



Honey bees Larval Toxicity Test, Single Exposure: OECD Testing Guideline 237

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 *in vitro* rearing of larvae grafted from honey bee colonies and observing effects until day seven (D7) of development (pre-pupation).

The study comprises a dose response test to examine toxicity to developing honey bee larvae. 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 course of the study the plates of larvae are placed in an incubator under tightly controlled and monitored temperature and humidity conditions.

This test is a single dose 'acute' study and the larvae are fed with test item dosed larval diet on day four (D4) only. On all other feeding days the larvae are fed undosed diet. For each test equivalent undosed 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 set out in the guidance document.

Mortality and other effects on normal development are recorded on D5, D6 and D7. The end point is larval mortality rate at D7 (72 hours post exposure). The 72 hour median Lethal Concentration/Dose (ED_{50}) with 95% Confidence Intervals, (and if possible an estimation of ED_{10} & ED_{20} values) and, if data allows, the No Observed Effect Dose (NOED) values are also estimated.

Test guidelines and references

OECD Test Guideline 237 (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.

