Notes from the Lab:

The Latest Bee Science Distilled

by Scott McArt

A seven-year study assessing pesticide residues in bee bread throughout the United States

Most beekeepers are concerned about pesticides but unsure how concerned they should be.

On the one hand, every commercial beekeeper who rents their bees for pollination of almond, blueberry, apple, or other high-value crops has a story about how some of their colonies have died fairly quickly after one or more pollination events. Given what we know about pesticide exposure during crop pollination, it's highly plausible those colonies died from harmful exposures.

But if a beekeeper doesn't do crop pollination, acute problems easily attributed to pesticide exposure are rare. Does that mean pesticide exposure is rare outside of crop pollination? Or, if exposure is common, are the types and levels of pesticides benign enough that they don't pose an acute risk to bees? What about sublethal risk? Is there reason to suspect normal day-to-day pesticide exposure influences the susceptibility of bees to varroa, nosema, brood diseases, or queen events where an immediate link to pesticides isn't necessarily clear? These are the topics for our forty-first Notes from the Lab, where we summarize **"Pesticides in Honey Bee Colonies: establishing a baseline for real world exposure over seven years in the USA,"** written by Kirsten Traynor and colleagues and published in ***Environmental Pollution*** **[2021].**

As most readers of this column are probably aware, there are thousands of published studies showing that when a bee is dosed with a pesticide, a high enough dose will kill the bee. Some readers may also be aware of the more recent literature showing that low doses of pesticides can have important sublethal effects on bees, including impacts on immunity, behavior, and reproduction. But a critically important point is that these studies measure the *toxicity* of a pesticide, which is only half the equation for understanding *risk* from that pesticide. We also need to understand *exposure*. In other words, **risk = toxicity x exposure**, so we need to understand toxicity and exposure to understand risk from pesticides.

Gaining an understanding of pesticide exposure to bees is difficult for two reasons. First, exposure occurs in many different contexts. For example, you can probably guess that exposure to pesticides will be greater if your hives are in an agricultural region compared to Glacier National Park. But what if you live next to a golf course compared to a small apple orchard? Or what if your hives are in the suburbs compared to a small organic farm? Can you predict in which of those settings your bees will encounter more pesticides? I'm guessing you can't. I certainly can't. Which means we need to test a lot of different contexts to fully understand when and where pesticide exposure will occur.

Second, there are currently several hundred different pesticides that are used throughout the United States, and you need to test for all of them (or at least a majority of them) to have an adequate understanding of exposure. As you might guess, that's expensive. Currently, multi-residue pesticide analyses are about $350 per sample. *$350 per sample!* That means analyzing 100 samples will cost more than a brand new Ford F-150 (**https://www.ford.com/trucks/f150/models/f150-xl/**).

Due to these facts, the number of exposure studies that exist is far less than the number of toxicity studies. Thus, when trying to understand risk from pesticides, we're frequently limited by our knowledge of exposure. To fill this gap, Traynor and

Lead author Kirsten Traynor

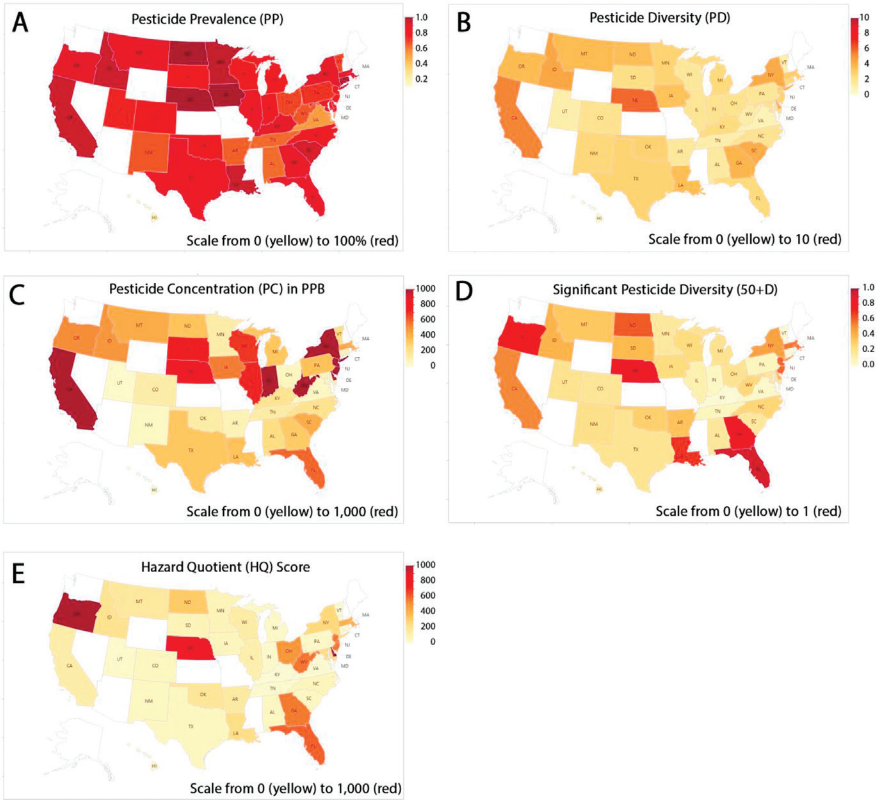

Fig. 1 *Pesticide exposure in bee bread samples collected as part of the National Honey Bee Disease Survey. (A) Pesticide prevalence: the percentage of samples in each state with one or more pesticide residues. Heat map shows range of positive, from yellow which indicates no samples are positive to dark red where all samples are positive; (B) Pesticide diversity: the number of pesticides found per sample, from a scale of 0-10 with yellow indicating an average of no detections and red indicating an average of 10 detections; (C) Pesticide concentration: the sum of all detected residues in a sample, with the mean displayed in parts per billion (ppb) per state on a scale of 0-1,000. We limited the max color scale of deep red to 1,000 ppb to illustrate which states regularly meet this threshold concentration, but if increased to 3,000 ppb then all states except NJ are shades of yellow to light orange; (D) Total number of pesticide detections contributing at least 50+ to the hazard quotient, a threshold equivalent to 0.5% of a honey bee LD50, used for eliminating trace residues that contribute negligibly to consumption risk; E) Mean hazard quotient (HQ) scores per state on a scale of 0-1,000, where 1,000 is our threshold of high risk.*

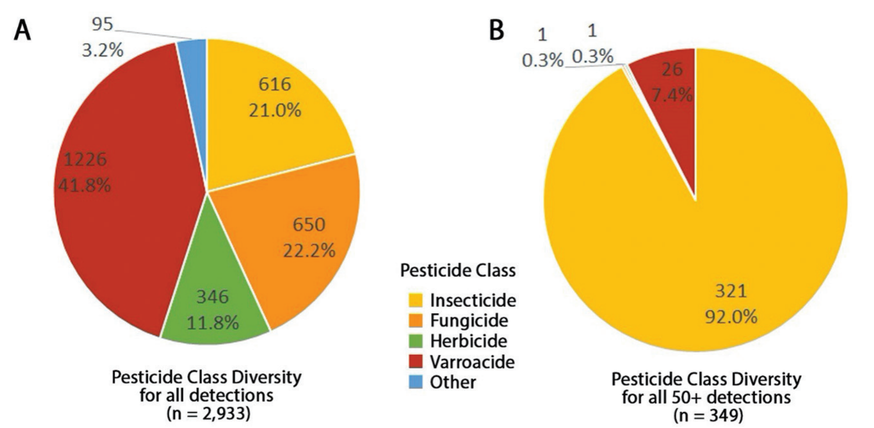

Fig. 2 *(A) Total number of pesticide detections by pesticide class; (B) Total number of pesticide detections contributing at least 50+ to the hazard quotient, a threshold equivalent to 0.5% of a honey bee LD50, used for eliminating trace residues that contribute negligibly to consumption risk. The 50+ diversity varied by U.S. state.*

colleagues set out to gain the broadest understanding possible of day-to-day pesticide exposure via pollen to honey bees in the United States. Over a period of seven years (2011-2017), they collected 1,055 bee bread samples from apiaries in 39 states and Puerto Rico (Figure 1). Samples were taken throughout the year (January-December) during normal apiary inspections as part of the National Honey Bee Disease Survey (**https://research.beeinformed.org/state_reports/**). The samples were analyzed for 175 pesticides and metabolites on average. In addition, at a subset of apiaries, hives were assessed for varroa (*n* = 1,048 apiaries), nosema (*n* = 1,034), virus presence (*n* = 1,015), and brood disease symptoms and queen issues (*n* = 151).

So, what did they find? Were pesticides common in bee bread? Yes. Pesticide residues were found in 82% of bee bread samples and the likelihood of detecting pesticides was always high, though it varied from state to state (Figure 1A). On average, 2.8 pesticides were found per sample, with some states having a greater number of pesticides per sample than others (Figure 1B). The highest average concentration of pesticides (measured in parts per billion, ppb) occurred in samples from New Jersey (mean = 2,942 ppb), Indiana (mean = 1,306 ppb), New York (mean = 1,239 ppb), Delaware (mean = 1,228) and California (mean = 1,110 ppb; Figure 1C).

Which pesticides were the most common? Varroacides (Figure 2A, red) and fungicides (orange) were the most common pesticides found in bee bread, followed by insecticides (yellow), herbicides (green), and other pesticides such as rodenticides (blue). The varroacides DMPF (breakdown product of amitraz; 45% of samples) fluvalinate (37% of samples), and coumaphos (32% of samples) were the most common individual pesticides. This should not be surprising since many beekeepers treat their colonies with amitraz and fluvalinate. Coumaphos residues are unfortunately still common in comb wax due to the long half-life of this pesticide, which can migrate from wax into bee bread.

The most common non-varroacide pesticides were the insecticide chlorpyrifos (16% of samples), the herbicide atrazine (12% of samples), and the fungicides azoxystrobin (8% of samples) and chlorothalonil (7% of samples). The relatively high prevalence of chlorpyrifos is notable since this organophosphate insecticide is highly toxic to bees. Neonicotinoids were found only rarely (2% of samples), though they were thought to carry significant risk when detected.

What about acute risk from the pesticides? Were exposure and toxicity high enough to cause acute poisoning of bees? Very rarely. Although pesticides were common in bee bread, acute risk was generally low. Overall, 5.4% of bee bread samples had a Hazard Quotient (HQ) level above 1,000 (equivalent to 10% of a honey bee LD50). Hazard Quotient varied by state (Figure 1E) with bee bread from Oregon, Delaware, Nebraska, and Florida posing the greatest acute risk to bees. Insecticides largely drove the results regarding acute risk (Figure 2B), particularly the organophosphate chlorpyrifos (13 detections above HQ = 1,000), neonicotinoid clothianidin (8), pyrethroids bifenthrin (6) and prallethrin (6), and carbamate carbaryl (6).

What about sublethal risk? Were there indications that pesticides could influence susceptibility to varroa, nosema, brood diseases, or greater frequency of queen events? Yes, yes, yes, and yes. Hazard Quotient was positively associated with varroa loads in colonies, indicating bees may be more susceptible to varroa infestations when greater risk from pesticides occurs. Number of pesticides and HQ from fungicides were each positively associated with nosema levels. In addition, HQ from fungicides was greater in colonies with brood diseases (American foulbrood, European foulbrood, sacbrood, chalkbrood, and/or snot brood) and colonies experiencing queen issues. These associations with fungicides have some precedent since previous studies have found fungicide exposure can cause nosema to proliferate in honey bees, though more experimental work is needed to understand the associations with brood diseases and queen issues.

Overall, the study by Traynor and colleagues provides excellent insight into day-to-day exposure of honey bees to pesticides via pollen. Furthermore, the Hazard Quotient calculations and associations between exposure and sublethal effects provide insight into acute and sublethal risk posed from the pesticides, respectively. As with any observational study, it's impossible to infer causation from correlations. But with correlations in hand, researchers can design manipulative experiments to further assess whether causation exists. In combination with exposure data, these studies are what inform regulatory decisions regarding pesticides, ultimately improving the safety of our environment for bees.

Until next time, bee well and do good work.

Scott McArt

Reference:

Traynor, K. S., S. Tosi, K. Rennich, N. Steinhauer, E. Forsgren, R. Rose, G. Kunkel, S. Madella, D. Lopez, H. Eversole, R. Fahey, J. Pettis, J. D.Evans and D. vanEngelsdorp. 2021. Pesticides in Honey Bee Colonies: establishing a baseline for real world exposure over seven years in the USA. *Environmental Pollution*. **https://doi.org/10.1016/j.envpol.2021.116566**

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