

Honey bee testing in Southern Europe: from the laboratory to the relevant crop in the field

Ingo TORNIER, Annette KLING, Andrea SCHUR

GAB Biotechnologie GmbH, Niefern-Öschelbronn, Germany

Abstract

The existing guidelines (BBA VI, 23-1, OECD Nos. 213 and 214, OEPP/EPPO No. 170) for honey bee (*Apis mellifera* L.) testing of plant protection products were mainly developed in the northern part of Europe. The test designs were adapted to the northern climatic zones and the bee specie (*Apis mellifera carnica* Pollm.), which typically come to its use. Since the last 2 – 3 years the request of testing the side effects of plant protection products on the honey bee in Southern Europe gets more important.

Therefore the LD₅₀ values of dimethoate in acute oral and contact toxicity tests were determined in Spain (Southern Europe) in comparison to Germany (Northern Europe) to evaluate the sensitivity of *Apis mellifera mellifera* L. and *Apis mellifera carnica*.

Furthermore tunnel tests and field tests in *Phacelia* (*Phacelia tanacetifolia* Benth.) were conducted in both regions. Differences in methodology and results will be presented.

Since the last update of the OEPP/EPPO No. 170 guideline the possibility is given to test pesticides in the relevant crop, on which the test product use is proposed. Adapted test methods for important crops in the South, e.g. Citrus, Melon, Tomato, Peach and Plum were developed and should be discussed.

Key words: *Apis mellifera*, Southern Europe, EPPO Guideline 170, laboratory test, LD50, relevant crop, semi-field, field testing.

Introduction

For the registration of plant protection products honey bee tests must be performed according to the Council Directive 91/414/EEC. The guidelines (OECD, No. 213 and No. 214 (1998), OEPP/EPPO Guideline No. 170 (3)) which were formulated to evaluate the risk of plant protection products on honey bees were mainly developed in the northern part of Europe and are adopted to the conditions existing in these countries. A great amount of plant protection products which are tested for registration are intended to be used also in the southern part of Europe in crops like citrus, peach, almonds, tomato. Since the climatic conditions in countries like Spain, Italy and South France are much different compared to Northern Europe, especially honey bee testing is influenced and the test design and the requirements to perform honey bee tests have to be modified. In this publication the main differences in conducting bee studies under laboratory, semi-field and field conditions in Northern Europe compared to Southern Europe will be described.

Laboratory tests

In the OEPP/EPPO and OECD guidelines for honey bee testing no special recommendation is given concerning the bee race which should be used in the tests. In Europe there are three bee races which could possibly be used for honey bee testing.

- *Apis mellifera carnica* Pollm.; the "grey bee" which is common in Northern Europe (e.g. Germany)
- *Apis mellifera mellifera* L.; the "dark bee" mainly distributed in Southern Europe (e.g. Spain)
- *Apis mellifera ligustica* Spin.; the "Italian bee", distributed in Southern Europe (e.g. Italy)

In order to investigate possible differences between

the two bee races *A. mellifera carnica* and *A. mellifera mellifera*, several tests were performed. Standard laboratory tests were conducted in Germany (*A. mellifera carnica*) and in Spain (*A. mellifera mellifera*) in order to determine the oral and contact LD₅₀/24h values of the toxic standard substance Perfekthion (a.i. dimethoate). These tests were performed according to the OECD guideline No. 213 / 214 (1998) with one control group and four doses of dimethoate. At each dose five replicates with 10 bees per replicate were tested. In the contact toxicity test a drop of 2 µL test solution was applied with a hand microapplicator to the thorax of the bees after they had been anaesthetised. In the oral toxicity test the bees starved for 2 hours and afterwards a quantity of 250 µL test solution was offered in Eppendorf cups to each cage of ten bees for a maximum of 6 hours. The LD₅₀ values with 95 % confidence intervals were calculated by means of a probit analysis using the statistic program SAS V8. The results are presented in the following tables.

The results of these tests showed that the LD₅₀/24h values of both bee races were within the accepted range described and published in the OECD guideline No. 213 / 214 (oral toxicity test: 0.10 to 0.35 µg a.i./bee; contact toxicity test: 0.10 to 0.30 µg a.i./bee). It can be concluded that there is no significant difference regarding the sensitivity of the two bee races *A. mellifera carnica* and *A. mellifera mellifera*.

Regarding the laboratory oral and contact toxicity test, it is recommended not to collect the bees in early spring and late autumn which is based on the assumption that the bee colonies are wintering and have no brood over wintertime. Due to the mild climatic conditions in Spain the bees are in brood over the whole year and young adult worker bees can be provided even in wintertime. Therefore honey bee laboratory testing is not restricted to the summertime season.

Table 1. Oral LD₅₀/24h values of dimethoate determined over the bee testing season 2002 in Germany with *A. mellifera carnica* and in Spain with *A. mellifera mellifera*.

Date	LD ₅₀ [µg a.i./bee]	95 % confidence limits
<i>Apis mellifera carnica</i>		
16/04/02	0.14	0.13 to 0.15
11/06/02	0.16	0.15 to 0.16
30/07/02	0.14	0.13 to 0.15
<i>Apis mellifera mellifera</i>		
12/03/02	0.12	0.11 to 0.14
14/05/02	0.10	0.10 to 0.11
25/06/02	0.14	0.13 to 0.16

Remark: accepted range according to the OECD guideline for the oral toxicity test: 0.10 to 0.35 µg a.i./bee.

Table 2. Contact LD₅₀/24h values of dimethoate determined over the bee testing season 2002 in Germany with *A. mellifera carnica* and in Spain with *A. mellifera mellifera*.

Date	LD ₅₀ [µg a.i./bee]	95 % confidence limits
<i>Apis mellifera carnica</i>		
16/04/02	0.21	0.19 to 0.23
11/06/02	0.21	0.20 to 0.22
30/07/02	0.14	0.11 to 0.16
<i>Apis mellifera mellifera</i>		
12/03/02	0.23	0.21 to 0.28
14/05/02	0.25	0.22 to 0.33
25/06/02	0.26	0.23 to 0.29

Remark: accepted range according to the OECD guideline for the contact toxicity test: 0.10 to 0.30 µg a.i./bee.

Cage tests

To evaluate the effects of an application of plant protection products on flowering crops while bees are foraging, a cage test can be performed. The treated plots are covered by a tunnel tent and bees are allowed to forage only in the covered space. In Northern Europe these tests are mainly conducted with the bee-attractive crop *Phacelia tanacetifolia*. During the last few years several cage tests were performed in Southern Europe mainly in Spain and Italy. Based on these experiences some differences can be concluded between performing cage tests in Northern and Southern Europe. As well as in the laboratory testing, different bee races are used in Northern and Southern Europe.

To guarantee uniform bee material in all treatments it is recommendable that the queens originate from one breeding line. But due to the absence of artificial insemination no sister queens are available in Spain. Therefore the test colonies should be critically inspected and chosen before start of the test.

P. tanacetifolia is a possible crop, also for cage tests in Southern Europe but it must be considered that the seeding should be performed much earlier in the year, compared to Northern Europe. The dry climatic conditions are requiring a weekly irrigation and a fertilisation to guarantee a good plant growth and to stimulate the nectar-flow. The experimental phase in Southern Europe normally starts between April and June compared to a testing season between May and August in Germany. The applications must be performed early in the morning to guarantee a good flight intensity and to avoid temperatures above 30°C during the application.

Besides *Phacelia* and rape, cage tests can also be conducted in the relevant crop, in which the use of the test substance is intended (e.g. citrus, apple orchards, melon, peaches, plum, cherry or sunflower).

Field tests

According to the OEPP/EPP guideline (2001) the field tests are carried out in the relevant crop or in another crop which is attractive to bees. For field testing of plant protection products, bee colonies are placed at the edge of a test field with flowering crops. As described above the bee-attractive crop *P. tanacetifolia* can be used as test plant in Southern Europe but also several relevant crops like citrus, apple, peach, plum, melon etc. are possible. The same differences as described above in the cage testing, concerning the bees (different bee race, no sister queens), the crop (early seeding, irrigation) and the test conduct (application early in the morning) is applicable for the field tests in Spain. In contrast to Germany, a toxic standard treatment can be included in the test, because the "bee density" is much lower in Spain and it can be guaranteed that only bees used for the test will be affected by the application and not the bees of other bee-keepers. But the test design, especially the assessments, must be adapted to the conditions in the relevant crop and generally in Southern Europe. In northern Europe the flight intensity is normally assessed as number of foraging bees/m² at present. In Southern Europe the flight intensity is much lower compared to Northern Europe and therefore it is reasonable to consider the time during the assessment as well and count the number of bees per m².

per minute. In the relevant crop like citrus, apple, peach, there are less flowers compared to a crop like *Phacelia* and a defined number of flowers should be observed per minute to be able to obtain consistent and comparable data.

Conclusion

Several laboratory studies, cage tests and field tests conducted in Spain and Italy showed that honey bee testing is possible in Southern Europe according to the recent guidelines. An adoption of the guidelines to the different conditions in these countries could be considered.

References

- COMMISSION DIRECTIVE 96/12/EC (amending Council Directive 91/414/EEC), 1996.- *Official Journal of the European Communities* 65: 20-37.
- OEPP/EPPO, 2001.- Guideline for the efficacy evaluation of plant protection products – Side effects on honeybees.- *OEPP/EPPO Bulletin*, PP 1/170(3) update 2000, 19-23.
- OECD, 1998.- Guideline 213: Honeybees, acute oral toxicity test.- *OECD Guidelines for the testing of chemicals*.
- OECD, 1998.- Guideline 214: Honeybees, acute contact toxicity test.- *OECD Guidelines for the testing of chemicals*.

Corresponding author: Dr Ingo Tornier, GAB Biotechnologie GmbH, Eutinger Strasse 24, D-75223 Niefern-Öschelbronn, Germany. E-mail: ingo.tornier@GAB-Biotech.de