



Honey bees Larval Toxicity Test, Repeated Exposure: OECD Guidance Document 239

This first tier laboratory test is designed to assess the potential risk to honey bee larvae (brood) feeding on contaminated larval diet to which they may be exposed due to adult workers foraging on crops or non-target plants treated with plant protection products. This is tested in the laboratory by dosing and rearing larvae grafted from honey bee colonies *in vitro* and observing effects until day 22 of development (*adult emergence*).

The study comprises a dose response test to examine toxicity to developing honey bee brood i.e. larvae, pupae and adults. Each test is usually run with a minimum of 5 dose rates per test item (usually based on the outcome of a range-finding test), although it is possible to run a limit test if required. There is a minimum requirement of 36 larvae (48 are set up as standard) per dose rate sourced from three separate colonies i.e. a minimum of 12 (but usually 16) per colony, per treatment group. On day one (D1) of the study, first instar (L1) synchronised larvae are grafted from the combs from three pre-prepared colonies and individually placed (grafted) into individual artificial cells held in 48 well-plates. The larvae are fed a standardised amount of artificial diet (made up from a mixture of royal jelly, sugars and yeast extract). Throughout the study the plates of larvae are placed in an incubator under tightly controlled and monitored temperature and humidity conditions.

For four days of the test, from D3 to D6, the larvae are fed with test item dosed larval diet. For each test equivalent control and toxic reference test units are set up to ensure

that the test organisms and test system are functioning and meet the test validity criteria. Chemical analysis is required for dose verification and demonstration of homogeneity and stability the test item in the dosed larval diet under **OECD 19**. This analysis is undertaken using methods validated according to **SANCO/3029/99 rev.4**.

From D4 to D8, mortality and other effects on normal development are recorded daily, they are also recorded again on D15. The adult bee emergence rate on D22 is recorded - the validity criteria being $\geq 70\%$ emergence in the undosed control plates. The end points are larval and pupal mortality rates (D8 and 15 respectively) and adult emergence at D22. For adult emergence on D22, the median Lethal Concentration/Dose EC_{50}/ED_{50} , (and if possible EC_{10}/ED_{10} & EC_{20}/ED_{20} values) will be estimated and if data allows, No Observed Effect Concentration/Dose NOEC/NOED will also be estimated.

Test guidelines and references

OECD Guidance Document 239 (July 2016): Honey Bee (*Apis mellifera*) Larval Toxicity Test Following Repeated Exposure.

OECD 19: Advisory Document of the Working Group on Good Laboratory Practice on the Management, Characterisation and Use of Test Items.

SANCO/3029/99 rev.4: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414.

FERA'S WORK IN BEE ECOTOXICOLOGY

Fera has the expertise and scientific resources to help partners test active ingredients or formulated products for their effects on bee survival, development and behaviour, enabling the development products that are safe for bees.

Fera's specialists are perfectly placed to meet data requirements and our services in bee ecotoxicology range from standard laboratory studies to bespoke higher tier studies to address specific risk assessment needs.

Fera works closely with and is co-located with the National Bee Unit, and we own 150+ colonies of honey bees managed directly by our highly skilled on site bee keeping team. These colonies are used to support our risk assessment work and R&D.

MORE ABOUT FERA

Fera is based at the National Agri-Food Innovation Campus near York, UK.

We work closely with plant protection and veterinary medicine product manufacturers to help develop effective, sustainable and safe chemical products that minimise ecosystem impacts and pollution, while maximising the beneficial effects for crops, plants and animals.

Combining the extensive expertise of our scientists with advanced resources and GLP-compliant laboratories, we provide valuable support to companies in their chemical evaluation and registration efforts.

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For more information and to book a test, call Fera on **+44 (0)300 100 0321**, email **sales@fera.co.uk** or visit **www.fera.co.uk/chemical-regulation**

