
Section 1 – Laboratory/Semi-field/Field

1.1 Current experimental advances from the French Methodological Bee Group. New improvement for future repro-toxicity tests

Hervé Giffard¹ (Marie Pierre Chauzat², Julie Fourier³, Sandrine Leblond⁴, Pierrick Aupinel⁵, Frank Aletru⁶, Jean Luc Brunet⁵, Jean Michel Laporte⁷, Cyril Vidau³)

¹Testapi, ²Anses, ³ITSAP, ⁴BASF, ⁵Inra, ⁶SNA, ⁷Syngenta

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Abstract

The French Methodological Bee Group was re-initiated in 2006 during neonicotinoids assessments by the authorities. Formerly managed under the Ministry of Agriculture (CEB), it is now committed to provide guidance and protocols to assessors regarding local or international methodologies. Public and private researchers work together with beekeepers, industrials and contract research organizations (CROs) toward providing adapted protocols for assessing honey bees (*Apis mellifera*).

Laboratory LD50 tests and semi-field experiments were set up during the 70s' and have been reviewed regularly under CEB 230, while new guidelines were initiated because of needs for new assessments.

The honeybee brood test under laboratory conditions (Inra 2005) and the adult bee 10-day chronic toxicity test (Itsap 2009) were initiated before being extended to the international level. Methodologies to assess the behavior of forager honeybees within tunnels as well as the measurement of hypopharyngeal glands (HPGs) are still under CEB230 only.

More recently the homing flight test was initiated in 2011 (ITSAP) and is undergoing ring-testing within 7 European laboratories.

Beyond assessing short-term effects in laboratory and mid-term effects in field or semi-field, the professional beekeeper organizations require means of assessing long-term effects of phytopharmaceuticals on colony development. Moreover, there have been discussions on evaluating the lifespan of drones and queens. As it is a too large investment for a single methodology, we now focus on the drone fertility as a first step. Later on, the lifespan of forager honeybees would be evaluated as to whether it is related to reductions in honey production if the lifespan is reduced by several days. Moreover evaluating queen longevity would require multi-year observations and would present difficulties to run under Good Laboratory Practice (GLP) standards.

Drone fertility

The objective is to determine a NOEC on the spermatogenesis of the drones (quality and quantity).

The current design uses laboratory and semi-field conditions for the exposure and assessments of the drone development. This two-way assessment is necessary to choose the most efficient method to collect sexually mature drones.

Frames of drone wax are introduced into dedicated colonies in order to provide the expected brood with sufficient drone cells. Then drones and newly emerged bees are introduced in different queenless nucleus colonies for adaptation in at least 3 modalities (control, positive reference toxicant and a test item).

In laboratory conditions the exposure begins with the feeding of nurse bees (syrup at different concentrations + water and pollen ad libitum) for 20 days similarly to LD50 exposure. In semi-field conditions the exposure begins with the introduction within tunnels where a feeder is supplied daily in each modality during the 20-day exposure period.

In 2019 the protocol has not yet been finalized but the process for the collection of mature drones is efficient; however, validity criteria are still under discussion. A guidance document is expected in 2021, after which the protocol could be transferred for ring-testing at OECD level. Results may help to determine if an expected concentration of chemicals in realistic exposure has an effect on the sexual maturation of honeybee drones.