Notes from the Lab: The Latest Bee Science Distilled

by Scott McArt & Dan Wixted

The "Controversy" Surrounding Pesticide Risk to Bees

This month's "Notes from the Lab" is a bit different than usual. Because the focus of the January issue of *ABJ* is on pesticides, we're going to highlight a topic rather than a single recent study. And whether you know it or not, it's the topic that's causing much of the "controversy" surrounding pesticides lately. This is the topic that many of those studies published in top journals, then interpreted for you via the popular press, often face criticism over.

The topic – pesticide risk to bees – is actually two topics. This is because any risk assessment for pesticides must take into account both *exposure* (i.e., how much pesticide bees are exposed to in the field) and *effects* (i.e., the impact of that pesticide on bees). Unfortunately, it's very hard to address both exposure and effects in a single study. Thus, more often than not, individual studies are published that assess either exposure *or* effects, then make assumptions about the other.

So, what's the problem? Can't *effects* studies trust the *exposure* literature (and vice versa)? This approach does work well sometimes. For example, hundreds of studies have been conducted over the past ~15 years regarding exposure to and effects from neonicotinoids (particularly imidacloprid, thiamethoxam and clothianidin). Thus, we have a good understanding of both neonicotinoid exposure to bees and effects of those neonicotinoids on bees across a range of environmental contexts. New studies therefore have a broad knowledge base to tap into when designing experiments and making inferences about pesticide risk.

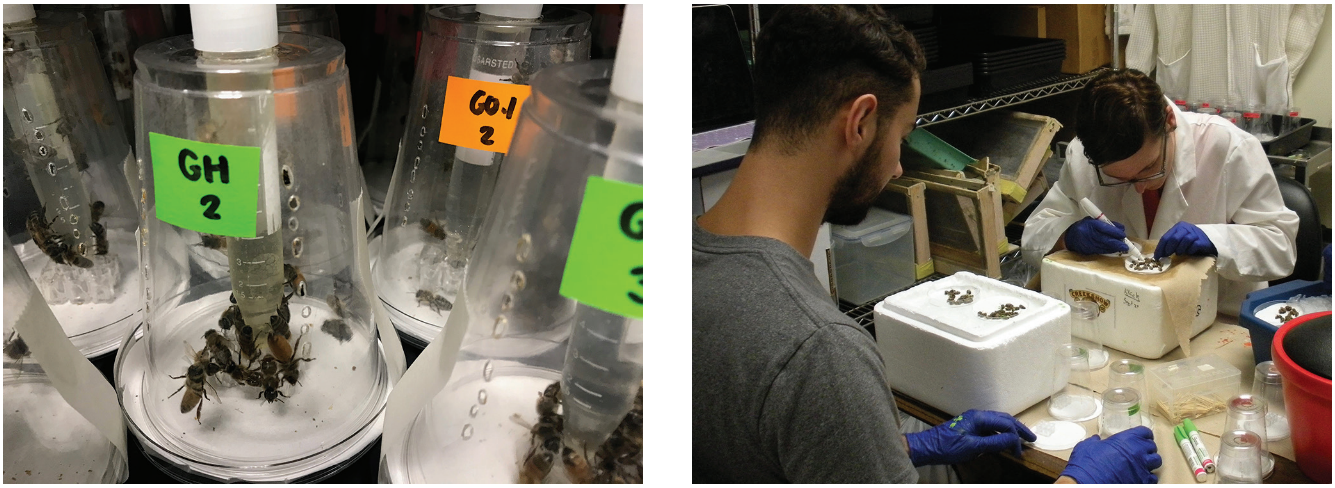
But, unfortunately, this broad knowledge base isn't available for all pesticides. In fact, it isn't available for most pesticides. Researchers know this and are very good at discussing the results from their studies appropriately. But most people read the popular press, not the original studies, and the popular press often extends inference beyond what a single study is capable of inferring. This is what can cause the "controversy" regarding pesticide risk to bees.

Our goal here is to illustrate how such controversy can occur using two examples that you've likely seen in the news lately. We are in no way critiquing the excellent science conducted in these studies (which we do believe is excellent). Instead, our goal is to point out that individual studies rarely assess pesticide risk, even though the popular press would have you believe they do. Thus, while the studies are very important contributions, they should be considered discussion starters, not discussion enders. In other words, each study finds very important results, but how those results should be interpreted regarding pesticide risk to bees requires further study.

"Common weed killer linked to bee deaths." This is the title of one popular press article highlighting the study titled **"Glyphosate perturbs the gut microbiota of honey bees,"** written by Erick Motta and colleagues and published in the journal ***Proceedings of the National Academy of Sciences* [115:10305-10310 (2018)].** It's quite an attention-grabbing title for a newspaper. Clearly, you might think, we've found that the weed killer glyphosate (an ingredient in most RoundUp® products) is responsible for the unsustainable losses of honey bee colonies currently experienced throughout the world. But what does the study really show?

Motta and colleagues conducted a pesticide *effects* study, showing that laboratory-based exposure of individual bees to 5-10 parts per million (ppm) glyphosate for 5 days (or 169 ppm glyphosate for 2 days) alters the bacterial communities in honey bee guts and can cause the bees to be more susceptible to a bacterial pathogen, *Serratia marcescens*. These are very interesting results and the level of mechanistic detail in the study is spectacular. At the same time, it's important to point out that inference regarding risk to bees depends on whether the exposure levels utilized for the effects assays (i.e., 5-10 ppm glyphosate exposure for 5 days, or 169 ppm exposure for 2 days) could be expected in the field.

What do we know about glyphosate exposure to bees in the field? Not much, unfortunately. Glyphosate



(L) *Bees being exposed to experimental treatments in the laboratory.* **(R)** *Erick Motta and Kasie Raymann marking experimental bees in the laboratory.* (Photographer: Kim Hammond, from Motta Study)

levels are rarely assessed in bee products (i.e., honey, pollen, wax) because the analysis is expensive (hundreds of dollars per sample) and requires different methods than standard multi-residue pesticide analyses. The study containing the best exposure data is titled **"Evaluating exposure and potential effects on honeybee brood (*Apis mellifera*) development using glyphosate as an example,"** written by Helen Thompson and colleagues and published in the journal ***Integrated Environmental Assessment and Management*** **[10:463-470 (2014)].** In this study, the authors found that glyphosate concentrations in nectar and pollen could be near the range manipulated by Motta and colleagues: >5 ppm in nectar collected 3.5 days after spraying, and >300 ppm in pollen collected 2 days after spraying. But it's worth pointing out that Thompson and colleagues' study was conducted in a greenhouse where the bees were restricted to foraging only on treated plants in full bloom, and field conditions that could reduce concentrations in nectar and pollen (e.g., precipitation, exposure to UV light) were minimized or absent. It's also worth pointing out that Thompson and colleagues went on to conduct effects assays using concentrations of glyphosate that were even greater than those used by Motta and colleagues; they found no effects of glyphosate on brood survival, development, or mean pupal weight. In contrast, Motta and colleagues focused on adult bees, which may be more susceptible to perturbations in microbiota, since gut bacteria are acquired from nurse bees in the hive in the first few days after adult emergence. Finally, the pH of the technical grade glyphosate that Motta and colleagues used in their assays is more acidic than field spray mixes; this may be a confounding factor since dietary pH is well-known to affect the gut microbiome of animals.

So, what does this mean? Does glyphosate pose a risk to bees or not? Hopefully the paragraphs above should tell you two things. First, the evidence from effects assays to date is mixed: there's some evidence that glyphosate may harm bees, and some evidence that glyphosate may not harm bees, at doses that may or may not be experienced in the field. Second, we need more data, especially regarding field exposure of bees to glyphosate. In toxicology, there's a famous saying: anything is toxic at a sufficient concentration. Yet we have virtually no knowledge of the concentrations of glyphosate that bees *commonly* encounter, or what they *sometimes* encounter in the field. Thus, it's difficult to design *effects* bioassays that accurately assess risk from glyphosate. In other words, Motta and colleagues' study is a very important conversation starter. Now, let's fill in the missing data so we can determine whether, where, and/or when glyphosate exposure poses a risk to bees.

The second study we'd like to highlight is titled **"Sulfoxaflor exposure reduces bumblebee reproductive success,"** written by Harry Siviter and colleagues and published in the journal ***Nature*** **[561:109-112 (2018)].** In this study, Siviter and colleagues document that exposure of bumble bees to 5 ppb sulfoxaflor for 14 days resulted in fewer males produced (and reproductive females produced, though sample size was low) over the colonies' lifetime. This study is similar to Motta and colleagues' study in that the authors conducted an *effects* assay and used the literature to make assumptions about exposure. It's a very nice study, and is important due to the current political and regulatory climate throughout the world. Specifically, usage of certain neonicotinoid insecticides (imidacloprid, clothianidin and thiamethoxam) was banned from agricultural use outside of permanent greenhouses in the European Union in spring 2018. Thus, there's great interest in determining which insecticides can potentially replace neonicotinoids as an effective crop protection tool while minimizing risk to bees. The popular press wasted no time latching onto Siviter and colleagues' study, with the title of one article claiming, **"New pesticide may harm bees as much as those to be replaced."**

So, does sulfoxaflor pose as much risk to bees as neonicotinoids such as imidacloprid, clothianidin and thiamethoxam? We need to answer this question in two ways. First, similar to glyphosate, we know very little about sulfoxaflor *exposure* to bees in the field. A single study, conducted by the U.S. Environmental Protection Agency (EPA), shows that pollen and nectar contains > 5 ppb sulfoxaflor at least 5 days post-spray on cotton plants that were in bloom during spraying. Thus, this study suggests that Siviter and colleagues' manipulation of 5 ppb sulfoxaflor exposure for 14 days may be reasonable.

However, second (and more importantly), how and when is sulfoxaflor actually used? This is critical information for assessing potential exposure. In their paper, the authors write, "To avoid a situation in which pesticides such as neonicotinoids are replaced by products that are similarly contentious, regulatory bodies should move towards an evidence-based approach that assesses both the lethal and sub-lethal consequences of novel insecticides such as sulfoxaflor on non-target organisms." We agree, and note that in the United States, the EPA has done just that. In 2016, the EPA amended the registrations of products containing sulfoxaflor by limiting their application to crops that are 1) not attractive to bees, 2) harvested prior to bloom, or 3) attractive to bees but can be treated only post-bloom (US EPA, 2016). The amended registrations also require buffer zones around treated areas under certain conditions to protect bees from pesticide drift. These restrictions appear on pesticide labels for sulfoxaflor-based products in the U.S. Thus, unless pesticide applicators break the law and spray sulfoxaflor-based products on bee-attractive plants during bloom (which are exactly the exposure data that Siviter and colleagues based their effects assay upon), the EPA concludes "exposure of bees on the treated field is not expected. Therefore, the potential for risk to bees potentially foraging for pollen and nectar on the treated field is considered highly unlikely." We agree with the EPA's assessment at this time.

This said, the EPA has recently granted a large number of emergency exceptions for sulfoxaflor usage to control specific cotton and sorghum pests. While the EPA must address risk to bees when granting such exemptions, there is concern that the protections provided in the exemptions are insufficient. Furthermore, sulfoxaflor is available in ~42 countries worldwide, and many countries have weaker regulatory frameworks than the EPA for agrochemicals and

Full colony placed into a field for the latter part of the experiment (Photo courtesy of Elli Leadbeater, from Siviter Study)

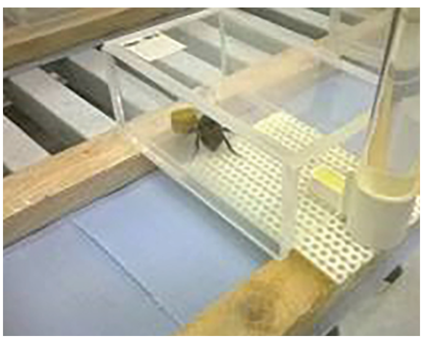
less effective means to enforce risk mitigation measures. In the absence of such measures, advice on product labels in other countries may not be sufficient to mitigate against environmental risk, for a variety of reasons listed in a report recently presented to the United Nations Human Rights Council (United Nations Human Rights Council, 2017).

So, what does this mean? Does sulfoxaflor pose a risk to bees or not? Similar to the glyphosate example, hopefully the paragraphs above should tell you that we need more data, especially regarding field exposure of bees to sulfoxaflor. Furthermore, while regulatory steps have been taken in the U.S. to minimize risk to bees from sulfoxaflor, this is not true everywhere. We suggest regulatory agencies in other countries follow the EPA's lead. However, the large number of exceptions granted by the EPA for sulfoxaflor usage in the U.S. need to be monitored to see if they result in substantial risk to bees.

Finally, as a systemic and environmentally persistent insecticide, sulfoxaflor certainly could pose a similar risk compared to neonicotinoids, regardless of usage restrictions. For example, our own data show that thiamethoxam contributes the greatest risk to bees during apple pollination in New York (McArt et al., 2017), yet thiamethoxam is not sprayed in apple orchards during bloom. Thiamethoxam exposure must therefore occur via other mechanisms, perhaps via contaminated wildflowers or persistence in apple blossoms from applications prior to bloom. Neonicotinoid persistence and contamination of wildflowers in field margins is a well-known source of exposure to bees (e.g. Botias et al., 2015). Until we understand these potential types of exposure to sulfoxaflor in the field, it's difficult to determine all potential routes of exposure and risk from sulfoxaflor usage. Therefore, we wholeheartedly agree with Siviter and colleagues' key statement, yet we recommend broadening it: To avoid a situation in which high-risk pesticides are used in a high-risk manner, regulatory bodies need to robustly assesses both field *exposure* and lethal and sub-lethal *effects* on non-target organisms.

In other words, similar to Motta and colleagues' study, Siviter and colleagues' study is an important conversation starter. Now, let's fill in the missing data so we can determine where and/or when sulfoxaflor exposure poses a risk to bees. There's nothing like good, hard data to take the controversy out of a topic.

Until next time, bee well and do good work,
Scott McArt & Dan Wixted



Bombus terrestris *queen rearing chambers (where the wild-caught queens started colonies and the sulfoxaflor treatment was initiated).* (Photos courtesy of Elli Leadbeater, from Siviter Study)

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