The Curious Beekeeper

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Running the Risk, Part II: The Higher Tiers of Honey Bee Risk Assessment and Comparisons between the U.S., Canada and Europe

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Toxicity Endpoints Defined

**LD<sub>50</sub>: Median Lethal Dose** - oral or contact dose at which 50% of test bees die, generally expressed in units of micrograms per bee.

**LC<sub>50</sub>: Median Lethal Concentration** - pesticide concentration in sugar solution (usually 50% sucrose) at which 50% of test bees die, generally expressed as parts per billion or micrograms per kilogram of solution (μg/kg). This is close to units of micrograms per liter of solution (μg/L), but not quite the same because a sugar solution is more dense than pure water.

**NOEC: No Observed Effect Concentration** - highest tested pesticide concentration in sugar water, pollen or royal jelly at which adverse effects other than mortality (reduced larval survival, impaired navigation or foraging efficiency, etc.) are not observed relative to a control group, expressed as μg/L. The performance of the control group is important in this context, and the experimental conditions and health of the control group are paramount to achieving a proper measurement of No Effect.

Mesh tunnels are used to confine bees to a treated area of bee-attractive plants (e.g., *Phacelia tanacetifolia*) for a Tier II semi-field study of pesticide toxicity to bees. Photo credit: Eurofins Agroscience Services EcoChem GmbH.

1) **Significantly reduced forage distance.** Much less energy is required to forage in a tunnel, which in turn reduces the daily food needs of the foragers and their corresponding intake of pesticide residues. This will result in an underestimate of the adverse effects of the pesticide.

2) **Too little forage for the colony.** The confined space limits the amount of contaminated nectar or pollen available, thereby reducing the impact of the pesticide on the colony.

3) **Ensuring that colonies utilize the resources brought in from the treated plants.** For accurate assessment of pesticide impacts on the colonies, it is essential that they are placed in the tunnel with minimal other food reserves to draw on in the hive. There is some evidence that bees faced with a choice of eating “clean” food or pesticide-contaminated food will choose the “clean” food preferentially, although the magnitude of this effect is likely dependent on the pesticide and the level of contamination.

4) **Ensuring that bees can return to the hive after foraging in the tunnel.** Hive placement is critical. Commercial beekeepers doing test pollination have discovered that hives placed on the ground of the enclosure quickly lose their forag-
ers into the tent tops, where most remain until they die. Tent studies to date have not taken this behavior pattern into account, yet it is easy to understand that if most of the foraging bees leave and do not return to the hive, the amount of pesticide brought back to the hive will be low.

5) **High variability.** The tunnel studies that have been done have highly variable results. This means that more replicates must be done to obtain statistically significant results.

A Cautionary Tale about Bees in Tunnels

A bee hard at work for commercial pollination of cherries. **Photo credit: Chris Jordan-Bloch/Earthjustice**

A beekeeper was pollinating cherry trees enclosed in a tunnel, and noticed that the hives were not doing well. After some close observation, he noticed that the foragers tried to fly up high to return to the hive, but ended up just flying to the top of the tunnel and staying there until they died, unable to make it back to the hive. Not only did the colonies lose a number of foragers, but they also did not have sufficient nectar and pollen to thrive. The bees were significantly weakened by the time spent in the tunnel, even with ample forage present. The cherry yield that year was abysmal because of poor pollination.

The next year, the beekeeper decided to try something different. He put the hives up high on a platform at the top of the tunnel. Concerned that the bees would starve like the previous year, he made sure to bring sugar syrup to feed them when he first went to check on the hives. To his surprise, when he opened the colonies, they were dripping with honey! The clever placement of the hives had allowed the foragers to find their way home. The result was also to the satisfaction of the grower—the cherry yields were so high that year the branches had to be supported to keep from breaking under the load of fruit.

**Tier III Field Studies: Difficult, Expensive, and No Guarantees**

If significant risks to bees are identified for a particular pesticide in the lower tier studies, a full field study (Tier III) may be required to clarify colony level impacts and uncertainties. Tier III studies involve placing a group of colonies near a treated crop and a control group in an area with no likelihood of exposure to the pesticide of interest, allowing the bees to forage freely, and then comparing the exposed and control colonies. As a result of the cost- and time-intensive nature of these studies, few Tier III studies have been conducted to date.

Most notable are the studies conducted by Scott-Dupree of the University of Guelph in Canada. These studies were aimed at assessing potential colony-level effects from bees foraging on canola planted from clothianidin-treated seed. While the concentrations of clothianidin and its breakdown product thiamethoxam in canola pollen and nectar are typically below the amount that would kill bees quickly (less than 50 μg/kg in nectar or pollen) concerns remain about the potential impacts on colony health of exposure to low levels of these pesticides over a longer period of time.

In the first attempt at the study in 2009, test colonies were placed in canola fields planted from untreated (control) and treated (exposed) canola seed. After bloom, the colonies were removed to a different apiary away from agricultural areas and followed for 130 days. Unfortunately, both the experimental design and the vagaries of nature made it impossible to draw definitive conclusions from this study. The primary issue was that clothianidin was detected not only in nectar from the exposed colonies, but also in nectar from the control colonies. The cross-contamination was not surprising, since the untreated and treated fields were separated by only 295 meters (less than two-tenths of a mile) at the closest approach, and the control bees were likely foraging in the treated fields. The untreated fields also suffered from planting difficulties and insect damage and were perhaps not as attractive to foragers as the treated field.

The study was attempted again in 2012. This time, more colonies were used, and the control and exposed groups were separated by 10 kilometers (about 6 miles). The researchers made more precise hive strength measurements and identified specific pollens in the hive to verify forage sources. Yet even with more careful planning and a substantial increase in resources, clothianidin was found in the pollen in the control hives in the second week of the study. What this observation indicates is that there were other sources of clothianidin in the control area—the researchers speculate it may have been sweet corn growing nearby.

Although the higher tier studies are ostensibly aimed at being "field-realistic" to obtain a better measure of risk, the prescribed test methods focus on exposure to a single pesticide. The actual reality in agricultural areas is that bees are exposed to many different mixes of pesticides over the course of a year, not just a single pesticide for only a few weeks. A recent comprehensive meta-analysis showed that on average, pollen from a single beehive contains 6.5 different pesticides, up to a maximum of 31 in a single hive. The Dupree studies, only clothianidin was measured in hive matrices, with even thiamethoxam (the primary degradation product of clothianidin, also toxic to bees) excluded from the analysis. For Tier III experiments to provide meaningful results, it is critical to take a broad view of the bees' environment and understand all of the factors that may affect colony health.

**Comparison of North American and European Approaches**

In the last several years, North American and European regulatory agencies—US Environmental Protection Agency (US EPA), Health Canada's Pest Management Regulatory Agency (PMRA), and the European Food Safety Authority (EFSA)—have been working to revise their pollinator risk assessment guidelines. Currently, much remains to be done to validate and harmonize the methods used for evaluating the potential risks of pesticides to bees.

Most notably, US EPA only requires the acute contact LD₅₀ test for adult bees for a pesticide registration. Canada's PMRA requires both acute contact and acute oral LD₅₀ tests for adult bees. In both countries, other tests may be conditionally required if regulators deem them necessary. To date, US EPA and PMRA have not required any additional tests, which leaves us with substantial data gaps in understanding pesticide toxicity to bees. In the recent 2014 US EPA guidance document, additional studies are recommended to support the evaluation of honey bee risk where pesticide exposure is considered likely, but these studies are not absolutely required.

**Larval toxicity tests are not required by US EPA for pesticide registration, and few larval toxicity studies are available in the literature. **Photo credit: Pesticide Research Institute.

In Europe, pesticide registration requires knowledge of pesticide effects on both adult and larval bees. EPSA's 2013 guidance document recommends a substantial amount of toxicity testing data be submitted for every pesticide, including:

- Acute oral and contact toxicity to adult bees, expressed as μg/bee (LD₅₀).
- Chronic oral toxicity to adult bees, including an assessment of the effects on hypopharyngeal glands (HPG) of nurse bees.

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bees, expressed as µg/bee per day (LC50 and NOEC for HPQ).
- Toxicity to larvae, expressed as µg/larva per development period (NOEC).
- Consideration of potential cumulative effects from exposure to mixtures of pes-
ticides.

The procedures for conducting the studies in the last three bullet points have not yet been fully developed or validated, so it will still be several years before this information is available, and even then it will only be required for new pesticides or those whose registration is being renewed.

**Recommendations for Improving US EPA Risk Assessments**

Expanding the data requirements for US EPA’s evaluation of pesticide effects on bees would go a long way towards increasing the body of available information needed to protect pollinators. At a minimum, US EPA should require:

For each registered pesticide:
- Both oral and contact acute LD₅₀ tests for both adult and larval bees for the pesti-cide and its primary degradation products.
- Both acute and chronic oral toxicity data for bees consuming contaminated pollen and nectar.
- An LD₅₀ study for all formulated pesticide products and any allowed tank mixes to better assess in-field exposures for bees.
- Both oral and contact LD₅₀ studies on another species of pollinator (bumble bees, leafcutter bees, or orchard mason bees), to assess potential differences in vulnerability between different species of bee and the potential for effects on native bees.

**US EPA’s risk assessment process does not currently consider effects on native bees. Photo credit: Pesticide Re-search Institute**

**Exposure considerations**
- Evaluation of risks associated with honey bee exposure to pesticides through contaminated drinking water sources (paddles, ponds, guttation water, and damp soil), an exposure source largely ignored in regulatory processes.
- Assessment of the prevalence of common mixtures of pesticide residues in honey bee colonies and evaluation of their toxicity to determine whether there are additive, synergistic or antagonistic interactions between co-formulated or si-multaneously applied pesticides.

**Conclusions**

The end result of the risk assessment process should be a better understanding of pesticide impacts and development of use restrictions or best management practices. Tier II and Tier III studies can help refine risk estimates, but they still have limitations. The new guidance documents from the US EPA, PMRA, and EFSA are a step in the right direction, and these agencies should fast-track data collection and test method development to better protect pollinators.

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**References**

9. Mullin CA, Frazier M, Frazier JL, Ash-