

Honey Bees and Bumble Bees

Welcome To Eurofins Agrosience Services

The toxic effects of plant protection products (PPPs) to bees (*Apis mellifera*) are tested following the latest OECD Guidelines. Eurofins Agrosience Services is experienced in conducting honey bee (*Apis mellifera*) and bumble bee (*Bombus terrestris*) laboratory and extended laboratory studies. Moreover, EAS has been involved in several international ring test groups working on the development of OECD guidelines for new test systems. Tests systems under development are larvae tests, adult honey bee chronic feeding tests, acute oral and contact toxicity tests with bumble bees and acute contact toxicity test with solitary bees.

Laboratory Honey Bee Acute Toxicity Tests

The acute oral and contact toxicity tests of PPPs on the honey bee (*Apis mellifera*) are performed according to the OECD guidelines (No. 213 and No. 214). Test sites in Germany, Spain and the USA allow acute toxicity testing all year round; which is a huge benefit to clients.

When a PPP is expected to be of low acute oral and contact toxicity to bees, a limit test may be performed using a dose of 100 µg a.i./bee in order to demonstrate that the LD₅₀ (lethal dose) is above this value. When the toxicity of a PPP is unknown, bees are exposed to a range of doses to determine the LD₅₀. To assess the acute oral toxicity of PPPs, honey bees are fed in the laboratory with the test item dissolved in sucrose solution. For the determination of the contact toxicity, the PPP dissolved in a carrier is applied to the thorax of the bees. Mortality is recorded daily for 48 hours but can be recorded up to 96 hours when it continues to rise by more than 10 %. The LD₅₀ values will be determined for each assessment throughout the entire test period.



Laboratory Honey Bee Chronic Toxicity

The chronic toxicity test was established several years ago and Eurofins Agrosience Services has a leading role in the development of a respective OECD Guideline. Newly hatched honey bees are exposed to treated sugar solution at different concentrations by continuous and *ad libitum* feeding over a period of 10 days. Mortality, behaviour and the food consumption will be determined daily throughout the test period. The main endpoints are the determination of the LC₅₀ (lethal concentration) and LDD₅₀ (lethal dietary dose) as well as the NOEC (no observed effect concentration) and the NOEDD (no observed effect dietary dose) at the end of the test, where possible.

Laboratory Bumble Bee Acute Toxicity Tests

Eurofins Agrosience Services is currently involved in the development of new guidelines for acute oral and contact toxicity tests with bumble bees (*Bombus terrestris*). The test systems are based on the OECD guidelines for honey bees, No. 213 and No. 214, but have to be adapted to accommodate the biology of bumble bees. Due to the absence of trophallaxis, group feeding is not possible, and acute oral toxicity has to be determined by the individual feeding of single bumble bees in Nicot queen breeding cages. For the assessment phase of oral and contact toxicity tests, bumble bees are kept under single housing conditions. The seasonal-independent breeding of bumble bee colonies, allow acute toxicity testing all year round in Germany and in Spain.

Extended Laboratory Tests

The conduct of extended laboratory bee trials is based on the EPA OPPTS 850.3030 guideline either in Spain or in Germany depending on the crop selected (vegetables, orchards and alfalfa in Germany; citrus in Spain). Honey bees (*Apis mellifera*) are exposed in the laboratory to residues of PPPs on leaves, aged under semi-field or field conditions. Through an exposure phase in the laboratory, different time intervals between the application date and start of bee exposure to the treated plant material can be evaluated. Along with the assessments of mortality, special attention is drawn to possible behavioural abnormalities of the bees compared to the control group.

Honey Bee (*Apis Mellifera*) Larval Toxicity Test

Toxicity effects to larvae of the honey bee (*Apis mellifera*) from single and repeated exposure of PPP can be assessed by our *in vitro* larvae tests. These tests are conducted in the laboratories in Niefern-Öschelbronn (Germany) and in the USA during the egg-laying period of the queen bee from mid of April until mid of August. For that purpose honey bee larvae from own colonies are grafted and transferred into cellular culture plates and exposed to untreated and treated diet containing the test item in a range of increasing doses. During the test, larval and pupal mortalities and the number of successfully emerged bees and signs of active food consumption can be assessed. The single exposure test design follows the OECD guideline 237. The repeated exposure test design bases on the latest draft OECD guidance document. Currently EAS is involved in international ring test groups and participates on the development of the OECD guideline for the repeated exposure test.

Honey Bee Stomach And Pollen Preparation

To determine the amount of residues carried into the hive by forager bees after PPP application, honey stomach preparations can be performed. Deep frozen samples of honey bees collected at intervals after application are sent to our laboratory where the bees will be dissected for the manual extraction of their honey stomach contents. The pollen loads can also be collected for analysis. The obtained samples can be either analysed at our facility or stored until shipment to the selected analytical laboratory.

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The Agrosience Group offers unparalleled expertise to the crop protection industry; with over 750 staff globally and more than 80 fully owned facilities across 25 countries, we are committed to developing and growing in order to meet the needs of the Agrosience industry.

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