# IMPLEMENTATION OF GLP PRINCIPLES IN "LECO" TESTING FACILITY - HAZARD ASSESSMENT OF PLANT PROTECTION PRODUCTS TOWARDS POLLINATORS

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**Abstract:** OECD Good Laboratory Practice principles have been elaborated to promote the quality and validity of testing data, used to establish the safety of chemical substances and products. Aiming the chemical substances assessment and other utilizations concerning the safety of humans and environment, needs that these principles to be pursued in the testing facilities were are carried out approved studies by the national authorities. In Romania the Good Laboratory Practice implementation and application is legalized by the regulations documents such as: HG nr.63/2002, HG nr.266/2006, HG nr 448/2007, Statutes/Regulation 440/2008. For GLP principles implementation in "LECO" testing facility have been designed several types of procedures and complying with their demands have leading to the quality results of the eco-toxicological tests. The aim of this study is showing the implementation modality of OECD Good Laboratory Practice demands, regarding some aspects which are directly involved in the performing of the toxicity study for the determination of certain substances/products in acute contact toxicity test on honeybees. The study aspects were: 1) measuring and control equipments existing in endowment of honeybee laboratory 2) laboratory glassware 3) reference and control tests substances 4) biological testing system represented by honeybees.

## INTRODUCTION

OECD Good Laboratory Practice (GLP) principles have been elaborated to promote the quality of testing data, used to establish the safety of chemical substances and products (OECD Guide no.1). A management concept said that laboratory studies must be planed, achieved, monitored, registered and reported. For chemical substances assessment and other utilizations concerning the safety of humans and environment needs, all these principles have to be pursued in laboratory were are carried out the studies.

Good Laboratory Practice implementation and application is legalized in Romania, by the regulations documents, such as: HG nr.63/2002, HG nr.266/2006, HG nr 448/2007, Statutes/Regulation 440/2008.

OECD member states seek the international harmonization of the testing methods and a good laboratory practice for avoiding the application of different implementation drafts which might impede the international trade of the substances. In our institute were assured the conditions to carry out certain impact studies complying the GLP, for plant protection products.

GLP principles implementation in "LECO", contributes to the achievement of some specific objectives of the project, ***Biological systems models for plant protection products risk assessment***

Documentation studies seek gathering the information becoming the foundation of several types of procedures: GP–general procedures, QSP–quality system procedures, SOP–standard operation procedures, WP–working procedures, TP–technical procedures, WI–working instructions.

For elaboration of operation procedures in "LECO" testing facility has been followed the conformity with quality system criteria for different types of procedures, having in view to optimize the the procedure regarding acute toxicity on honeybees.

**MATERIAL AND METHODS**

In GLP studies on honeybees it were used the following:

- Measure and control equipments
- Laboratory glassware,
- Testing and reference substances,
- Testing biological system.

The working method tried in the studies achievement for the risk assessment of plant protection products on honeybees is presented in OECD Guide for the chemical substances (13 and 14) testing and the Romanian version in Regulation 440/2008, the method presented in section C – Eco-toxicity determination method, C17. Bees – Acute contact toxicity test.

**RESULTS**

In a prior study (*Mincea Carmen and col., 2011*), has been presented the procedures applied in IT "LECO" to perform eco-toxicological tests complying OECD GLP principles adjusted to the practical conditions of the facility.

For the eco-toxicological tests has been designed working plans which present the interaction between different types of procedures directly participating in the study on going, for getting a larger image of the different aspects described in procedures. These working plans lead to the achievement of quality results, to the increasing of personal working efficiency, ensuring a direct intervention to solving problems that can appear during the testing process. The draft describing the interaction between the procedures directly involved in the determination of acute contact toxicity studies of plant protection products on honey bees is presented in Figure 1.

**Measure and control equipments**

Endowment with laboratory equipment complying with Good Laboratory Practice demands has been one of the main phases achieving the necessary conditions for GLP certifying the methods used for the environmental risk assessment of plant protection products.

To ensure the results quality, the laboratory is equipped with performing measure and control equipments and own the procedure "Equipments and Materials". This procedure describes the general methodology, maintenance, calibrated apparatus and equipment used in risk assessment studies. By the management decision, at the testing facility has been designated a person in charge with equipments. Metrology Responsible (MR) actualizes "Equipment list of GLP testing facility" supplying information about the equipment/apparatus state, inventory number and their location.

The list of apparatus/equipment needed on honeybees studies are: BINDER acclimation room, BURKARD micro applicator, KERN type analytical and technical balances, thermometer and hygrometer, honeybees test cages, $CO_2$ gas cylinder.

For the best facility functioning, for each equipment and apparatus, the metrology responsible prepares a file that includes:

- Equipments/apparatus evidence card/note, where are mentioned certain apparatus information complying with the demands written in the SOP-01 GLP "Equipments and materials"
- Technical book or/and equipments/apparatus utilization instructions/directions and any other kind of referring documentation;
- Equipments/apparatus working/operating instructions;
- Calibration bulletins, interventions reports issued by the competent authorities:

Maintenance working demands are written in the books of technical instructions and are executed either by testing facility personal, or by specialized organizations to which IT establishes a contract. The metrology responsible plans a "Maintenance and reparations yearly program" and checking their application.

To ensure the measurements traceability, all equipments/apparatus are submitted to the general analyses carried out by national standards metrology control and a testing facility quality control.

Equipments/apparatus are identified and submitted to the calibration and metrology control operations complying with "Yearly program for calibration and metrology control". This program includes initial and periodical calibrations and metrology controls and it is prepared, applied and followed by the metrology responsible.

The equipment submitted to metrology calibration/control must be visible marked and labeled regarding the metrology calibration/control state, with the metrology mark as "Validity up to … ". The expired metrology validity for the apparatus which are not used is adequately and visibly labeled: "Out of use".

An additional factor which might modify/influence the final study results could be the modality of laboratory maintenance and glassware utilization.

Laboratory glassware has to be permanently kept in perfect cleaning state to avoid interference with the study results.

During the study must be used the same set of laboratory glassware if it is necessary such kind of equipments. Laboratory glassware destined to liquids volumetric measurement such as pipettes, balloon flask, burette etc. complying with GLP are calibrated.

**Testing and reference substances**

GLP requests regarding testing and reference substances are described in the procedure "Characterization and identification of the testing and reference substance".

In testing facility there is a person who has the responsibility for testing and keeping substances/products reception, named Receptionist (R).

At the reception delivered testing/reference substance must have enclosed certain documents: (i) Quality Certificate and/or Analyze Bulletin where are specified the product characteristics, (ii) Delivery Notification where is specified the product type and quantity, and (iii) Safety Data Note where are specified the sample characteristics (needed both for testing process as well as for risk assessing of handling the sample on the human health and environment), safety keeping conditions, handling, safety personal measures, measures in the case of accidental loss etc.

All annexed documents for the testing/reference substance are kept by the Receptionist. The samples which are not fulfilling these requests are rejected.

**Biological testing system**

To accomplish the GLP demands regarding the testing biological system – honeybees, must be followed the procedures described in "Testing systems supplying" and "Maintenance and control of honeybees testing biological system". According to the OECD Regulation 440/2008 the recommendation is *Apis mellifera* L. as testing species. There are selected young adult healthy honeybees breaded from the same queen, with known physiologic and historic conditions. The honeybees' collection must be done in the same morning with the experiment or the evening before testing and are kept in the experimental conditions

The honeybees purchasing are done complying the procedure described in "Testing systems supplying" from certified suppliers and kept in acclimation room of laboratory until testing. At the delivery the purchased honeybees must have annexed a quality certificate to certify the health condition.

GLP principles demand the health condition characterization of testing biological systems. The assessment of health condition biological systems is carried out separately for each system because each of them has distinctive evidence and acceptance testing criteria.

The honeybees testing biological system evidence is kept in "The evidence register of honeybees testing biological system" Annex regarding "Testing systems supplying" where are specified also the environmental conditions (temperature and humidity)

According to the procedure "Maintenance and control of honeybees test biological system" the honeybees transportation is made in stain steel or plastic cages, the transfer from the bee colony frame into the cages is made by brushing. The acclimation is carried out in dark, at $25\pm2^0$C temperature and 50-70% humidity. Honeybees are fed with a 50% sucrose solution in distillated water during the testing process. The food is supplied without restrictions.

The OECD Good Laboratory Practice principles implementing in "LECO" testing facility represents the guaranty in achievement of good quality results in contact acute and ingestion toxicity determination studies of certain substance/products against honeybees.

## CONCLUSIONS

- The measurements and control equipments/apparatus from Honeybees Testing Laboratory used in GLP studies, comply with the OECD principles regarding calibrations, metrology and technical intermediary controls etc. according to the description in the procedure "Equipments and Materials".
- Laboratory glassware has to be kept in perfectly hygienic condition, in GLP studies it is used only calibrated glassware.
- Testing/reference substances are permitted in the Testing Facility only with the following documents: Quality Certificate and/or Analyze Bulletin, Delivery Notification, Safety Data Note. All the demands regarding the testing/reference substances are described in the procedure "The testing/reference substances identification and characteristics".
- At the purchasing of the testing biological system, respectively honeybees, has to be attached a quality certificate where is certified the honeybees health condition. All the other GLP demands regarding testing system supply are described in procedure "Testing systems supply" and "Maintenance and control of honeybees test biological system" .
- The activities presented in this study aimed one of the main purpose of the project – obtaining of GLP certificate for the methods used in the determination of environmental risk assessment of plant protection products and its active ingredients.

## REFERENCES


- Government decision no. 63/2002 regarding approval of Good Laboratory Practice Principles.
- Government decision no. 266/2006 to amending and completing Government Decision no. 63/2002
- Decision no. 448 on 16/05/2007 to Government decision no. 63/2002 regarding approval of Good Laboratory Practice Principles, as well as the inspection and control of its applications on chemical substance testing.
- OECD series referring to Good Laboratory Practice and monitoring the conformity.

Number 1 OECD principles of Good Laboratory Practice (1997 revised)
OECD Guides for chemical substances laboratory testing

- EC Regulations no. 440/2008 of the Comity establishing the testing methods based on the EC Regulation no. 1907/2006 of European Parliament and the Council regarding chemical substances registration, assessment, permission and restriction (REACH) C-Methods section for eco-toxicity determination.
- Mincea Carmen, Elena Hera, Alexandra Pasareanu, 2011, RJPP, vol. IV

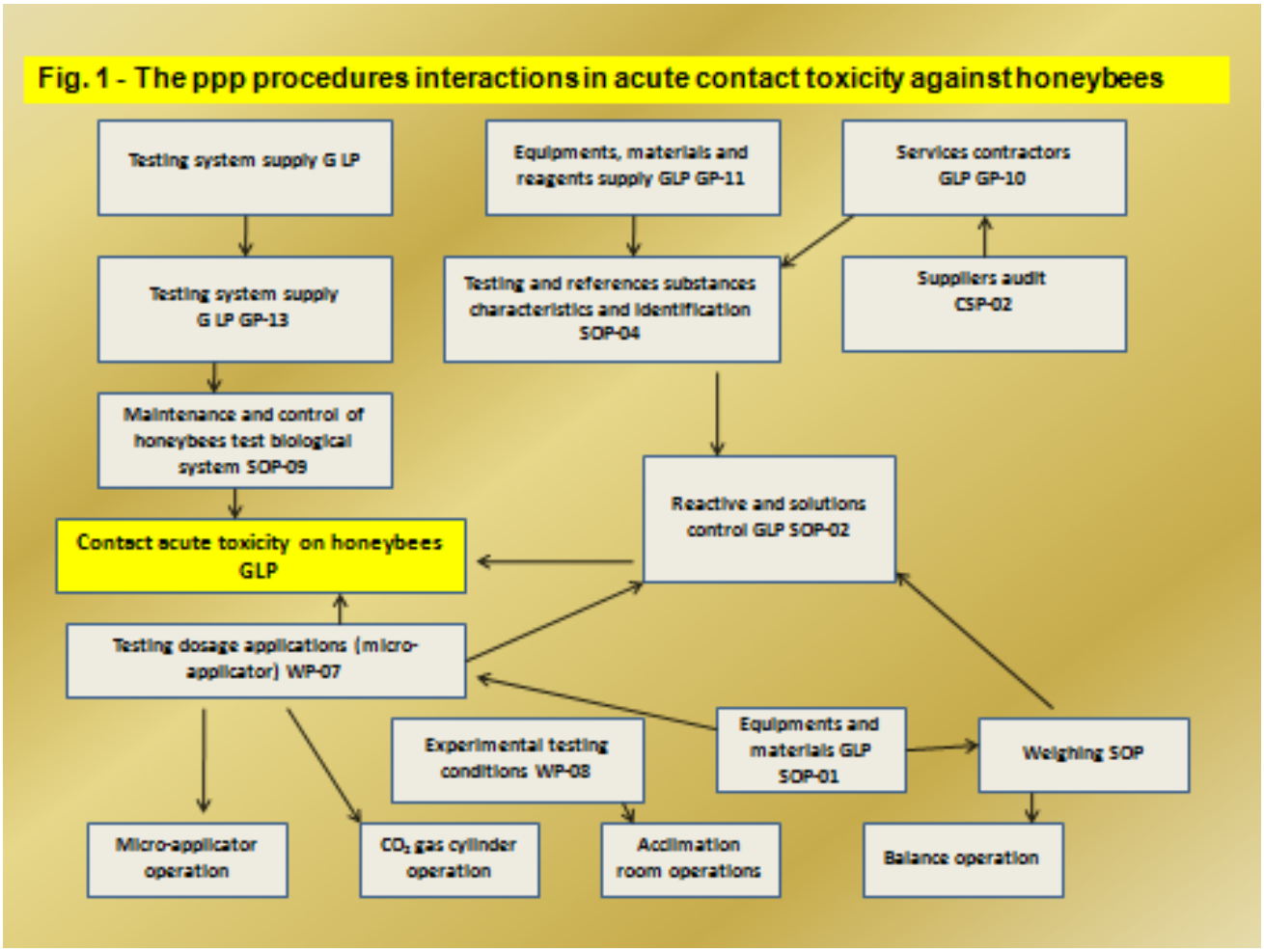
Fig. 1 - The ppp procedures interactions in acute contact toxicity against honeybees
Testing system supply G LP
Equipments, materials and reagents supply GLP GP-11
Services contractors GLP GP-10
Testing system supply G LP GP-13
Testing and references substances characteristics and identification SOP-04
Suppliers audit CSP-02
Maintenance and control of honeybees test biological system SOP-09
Reactive and solutions control GLP SOP-02
Contact acute toxicity on honeybees GLP
Testing dosage applications (micro-applicator) WP-07
Experimental testing conditions WP-08
Equipments and materials GLP SOP-01
Weighing SOP
Micro-applicator operation
$CO_2$ gas cylinder operation
Acclimation room operations
Balance operation