

**July 20, 2020**

**To:**

**From: David Crow, Laurie-Ann Flanagan, David Beaudreau**

**Re: Webinar: Advancing the Science of Assessing Risks to Bees from Pesticides**

On July 21, the EPA’s Office of Pesticide Programs hosted the first webinar in a series of many on designing and conducting bee studies to provide background on how EPA regulations on insecticides, pesticides, etc. are determined.

**Summary:**

The webinar consisted of [four presentations](https://handouts-live.s3.amazonaws.com/2df2ecd6887c4921af8bfa69b517a7ef?X-Amz-Algorithm=AWS4-HMAC-SHA256&X-Amz-Date=20200721T145755Z&X-Amz-SignedHeaders=host&X-Amz-Expires=86400&X-Amz-Credential=AKIAJICNIQWVMWBRIUMQ%2F20200721%2Fus-east-1%2Fs3%2Faws4_request&X-Amz-Signature=21640367ddf10e98eae0cf502c2408cc6621d3c9daf206fc8de213d921223169) from distinguished researchers and ecotoxicologists, ranging in discussion of testing procedure standardization, to promising results from recent studies, to the general taxonomy of different species of bees. Dr. Daniel Myers, Senior Compliance Officer with EPA’s Office of Enforcement and Compliance, discussed Good Laboratory Practices (GLP) within the EPA specifically with respect to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Dr. Schmehl, Senior Scientist, Ecotoxicologist and pollinator specialist at Bayer Crop Science, spoke about the development of a bioassay for larval toxicity testing. Dr. Helen Thompson discussed the surrogate species testing approach utilized by several international regulatory agencies, as well as the EPA, and Dr. Ivo Roessink, Ecotoxicologist specialist at Wageningen University and Research, detailed his research on acute toxicity testing with the solitary mason bee.

**Good Laboratory Practice:**

Dr. Myers began by defining GLP as the mandates that assure the quality, validity, and integrity of facilities and scientific studies that support regulatory decisions by federal agencies such as the EPA via FIFRA and the Toxic Substances Control Act (TSCA). Studies submitted to EPA adhering to GLP regulations must cover the study personnel; facility and equipment; standard operating procedures; documentation; test, control, and reference substances; the final report; and archived records. He discussed how GLP inspections are carried out in the forms of on-site facility inspections or data audits, and concluded by categorizing the available non-compliance responses at the EPA’s disposal as: regulatory action, civil action, and criminal action, depending on the degree of non-compliance.

**Development of a Bioassay for Larval Toxicity Testing:**

Dr. Schmehl began by describing the types of stress that pollinating bees may be exposed to in order to frame his later discussion of risk and toxicity when studying bioassays. He defined the risk term considered in a pesticide safety assessment as a function of toxicity and exposure, indicating his specialization in examination of the former. He noted that laboratory toxicity studies are designed to quantify the dose-response relationship following exposure of an organism to a pesticide, highlighting the importance of the LD50 level, at which 50% of a population tested experience the effects of a lethal dose. He underscored the difficulty of replicating a baseline control of larvae needed for quality assurance due to the inconsistent performance, leading the Pollinator Research Task Force to sponsor a project to validate the proposed method for larval toxicity testing. The method developed involves extracting larvae from a hive and transferring them to an in vitro control plate where they develop through to the pupation state, and then to full adulthood. The proposed validation protocol set out to evaluate the control test performance, evaluate the 2% acetone solvent test performance, and determine variation in sensitivity to dimethoate (the reference toxicant) across participating laboratories using different concentrations. He presented the performance data from the international data collection effort, showing a mean control survival rate exceeding 70% (the control evaluation threshold) from the overwhelming majority of labs in North America, China, and Europe, and an insufficient survival rate using a solvent. The validation procedure was determined to be a reliable method for quantitative risk assessment to be used by regulatory agencies across the world, evidenced by consistent and replicable results across multiple labs and low variation in toxicity to the reference toxicant.

**Surrogate Species Testing:**

Dr. Helen indicated that of the 20,000 species of bees, only a few of those exhibit the ideal characteristics for a suitable surrogate species which include: representativity of the broader genus, availability, ease of manipulation in a laboratory setting, consistency in response, and the ability to test all life stages: acute and chronic, and higher tier. She noted that the European honeybee is the most common surrogate honeybee, but that despite the consensus of a specific species, there remain inconsistencies between applications of dosage, temperature, and body weight. She summarized a recent study which covered the sensitivity to contact dosage of 28 viable surrogate species adjusted for bodyweight, which concluded that bees of the *Apis* genus were the most sensitive, and those of the *Bombus*, *Osmia,* and *Trigona* genus’ ranked among the more resilient. She then described what the levels of sensitivity mean in the context of default exposure estimates at Tier 1 EPA standards and how the EPA is able to refine their exposure estimations and develop more realistic exposure scenarios for risk assessment trials in light of the recent discoveries. She concluded that the honeybee is a relatively sensitive species, but easily fulfills the other criteria needed for surrogate testing use, despite the outstanding resilience of *Apis*.

**Acute Toxicity Testing with the Solitary Mason Bee (*Osmia* spp)**:

Whereas Dr. Helen mentioned that 95% of bee species are solitary, making them non-representative of the pollinators about which the EPA is primarily concerned, Dr. Roessink spoke about his efforts in testing one such solitary species to isolate optimal techniques for toxicity testing. He outlined the procedure for acute testing of this species, detailing the climate conditions, dose-response design, test item, dose range, end points, and trial sizes. He noted the observed increase in control survival when using group housing to store the test species compared to individual housing, likely due to the bees augmented ability to find the habitat feeder when housed with others. He then discussed the challenges in identifying an appropriate wetting agent to carry the test substance. His study concluded that group housing, using TritonX as a wetting agent, applied via either wick feeder or a synthetic petal provided optimal results for acute toxicity contact tests.