degree of different activities in order to insure the adequate development of the studies. To fulfil the GLP demands and minimize the disturbances that could interfere with the results validity, during the eco-toxicological testing process, the testing facility contains the following compounds:

- Area for *Daphnia* breeding
- Area for the fish reception and acclimation
- Area for the food storage
- Areas intended to inappropriate testing systems discharging
- Area designed for the water supply equipment
- Area for fish and *Daphnia* tests
- Area for the samples/counter-samples reception and storage
- Weighing scales room
- Areas intended for the storage of the rejected testing systems
- Ancillary areas (office, conference hall, staff lockers, bathroom)

#### 2) *Material and technical basis achievement*

In order to achieve the experimental pattern of the biological reconstituted systems with fish and *Daphnia*, the testing facility has been equipped with:

- *Advanced equipment*: acclimation tanks, testing aquariums, automatized plant for preparation and distribution of three water types used in the studies (demonized, reconstituted and thermostated reconstituted water), oxygen-meter, scales, appropriate equipment for the control and adjustment of the environmental conditions (solved oxygen concentration, temperature, humidity, etc.), and usual laboratory vessels (glasses, cylinders, pipettes, balloon flash etc.)
- *Consumables*: food for the testing systems, sanitary materials for cleaning maintenance,
- *Acclimation tanks and aquariums*

The testing facility is equipped with two fish acclimation tanks and three series of aquariums where are carried out the eco-toxicology studies. The tanks are made of plastic and have a capacity of 650 l. (Fig.1). Each acclimation tank is intended for a certain exposure type. In the tanks there is a fluxmeter set on the pipe that supplies the reconstituted water, set to supply a constant and measurable water flow during the entire acclimation period.

Those three series of aquariums where the tests are carried out are glass made, each with a capacity of 20 l (Fig.2). The following series are designed to the three different exposure methods of tested product:

- 7 aquariums are meant for testing in static conditions
  - 7 aquariums are meant for testing in continuous flux conditions
  - 14 aquariums are meant for the testing in semi-static conditions
- *Water plant equipment*

One of the main conditions for the performance of an eco-toxicological testing on aquatic organisms is the conformity with the demands regarding water used for tests. In this respect the two documents "OECD Guidelines and GD 490/2002" underline the precise conditions must fulfill the water used in eco-toxicological testing. For the obtaining of the reconstituted water which has to fulfill the settlements in force, it has been designed a modern equipment, a true plant that supplies the water at the needed quality for the performance of the eco-toxicological testing.

The plant has four sections with different functions (Fig.3).

- Section for deionization water production (bi-distilled water)
- Section for reconstituted water production
- Section for thermostatic reconstituted water
- Control section (computer)

The deionization water production is based on the reversible osmosis technique and it is the main water for all procedures carried out in the testing facilities and used for the preparation of the concentrated saline solution and reconstituted water. This reconstituted water is used for the tanks and aquariums rinsing, the aquatic organisms breeding, and testing, as well as the for the habitual laboratory use. The deionization water equipment is cooper free according to the regulations in force.

For the reconstituted water production first it is recommended the preparation of a concentrated saline solution (CSS) by solving in deionization water a quantity of salt in a fixed proportion (according to GLP).

The reconstituted water is obtained from serial linear dilutions of the concentrate saline solution (CSS) in deionization water (DEIO) providing a 0.5 g/l saline concentration, according to the regulations in force. The dilution method for reconstituted water preparation was chosen to optimize the reconstituted water volume and for maturation time control. The linear dilution in predetermined volumes will allow the maturation time to be longer than 12 hours, with a reconstituted water production of 450 l/h, higher than the real one which is between 280 and 350 l/h. The maturation time is in compliance with the regulations recommending a minimum stationary period of 12 hours.

The thermo stating of the water is accomplished by direct cooling using a thermo-regulator unit able to keep the water temperature between 10 and 22°C, a temperature that is displayed together with the pH value on the control system screen. This thermo-regulator unit is able to ensure a continuous water flow at minimal/low temperature (10 °C), delivering about 650 l/h. The plant is equipped with pipelines network so that each water type supply each aquarium including the acclimation tanks with traced point for each use. Section for plant control (fig.4) commands:

- Sensors for main parameters' control
- Conductibility
- pH
- Temperature
- Light
- Sensors for deficiencies detection
- Own electric power source
- Device for automatic reset

### 3) *The accomplishment of the requested environmental conditions*

OECD Guideline and GD 490/2002 precisely establish the environmental conditions to pursue during the eco-toxicological testing process, conditions that represent in fact the quality criteria which validate these tests. The most important environmental conditions are related to light, dissolved oxygen concentration, temperature, and water pH.

Thermostatic of the water in the testing aquariums at the temperature of 20-24<sup>0</sup> C, it is performed with the aid of a thermo-regulators working in one or two aquariums (fig. 4). The temperature is monitored automatically and also manually using a calibrated thermometer during the acclimation and growth period as well as along the entire period of the biological systems testing. Room temperature is kept at 20 ± 2°C by an air conditioner. The light conditions for acclimation and fish testing are made by means of day lighting fluorescence lamps. For their turning on there is a device controlling the photo-period, enabling the two transition periods during the 24 h day, to simulate the smooth transition from night to day and reverse. The light phase of the room is adjustable from the control station. The tanks and aquariums light intensity is also checked by a certified lux-meter.

In the aquariums the dissolved oxygen concentration and water pH can be determined by etalon devices. These data are specific for each testing system and they are mentioned in the specific operation procedures.

### 4. *Accomplishment of the specific operation procedures*

For the accomplishment of the specific operation procedures of the reconstituted biological systems with aquatic organisms, in this stage they were set some procedure rules as it follows:

*a. Materials and utilities method of supply*, as well as the responsibilities of the involved personnel in this activity. The procedure established rules that are in fact the requirements the equipments, food, water and hygienic-sanitary materials must fulfill.

*b. Maintenance and control of the biological systems with fish and Daphnia .*

The procedures established rules related to testing species selection, testing systems supply, handling the biological systems, food supply, providing the proper environmental conditions, fish management in acclimation room, reference substance selection for *Daphnia*, tanks, aquariums and testing vessels marking, cleaning and hygienic measures for testing and acclimation areas, discharge of the used testing biological systems, etc



Fig.1 Fish acclimatization tanks



Fig.2 Aquariums for eco-toxicological testing performance

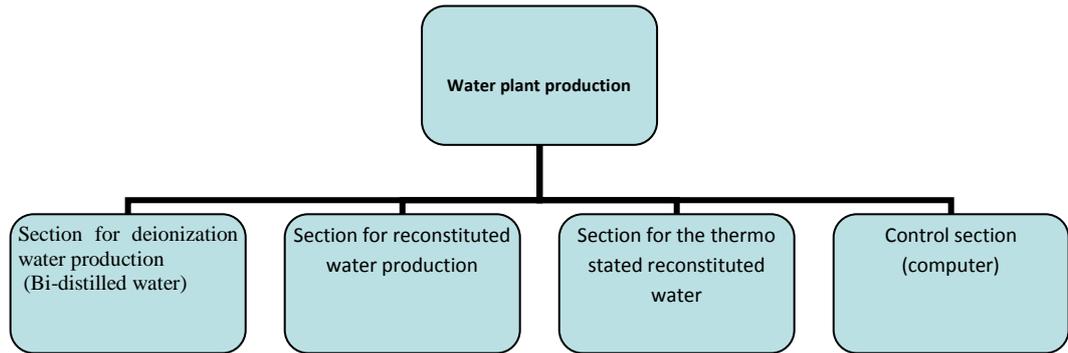


Fig.3. A part of the water plant production and some images of different sections



Fig.4. Plant control section



Fig.5. Thermal regulators

## CONCLUSIONS

- They were organized the testing facilities;
- It was provided the technical material base for the two proposed biological system;
- It was accomplished the production of deionized, reconstituted and thermostatic water;
- It was provided the appropriate equipment for the three types of tests;
- They were fulfilled the environmental conditions necessary to carry on the activity of risks assessment;
- They were accomplished the specific operation procedures for the two biological systems.

## **REFERENCES**

GOVERNMENT DECISION No 63/2002 approving the Good Laboratory Practice Principles  
GOVERNMENT DECISION No 490/2002 - Methods for eco-toxicity determination/measurement;  
GOVERNMENT DECISION No 266/2006 amending and modifying the Government Decision No. 63/2002  
OECD No 1- Good Laboratory Practice Principles (1997 revised)  
OECD series referring to GLP and conformity monitoring