Honey Bee Larval Toxicity Test Protocol Development Summary
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A recent effort by the Pollinator Research Task Force[1] (PRTF) validated the use of a method for evaluating the chronic toxicity of a compound (*e.g.*, a pesticide) to an immature honey bee for use in a risk assessment. Substantial data on the honey bee toxicity (*i.e.*, what level causes an effect) and exposure (*i.e.*, what concentration and amount they encounter in the environment) on both the adult and immature stages of development are required prior to conducting a thorough risk assessment for a pesticide. Currently, established methodology exists for measuring acute oral and contact toxicity of a pesticide on adult honey bees, and for measuring acute toxicity on honey bee larva. Developing a robust study design for evaluating chronic exposure of a compound to immature honey bee development has been challenging, due to high mortality in the controls (control = no pesticide present).

The University of Florida recently developed and published modifications[2] with the intention of improving test performance to previously published rearing methodology[3] The PRTF organized a ring test, where 15 independent laboratories on three continents and across government, academia, and industry[4] followed the same testing protocol to: 1) Determine if the test performance is robust across different geographic regions and different laboratory personnel; and 2) Identify any limitations associated with the methodology. The current control performance criteria for a valid test is ≥ 85% survival at the end of the larval development and ≥ 70% survival through adult emergence.

Thirteen trials (81.3%) satisfied the validity criteria and the test design's performance was determined adequate for regulatory testing. The toxic reference chemical (used to ensure the test organism responds to a known honey bee toxin) used in the test resulted in a consistent response. However, the use of solvents for testing proved to be problematic. Acetone is historically used as a solvent in regulatory testing to successfully place a test compound into solution, but the honey bee larvae can be particularly sensitive to acetone by itself. Current efforts in Europe and the US are aimed at identifying alternative solvents for use in honey bee testing that provide good solubility while simultaneously not differing in survival when compared to the control. In conclusion, the ring test demonstrated that the methodology for evaluating the cumulative exposure of a potential toxin on developing bees can be successfully conducted.

[1]The Pollinator Research Task Force (PRTF) was formed in January 2016 and is comprised of ten pesticide registrants (Arysta LifeScience, BASF Corp., Bayer Crop Science LP, Dow AgroSciences LLC, DuPont Crop Protection, FMC Corp., Mitsui Chemicals Agro Inc., Monsanto Co., Syngenta Crop Protection LLC and ValentUSA Corp.) with the focus of mining and generating data to refine and improve pollinator risk assessments in North America and globally where applicable.

[2]Daniel[2]Daniel R Schmehl, Hudson V V Tomé, Ashley N Mortensen, Gustavo Ferreira Martins & James D Ellis (2016): Protocol for the in vitro rearing of honey bee (Apis mellifera L.) workers, Journal of Apicultural Research, DOI: 10.1080/00218839.2016.1203530

[3]Karl[3] Crailsheim, Robert Brodschneider, Pierrick Aupinel, Dieter Behrens, Elke Genersch, Jutta Vollmann & Ulrike Riessberger-Gallé (2013) Standard methods for artificial rearing of Apis mellifera larvae, Journal of Apicultural Research, 52:1, 1-16, DOI: 10.3896/IBRA.1.52.1.05

[4]The participating laboratories for the ring test are as follows:

- BASF (Germany, Nicole Hanewald, Stephanie Royer);
- Bayer Crop Science (Germany, David Gladbach, Dunja Przygoda);
- Biochem Agar (Germany, Katharina Kleebaum, Kathrin Scheller);
- Eco-Saf (China, Chi Wu);
- Eurofins (Germany, Jakob Eckert, Sophia Oberrauch);
- Eurofins (USA, Ming Hua Huang, Jing Zhai);
- Ibacon (Germany, Alexander Ehmke, Stephan Schmitzer)
- Innovative Environmental Services (Switzerland, Stefan Kimmel);
- LAVES Niedersachsen (Germany, Dorothee Luken);
- Michigan State University (USA, Zachary Huang);
- Nanjing Institute of Environmental Science (China, Yuangqing Bu);
- Smithers Viscient (USA, James Hoberg, Michael Patnaude);
- Syntech Research (France, Line Deslandes, Eric Ythier);
- Syntech Research (USA, Houston Howerton, Jared Leonard); and
- University of Florida (USA, Jamie Ellis, Ashley Mortensen).

All participating laboratories and contact personnel are listed in alphabetical order by organization and last name.