UMass Extension

2018 Massachusetts Hobbyist Health Survey Report:

Pesticide residues, *Varroa* mites, *Nosema*, and viruses

UMass Honey Bee Extension Program

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Acknowledgements

This project would not have been possible without the efforts of many people. Thanks to David Saleh, who coordinated with all beekeepers in Eastern MA, and collected samples from their hives. Thanks also to Nicolas Baert for conducting the pesticide residue analysis, and Scott McArt for sharing pesticide resources. Finally, thanks to all the beekeepers who generously allowed us to sample their hives, in order to collect data that will help us improve honey bee health in the long run.

1. OVERVIEW

Declines in bee health are due to a network of interacting factors, including pathogens, poor nutrition and pesticides (Spivak et al. 2010). The neonicotinoid class of insecticides in particular has been shown to have lethal and sub-lethal toxicity to bees and is of particular concern to beekeepers and the public (Decourtye and Devillers 2010). Pesticides applied to agricultural landscapes enter hives when foraging bees collect contaminated pollen, nectar and water, or come into direct contact with pesticides. Bees are also exposed to pesticides that are inserted into the hive by beekeepers to control parasites. Pesticides in the hive can accumulate in wax in addition to being found in pollen and nectar.

Massachusetts is home to over 4,000 beekeepers, the vast majority of whom maintain fewer than five hives. It is also home to a large pollinator-dependent cranberry industry, as well as numerous small-scale vegetable farms. In order to assess pesticide risk and disease levels for Massachusetts colonies, we collected bee, wax and beebread (stored pollen) samples twice during summer 2018 from 40 hobbyist apiaries. Wax and beebread samples were assessed for pesticides, and bee samples were screened for *Varroa* mites, viruses and the gut parasite *Nosema*. At the direction of the Massachusetts Department of Agriculture (MDAR), we included only beekeepers with fewer than five colonies, since pesticide levels among larger-scale operations are already documented through the USDA APHIS annual survey, and since small-scale beekeepers are most representative of the Massachusetts beekeeping industry.

This report presents results of the pesticide and disease analysis. We tested 266 pesticides from nine chemical classes. We then calculated a hazard quotient for detected pesticides using established honey bee toxicity metrics, which we compared to risk thresholds established by the EPA. We also determined the most frequently detected pesticides and those with the highest concentrations. Finally, we mapped high-risk pesticide detections, and assessed correlations between key pesticides and disease (*Nosema*, *Varroa* and key viruses).

Several Eastern states recently surveyed pesticide levels in pollen, including Connecticut (Stoner and Eitzer 2013), Maine (Drummond et al. 2018), Virginia (Gooley, Gooley and Fell 2018) and New York (McArt et al 2017). New York also recently surveyed pesticide levels in wax (Mullen et al. 2016). In 2016, researchers at Harvard tested neonicotinoid levels in pollen and honey from across Massachusetts and found that nearly three quarters of all samples contained at least one detectable neonicotinoid (Lu et al. 2016). The present study is unique in that it assesses wax as well as pollen, tests >200 different pesticides, and also incorporates *Nosema*, *Varroa* and viruses.

The next section of the report (methods) describes our experimental design and sampling process, followed by results and then a brief discussion that considers the implications of our findings and compares results to other statewide surveys.

2. METHODS

Sample Design and Data Collection. In spring 2018, 40 beekeepers were selected by the Massachusetts chief apiary inspector. Only beekeepers with more than two and fewer than five hives were considered. Apiaries were located in 12 out of 14 counties in Massachusetts: Worcester-7, Hampden-6, Middlesex-5, Hampshire-5, Berkshire-3, Essex-3, Bristol-3, Norfolk-3, Plymouth-2, Franklin-1, Suffolk-1 and Barnstable-1. See **Figure 1** for a map of sampled apiaries.

Samples were collected by the UMass Amherst Honey Bee Extension Educator, and an apiary inspector hired by UMass Amherst. See **Figure 2** for images of the sampling procedure. Two hives were sampled per beekeeper. Hives were sampled twice: first in June or July (June 3 – July 20), and again in August or September (Aug 1 – Sept 27). During each visit, hives were assessed for size, queen presence and brood health. For each hive, inspectors removed 16 cells of beebread (stored pollen) and gathered one walnut-sized piece of wax comb (new wax from one hive per apiary; old wax from the other). They then selected a frame of open brood from the center of the hive and shook the bees from the frame into a plastic bin. They scooped out a half-cup of bees for virus testing (bees were poured into a ventilated shipping box containing water and queen candy) and a quarter-cup of bees for *Nosema* and *Varroa* testing (bees were placed in a jar filled with alcohol). To provide participating beekeepers with immediate information about mite levels, an alcohol wash was performed on site, and all bees, mites and alcohol were returned to the original container, and later shipped to the USDA lab for *Nosema* and *Varroa* testing.

Live bees were shipped as soon as possible to the University of Maryland Honey Bee Lab, where they were stored at -80° C and assessed for nine viruses: acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), deformed wing virus (DWV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), Lake Sinai virus 2 (LSV2), slow bee paralysis virus (SBPV) and Varroa destructor virus (VDV).

The alcohol samples were stored in a refrigerator (1.6° C), and later sent to the USDA Beltsville Disease Diagnostic lab for *Nosema* and *Varroa* analysis. The wax and pollen samples were stored in a freezer (-18° C), and later shipped to Cornell University.

A quick note: typically, stored pollen is called “beebread” to distinguish it from pollen gathered via pollen trap. However, for the sake of simplicity, we will refer to our stored-pollen samples as “pollen” in this report.

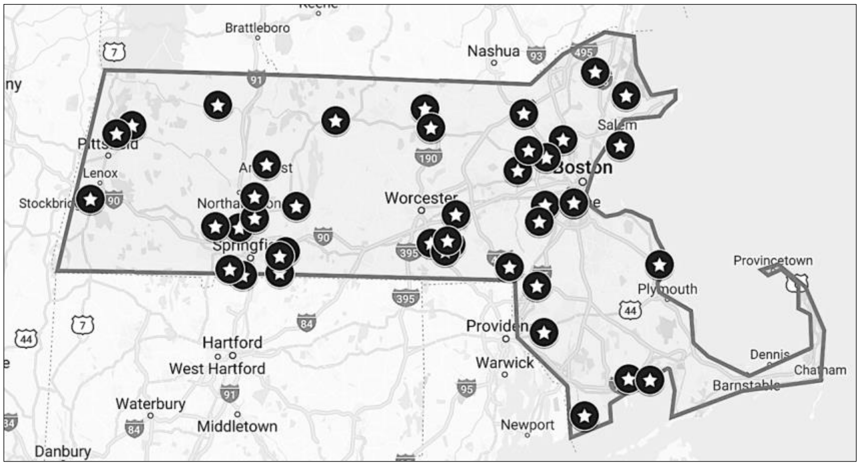


Figure 1. Map showing the locations of the 40 sampled apiaries.

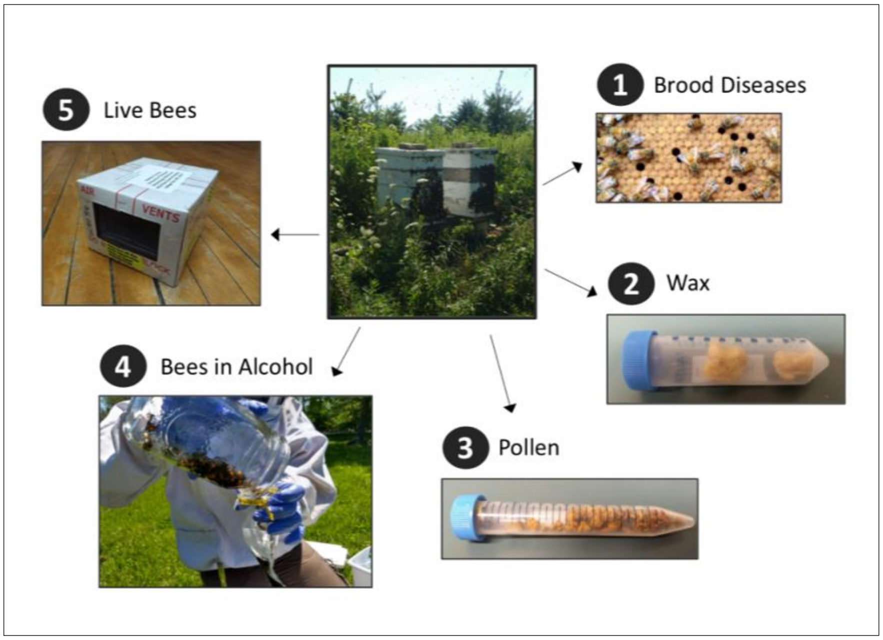


Figure 2. Diagram showing samples collected per hive: 1. Brood disease assessed, 2. Wax sample (for pesticides), 3. Pollen sample (for pesticides), 4. Bees in alcohol (for *Nosema* and *Varroa*), 5. Live bees (for virus testing).

Pesticide Analysis. Wax and pollen samples were processed by Dr. Nicolas Baert at the McArt Lab and Chemical Ecology Core Facility at Cornell. They were tested for 266 chemical compounds used in agriculture: 100 insecticides, 81 herbicides, 71 fungicides, 5 plant growth regulators, 2 herbicide safeners (used to reduce the impact of herbicides on crop plants), 2 rodenticides, 1 synergist, 1 food preservative, and 3 miticides.

For each chemical, the lowest detectable concentration (Level of Detection (LOD)) was recorded. For chemical detections with levels above the minimum quantifiable concentration (Level of Quantification (LOQ)), the concentration in ppb was recorded. For detections above the LOD but below the LOQ, the concentration was listed as the LOD. It is important to note that the LOD levels used by the McArt lab are much lower than those used by the USDA for the annual APHIS survey. For instance, McArt lab can detect above 0.18ppb for imidacloprid;

USDA APHIS can only detect above 5ppb. For fipronil, the McArt LOD can detect above 0.18ppb while the USDA LOD is 25 ppb. See **Appendix A** for a full list of pesticides tested, including the LOD for wax and pollen.

In order to assess pesticide toxicity to honey bees, we used LD_{50} values compiled by Scott McArt using information provided in Sanchez-Bayo & Goka (2014), the Tomlin Pesticide Manual, the ECOTOX database of the U.S. Environment Protection Agency (http://cfpub.epa.gov/ecotox/) and the AgriTox Database of the French government (http://www.agritox.anses.fr/index.php). LD stands for "Lethal Dose". The oral LD_{50} is the concentration at which a chemical will kill half of honey bees that consume it; the contact LD_{50} is the concentration at which a chemical will kill half of honey bees that come into physical contact with it. The contact and oral LD_{50} for all detected chemicals can be found in **Table 1**.

According to US EPA standards, compounds with an $LD_{50} < 2$ µg/bee are considered highly toxic, those with an $LD_{50} > 2$ and < 10.9 are considered moderately toxic, and those with an $LD_{50} \geq 11$ µg/bee are considered practically non-toxic (OCSPP Guideline 850.3030; US EPA 2012).

Hazard Quotient. To evaluate the health risk of pesticide residues in wax and pollen samples, we calculated a Hazard Quotient (HQ), which is a ratio of exposure to toxicity (Stoner and Eitzer 2013):

$$\boldsymbol{Hazard\ Quotient\ (HQ)} = \frac{\boldsymbol{Exposure}: detected\ concentration\ (\mu g/g)}{\boldsymbol{Toxicity}: LD50\ (\mu g/bee)}$$

We calculated this hazard quotient in two ways: first, we computed an *individual hazard quotient* for each instance of a pesticide detection. For instance, if we detected imidacloprid in a pollen sample, we would divide the detected imidacloprid concentration by imidacloprid's LD_{50}. This would allow us to assess the risk of that particular pesticide in that particular sample.

Second, for all wax and pollen samples, we calculated a *total hazard quotient* (THQ). This is the sum of all of the individual hazard quotients for *all* pesticides detected in that sample. This allows us to approximate the cumulative risk of pesticides detected in that sample, although it does not account for potential synergistic (non-additive) interactions between pesticides (Pilling and Jepson 1993, Schmuck Stadler and Schmidt 2003).

HQ values (both *individual* and *total*) help us to make sense of detected pesticide concentrations in two ways. First, they are intuitive to interpret: an HQ value of 1 means that the detected concentration is equivalent to the LD_{50}, meaning that it is enough to kill 50% of the bees that contact it. An HQ of <1 indicates that the detected concentration is lower than the LD_{50}, and HQ > 1 indicates that the concentration is greater than the LD_{50}. Second, HQ values allow us to compare pesticide concentrations to thresholds established by the EPA. The EPA has determined that honey bees are at risk of acute pesticide poisoning when they are exposed to pesticide concentrations at or above 40% of the LD_{50} (i.e. HQ = 0.4). This 40% threshold is referred to as the acute Level of Concern (LOC) (USEPA, 2014).

Note that before calculating the hazard quotient, we had to convert the pesticide concentration values from ng/g (or ppb – "parts per billion"), to µg/g by dividing the concentration values by

1,000 (1,000 ng = 1µg). The HQ can be calculated using the oral or contact LD_{50} value (both are included in **Table 1**). In the tables and figures that summarize HQ values for one pesticide across all positive samples, we use the **average concentration** (among positive detections) for a given compound as a measure of exposure (i.e., HQ = average detected concentration across positive samples/LD_{50}).

It is also important to note that the hazard quotient that we use here is not exactly the same as the risk quotient used by the EPA for pesticide testing. Both are a ratio of bee pesticide exposure to toxicity. However, the EPA estimates bee exposure (the upper value in the equation) based on pesticide application rates, using a formula derived from field studies (USEPA, 2014). In contrast, we use the detected concentrations of pesticides in wax or pollen as a measure of exposure, similar to Stoner and Eitzer 2013, McArt et al. 2017 and Mullen et al. 2017. The EPA-designated level of concern of 0.4 was used by McArt et al. and Mullen et al. to interpret their results, and we think that it is also a meaningful tool for interpreting our results here.

Correlation Analysis. A correlation analysis was conducted on eight key variables that were found in a relatively high number of samples. These included the following hive health parameters: ***Varroa*** mite levels (# mites/100 bees), ***Nosema*** levels (million spores/bee), **DWV** levels (# of viral copies detected) and **VDV** levels (# of viral copies detected). DWV and VDV were chosen because they were the most commonly detected viruses (found in 47% and 71% of samples, respectively). We selected four commonly detected pesticides: **imidacloprid** (detected in 19.5% of samples), which is of particular interest because it was the most commonly detected neonicotinoid and is highly toxic to bees; the insecticide **fipronil** (detected in 12.6% of samples), which is also high toxicity to bees; the beekeeper applied miticide **coumaphos**, which was the most commonly detected compound (in 67.3% of all samples, and 94% of wax samples), and the synergist **piperonyl butoxide (PBO)**, which was the second most commonly detected compound (in 57.9% of samples). Pesticide data were analyzed separately for pollen and wax. Correlations for all eight variables were analyzed separately for each time period. The correlation table was generated using the R package *corrplot.*

Data Formatting. Some data samples were lost and not included in analysis. One pollen sample was lost for pesticide analysis, and two mite samples were crushed and removed from the analysis. The mite levels for one sample during the August/September period were a clear outlier (>5 standard deviations above the mean), and so also removed from the analysis. For the correlation analysis, all samples from both sampling periods for those three apiaries were removed to make equivalent sample sizes across sampling periods. The virus data for deformed wing virus (DWV) and varroa destructor virus (VDV) contained a large range in values (from 0 to 1.05E+11) and was strongly left skewed. We transformed these values using a log transformation. For the pesticides coumaphos and piperonyl butoxide (PBO), the data were also strongly left-skewed. Most concentration values were low, but a handful were much higher. For instance, for coumaphos, there were only five detections out of 107 with concentrations greater than 15ppb, but those five values ranged from 23 to 108ppb. For PBO, there were only three concentrations greater than 15ppb, which ranged from 18-47ppb. For both of these compounds, all samples with concentrations >15ppb were changed to 15ppb to avoid having a small number of samples have excessive influence on results.

3. RESULTS

Hive Health Results

Varroa. As expected, *Varroa* levels were higher in late summer (avg. 1.47 mites/100 bees) than early summer (avg. 0.36 mites/100 bees; **Figure 3**). This is lower than the numbers reported by the most recent APHIS survey data (avg. 2 mites/100 bees in June, and 3.2 in August of 2016) (BIP - APHIS Survey State Reports). See **Figure 4** for a comparison of average mite levels across regions, noting that regions contain different numbers of beekeepers, so this comparison must be made with caution. When we mapped high *Varroa* mite detections (above the treatment threshold of 2-3 mites/100 bees), we found no clear geographic patterns, which is not surprising given that mite levels are so strongly tied to beekeeper management (mite treatment applications). It is important to point out that we recorded mite levels in two ways – using a field alcohol wash, and by sending the same sample to the USDA lab. Figures 3 and 4 show the results from the USDA lab analysis. However, field and lab mite levels did not always align (R_2 = 0.4858, indicating that less than half the variation in lab levels was explained by field counts; see **Figure 5**). In general, more mites were detected in the field. This could be because some mites were lost after the alcohol wash, or because the number of bees in each sample was under-estimated (in the field, we estimated 150 bees per ¼ cup sample; in the lab, the number of bees was counted). These results suggest that caution should be used when conducting a field alcohol wash before sending a sample for *Varroa* analysis.

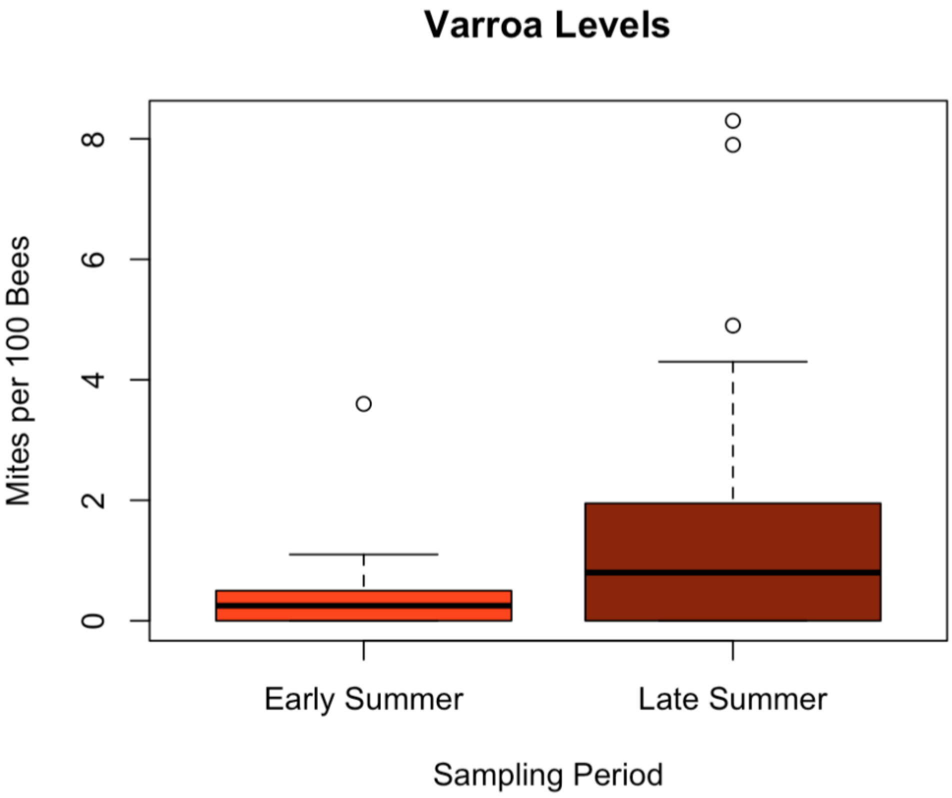


Figure 3. Varroa levels (mites/100 bees) by sampling period. Central black bars indicate the average mite level (i.e. mean) for that sampling period. The box borders 50% of the data (25% above the mean and 25% below). The dots indicate outliers.

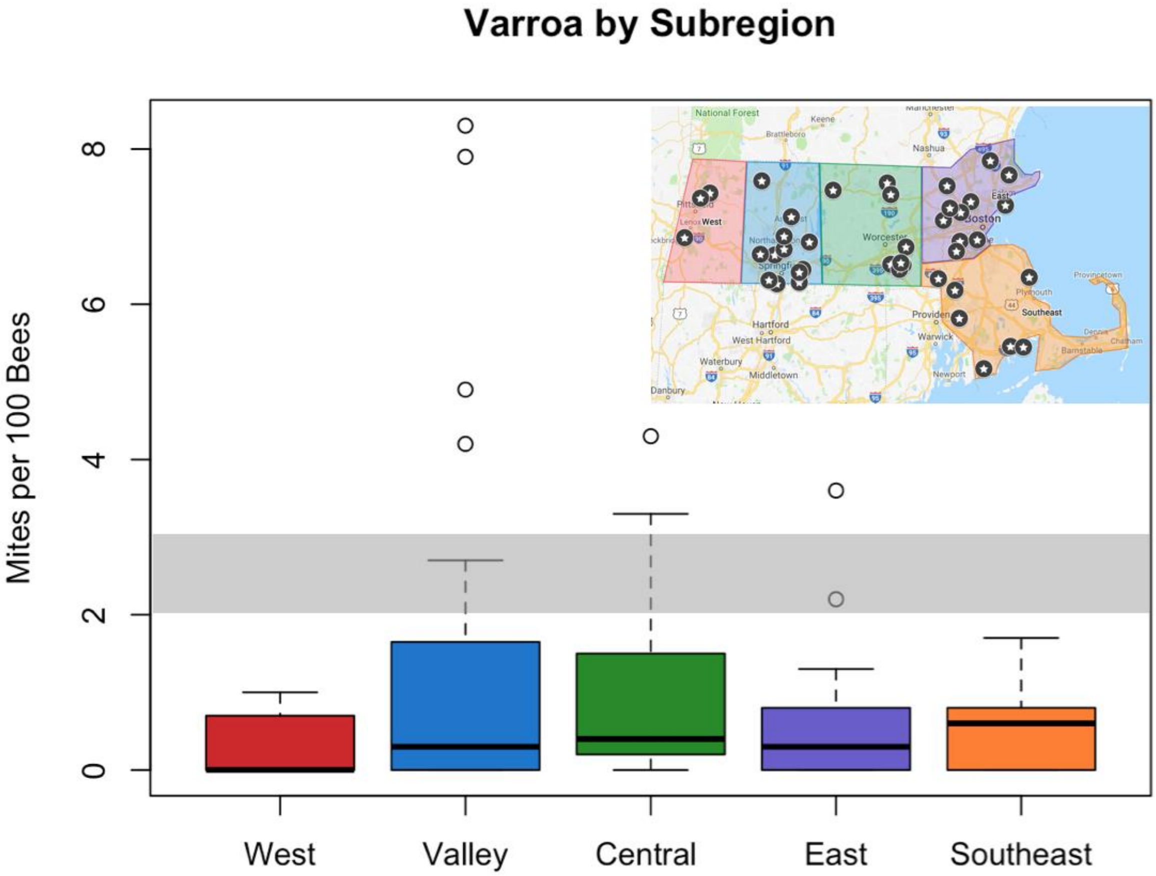


Figure 4. Varroa levels by subregion across all sampling time periods. The treatment threshold of 2-3 mites/100 bees in indicated in gray.

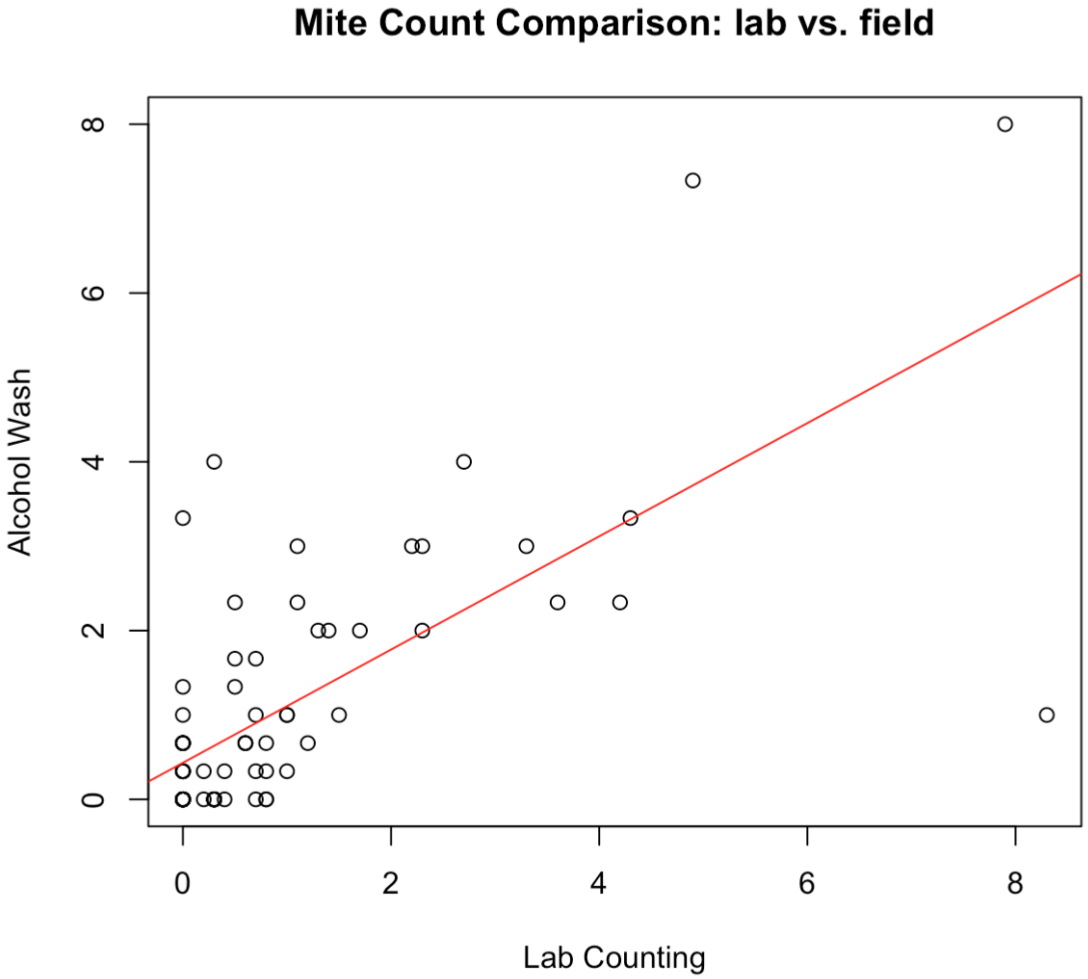


Figure 5. Comparison of mite levels detected in the lab vs. field alcohol wash. $R_2 = 0.4858$, $P < 0.05$. Alcohol Wash = 0.67093*Lab Count + 0.43327.

Nosema. The average *Nosema* level was 0.413 million spores per bee in early summer, and 0.248 million spores per bee in late summer. This is slightly higher than the levels detected in the 2016 USDA APHIS survey, which found an average of 0.3 million spores in June and 0.2 million in August (BIP - APHIS Survey State Reports). Overall, *Nosema* was detected in only 11 out of 40 apiaries in early summer, and eight out of 40 in late summer. In each period, only about half of apiaries with a detectable infection had a spore concentration above the treatment threshold of 1 million spores per bee **(Figure 6**). When we mapped *Nosema* detections above 1 million spores per bee, there was no clear geographic trend.

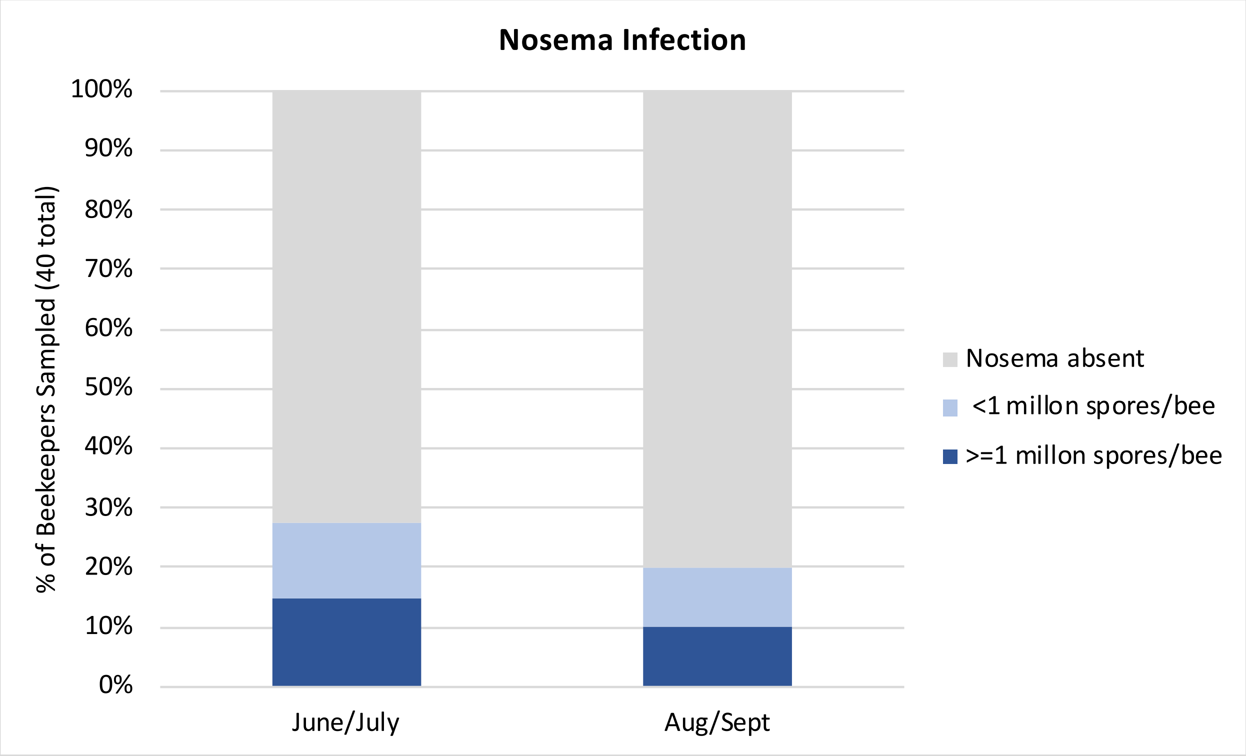


Figure 6. Percent of apiaries with detected *Nosema* infections.

Viruses. Of the nine viruses tested, only five were found in any sample: Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Israeli Acute Paralysis Virus (IAPV), Lake Sinai Virus 2 (LSV2) and Varroa Destructor Virus (VDV). The most commonly detected were VDV (67.5% of samples in early summer, and 75% in late summer) and DWV (35% of samples in early summer and 65% in late summer). The next most common were IAPV (12.5% of samples in both periods), LSV2 (35% in early summer; 7.5% late summer) and CBPV (5% and 10% respectively) (**Figure 7**).

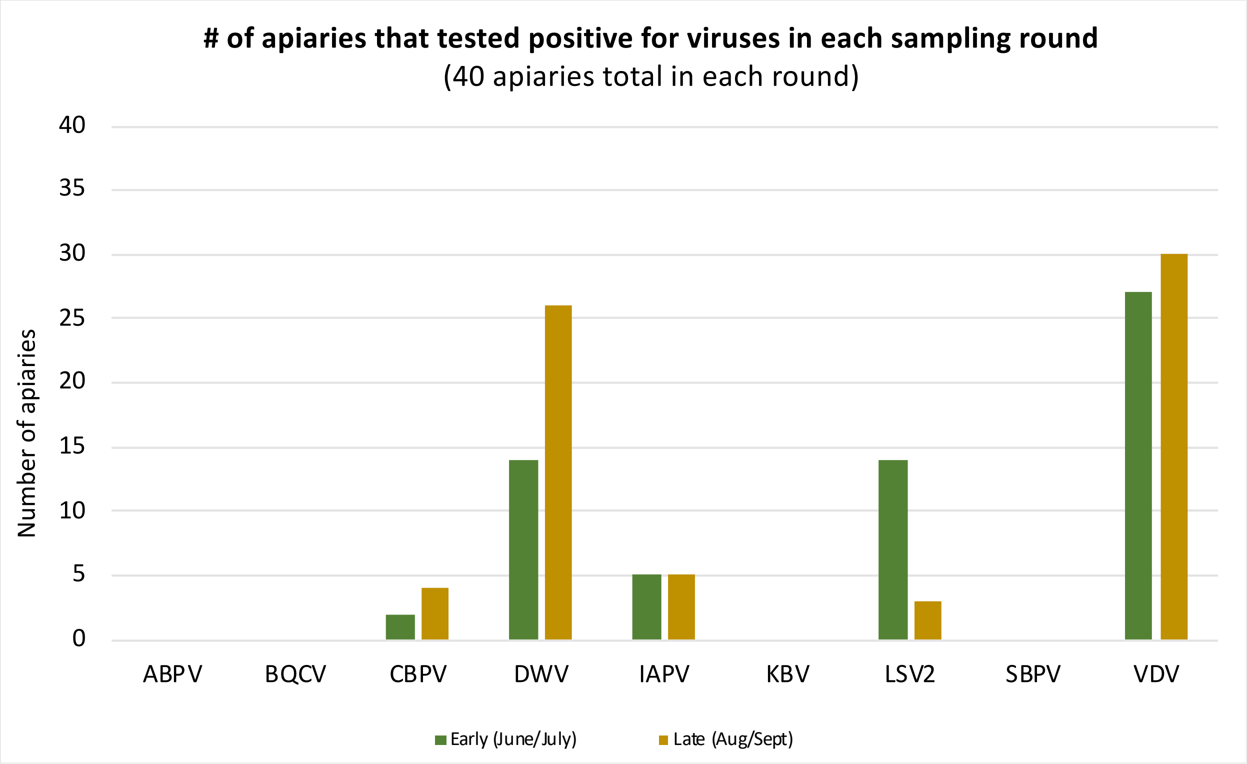


Figure 7. Number of apiaries testing positive for select viruses in each sampling round (early vs. late).

Pesticide Results

Overall. Of the 266 compounds tested, 208 were not detected in any samples, including all of the rodenticides, herbicide safeners, plant growth regulators and food preservatives. There were 58 compounds detected in at least one sample (**Table 1)**. For short descriptions of the top 10 most frequently detected pesticides, as well as all pesticides detected in concentrations above the level of concern, see **Appendix B**.

Distribution of results. The pesticide results were relatively dispersed, with many of the pesticides found in only a few samples. 98% of samples tested positive for at least one pesticide (all of the wax samples, and all but three of the pollen samples). However, over 70% of all compounds tested (41 out of 58) were found in fewer than 5% of samples. Only 12 pesticides were found in at least 10% of samples, and only four were found in at least 20% of samples (**Table 1**). There were on average 3.87 pesticides detected per pollen sample (range 0 -13) and 3.81 detected wax sample (range 1 -10). See **Figure 8** for a histogram of the number of pesticides detected per sample.

Compound	**Compound Type**	**Contact LD50 (ug/bee)**	**Oral LD50 (ug/bee)**	**Pollen**									**Wax**								
				Level of Detection (ng/g)	Level of Quantification (ng/g)	% Samples Positive	Concentration (ng/g)*		Mean Contact HQ**	Mean Oral HQ**	# Samples where HQ > LOC***		Level of Detection (ng/g)	Level of Quantification (ng/g)	% Samples Positive	Concentration (ng/g)*		Mean Contact HQ**	Mean Oral HQ**	# Samples where HQ > LOC***	
							mean	*max*			*Contact*	*Oral*				*mean*	*max*			*Contact*	*Oral*
Coumaphos	Miticide	20.3	4.6	0.23	0.70	40.5	2.5	11.9	0.0001	0.0005	0	0	0.18	0.53	93.8	6.9	108.3	0.0003	0.0015	0	0
Piperonyl butoxide	Synergist	11	/	0.05	0.14	34.2	1.8	9.2	0.0002	/	0	0	0.04	0.11	81.3	3.2	47.0	0.0003	/	0	0
Fenpyroximate	Miticide	11	118.5	0.09	0.28	5.1	1.8	3.5	0.0002	0.0000	0	0	0.07	0.21	41.3	3.3	39.6	0.0003	0.0000	0	0
Azoxystrobin	Fungicide	200	25	0.04	0.11	24.1	0.2	1.3	0.0000	0.0000	0	0	0.03	0.08	17.5	0.3	1.7	0.0000	0.0000	0	0
Imidacloprid ^	Insecticide	0.044	0.004	0.23	0.70	22.8	1.3	4.4	0.0287	0.3156	0	6	0.18	0.53	16.3	1.4	6.5	0.0323	0.3553	0	4
Atrazine	Herbicide	97	/	0.12	0.35	29.1	0.5	2.4	0.0000	/	0	0	0.09	0.26	5.0	0.2	0.5	0.0000	/	0	0
Fipronil sulfone	Insecticide	0.006	0.001	0.47	1.40	16.5	0.5	0.5	0.0778	0.4667	0	13	0.35	1.05	16.3	0.6	3.6	0.1001	0.6004	1	1
Carbaryl	Insecticide	0.232	0.15	0.09	0.28	26.6	1.3	9.8	0.0058	0.0089	0	0	0.07	0.21	3.8	0.8	1.0	0.0033	0.0052	0	0
Metolachlor	Herbicide	110	110	0.14	0.42	22.8	1.1	5.9	0.0000	0.0000	0	0	0.11	0.32	2.5	0.1	0.1	0.0000	0.0000	0	0
Fipronil	Insecticide	0.006	0.001	0.23	0.70	8.9	2.0	4.4	0.3359	2.0156	3	4	0.18	0.53	16.3	1.0	7.6	0.1629	0.9773	1	4
Chlorantraniliprole	Insecticide	0.706	0.0274	1.17	3.50	15.2	14.3	71.4	0.0203	0.5237	0	5	0.88	2.63	8.8	1.9	3.9	0.0027	0.0697	0	0
Boscalid	Fungicide	200	166	0.23	0.70	16.5	13.1	52.5	0.0001	0.0001	0	0	0.18	0.53	6.3	1.9	6.3	0.0000	0.0000	0	0
Acetamiprid ^	Insecticide	7.9	14	0.05	0.14	6.3	2.6	5.2	0.0003	0.0002	0	0	0.04	0.11	7.5	0.1	0.1	0.0000	0.0000	0	0
Pyraclostrobin	Fungicide	100	73	0.05	0.14	8.9	3.2	9.6	0.0000	0.0000	0	0	0.04	0.11	3.8	0.9	1.8	0.0000	0.0000	0	0
Thiophanate-methyl	Fungicide	100	100	0.70	2.10	10.1	52.6	414.2	0.0005	0.0005	0	0	0.53	1.58	1.3	0.5	0.5	0.0000	0.0000	0	0
Cyprodinil	Fungicide	100	100	0.23	0.70	3.8	21.3	59.8	0.0002	0.0002	0	0	0.18	0.53	7.5	1.9	7.2	0.0000	0.0000	0	0
Tebufenpyrad	Insecticide	6.8	1.8	0.19	0.56	0.0							0.14	0.42	10.0	1.4	3.0	0.0002	0.0008	0	0
Fluxapyroxad	Fungicide	100	110.9	0.09	0.28	6.3	1.8	4.9	0.0000	0.0000	0	0	0.07	0.21	2.5	0.8	1.4	0.0000	0.0000	0	0
Trifloxystrobin	Fungicide	200	200	0.05	0.14	3.8	16.8	49.8	0.0001	0.0001	0	0	0.04	0.11	5.0	0.2	0.4	0.0000	0.0000	0	0
Thiabendazole	Fungicide	4	34	0.07	0.21	2.5	0.1	0.1	0.0000	0.0000	0	0	0.05	0.16	6.3	0.9	1.7	0.0002	0.0000	0	0
Clothianidin ^	Insecticide	0.039	0.004	0.23	0.70	6.3	0.4	1.0	0.0101	0.0987	0	0	0.18	0.53	0.0						
Clomazone	Herbicide	100	85.3	0.07	0.21	6.3	0.1	0.4	0.0000	0.0000	0	0	0.05	0.16	0.0						
Spinosyn A	Insecticide	0.003	0.057	0.23	0.70	5.1	0.7	1.3	0.2315	0.0122	1	0	0.18	0.53	1.3	0.8	0.8	0.2638	0.0139	0	0
Fluopyram	Fungicide	100	102.3	0.07	0.21	5.1	1.3	2.2	0.0000	0.0000	0	0	0.05	0.16	1.3	0.2	0.2	0.0000	0.0000	0	0
Dithiopyr	Herbicide	81	/	1.63	4.90	5.1	3.3	8.2	0.0000	/	0	0	1.23	3.68	0.0						
Thiamethoxam ^	Insecticide	0.024	0.005	0.19	0.56	3.8	0.2	0.2	0.0078	0.0373	0	0	0.14	0.42	1.3	0.1	0.1	0.0058	0.0280	0	0
Fenbuconazole	Fungicide	292	/	0.47	1.40	3.8	8.0	11.5	0.0000	/	0	0	0.35	1.05	1.3	0.4	0.4	0.0000	/	0	0
Fenhexamid	Fungicide	207	1.7	1.17	3.50	1.3	12.4	12.4	0.0001	0.0073	0	0	0.88	2.63	3.8	3.1	4.3	0.0000	0.0018	0	0
Penthiopyrad	Fungicide	312	385	0.05	0.14	1.3	0.0	0.0	0.0000	0.0000	0	0	0.04	0.11	3.8	0.1	0.2	0.0000	0.0000	0	0
Metrafenone	Fungicide	100	114	0.23	0.70	3.8	171.5	513.1	0.0017	0.0015	0	0	0.18	0.53	0.0						
Pyrimethanil	Fungicide	100	100	0.23	0.70	3.8	1.8	2.4	0.0000	0.0000	0	0	0.18	0.53	0.0						
Cyazofamid	Fungicide	100	/	0.35	1.05	3.8	1.0	1.4	0.0000	/	0	0	0.26	0.79	0.0						
Difenoconazole	Fungicide	100	177	0.23	0.70	3.8	0.8	1.4	0.0000	0.0000	0	0	0.18	0.53	0.0						
Fluopicolide	Fungicide	100	241	0.12	0.35	2.5	45.4	88.3	0.0005	0.0002	0	0	0.09	0.26	1.3	0.4	0.4	0.0000	0.0000	0	0
Bifenazate	Insecticide	7.8	141	0.19	0.56	2.5	1.3	1.5	0.0002	0.0000	0	0	0.14	0.42	1.3	0.8	0.8	0.0001	0.0000	0	0
Chlorpyrifos	Insecticide	0.01	0.051	0.47	1.40	2.5	1.3	2.1	0.1266	0.0248	0	0	0.35	1.05	0.0						
Methomyl	Insecticide	0.16	0.24	0.23	0.70	2.5	5.2	9.3	0.0327	0.0218	0	0	0.18	0.53	0.0						
Tebuthiuron	Herbicide	100	/	0.02	0.07	2.5	0.3	0.4	0.0000	/	0	0	0.02	0.05	0.0						
Napropamide	Herbicide	/	113.5	0.05	0.14	2.5	2.2	2.8	/	0.0000	0	0	0.04	0.11	0.0						
Etofenprox	Insecticide	0.015	0.024	0.12	0.35	1.3	8.1	8.1	0.5426	0.3392	1	0	0.09	0.26	1.3	1.5	1.5	0.0993	0.0620	0	0
Tebuconazole	Fungicide	200	83	0.47	1.40	1.3	1080.7	1080.7	0.0054	0.0130	0	0	0.35	1.05	1.3	0.4	0.4	0.0000	0.0000	0	0
Dimethomorph	Fungicide	55	32	0.23	0.70	1.3	198.7	198.7	0.0036	0.0062	0	0	0.18	0.53	1.3	0.2	0.2	0.0000	0.0000	0	0
Quinoxyfen	Fungicide	79	316	0.14	0.42	1.3	4.5	4.5	0.0001	0.0000	0	0	0.11	0.32	1.3	0.4	0.4	0.0000	0.0000	0	0
Methoxyfenozide	Insecticide	100	100	0.19	0.56	1.3	1.1	1.1	0.0000	0.0000	0	0	0.14	0.42	1.3	0.5	0.5	0.0000	0.0000	0	0
Dinotefuran ^	Insecticide	0.047	0.022	0.70	2.10	1.3	10.4	10.4	0.2208	0.4717	0	1	0.53	1.58	0.0						
Methiocarb-sulfoxide	Insecticide	0.29	0.47	0.05	0.14	1.3	0.2	0.2	0.0006	0.0004	0	0	0.04	0.11	0.0						
Flubendiamide	Insecticide	200	200	0.70	2.10	1.3	109.4	109.4	0.0005	0.0005	0	0	0.53	1.58	0.0						
Fenamidone	Fungicide	47.1	159	0.23	0.70	1.3	1.0	1.0	0.0000	0.0000	0	0	0.18	0.53	0.0						
Propiconazole	Fungicide	50	77	0.70	2.10	1.3	0.7	0.7	0.0000	0.0000	0	0	0.53	1.58	0.0						
Cyflufenamid	Fungicide	100	100	0.09	0.28	1.3	0.5	0.5	0.0000	0.0000	0	0	0.07	0.21	0.0						
Triadimenol	Fungicide	/	/	0.23	0.70	1.3	0.2	0.2	/	/	0	0	0.18	0.53	0.0						
Neburon	Herbicide	/	/	0.07	0.21	1.3	3.2	3.2	/	/	0	0	0.05	0.16	0.0						
Imibenconazole	Fungicide	200	125	0.47	1.40	0.0							0.35	1.05	1.3	0.4	0.4	0.0000	0.0000	0	0
Hexythiazox	Insecticide	200	/	0.47	1.40	0.0							0.35	1.05	1.3	0.4	0.4	0.0000	/	0	0
Amitraz	Miticide	50.0	/	0.05	0.14	0.0							0.04	0.11	1.3	0.0	0.0	0.0000	/	0	0
Prometryn	Herbicide	96.69	/	0.05	0.14	0.0							0.04	0.11	1.3	0.0	0.0	0.0000	/	0	0
Metosulam	Herbicide	/	/	0.23	0.70	0.0							0.18	0.53	1.3	0.2	0.2	/	/	0	0
Terbumeton	Herbicide	/	/	0.05	0.14	0.0							0.04	0.11	1.3	0.0	0.0	/	/	0	0

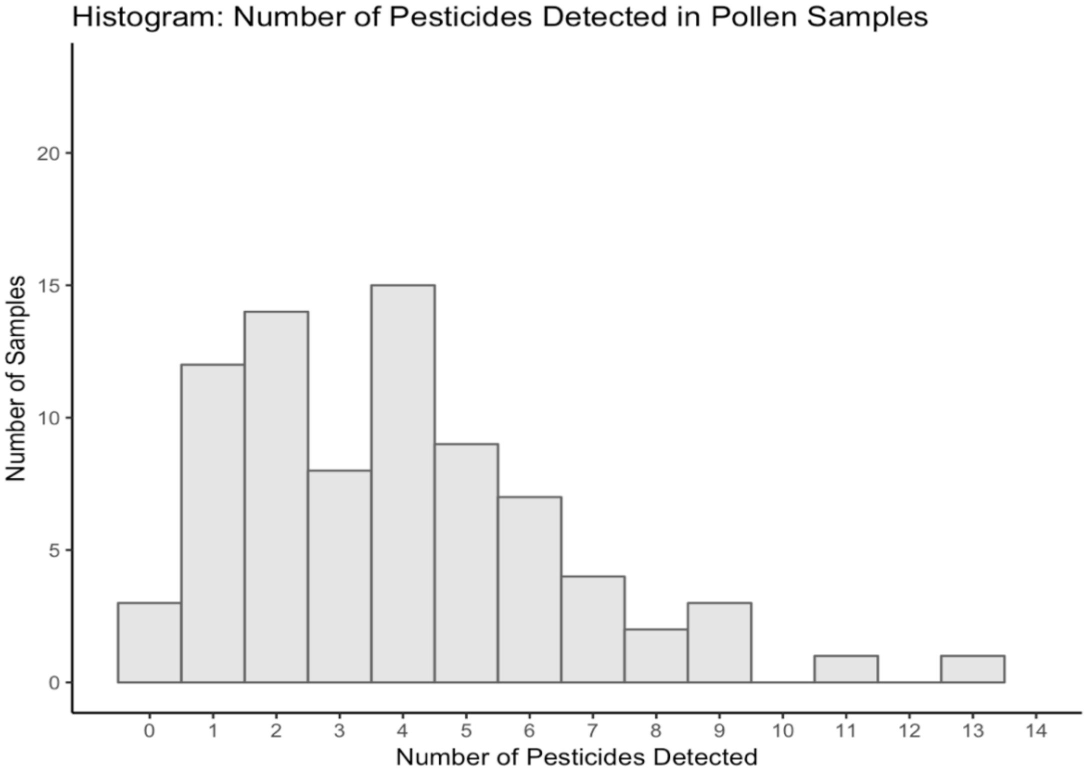
Table 1. Summary of pesticides detected, ordered from most to least frequently detected overall. Each compound type is highlighted in a different color for ease of interpretation. For the LD50 columns, values below 2 (highly toxic) are highlighted in red. Values between 2 and 11 (moderately toxic) are highlighted in yellow. For the contact and oral HQ columns, values above the EPA level of concern (0.4) are highlighted in red. A slash (/) in the LD$_{50}$ column indicates that the information was not available. *Note that the LD50 values are given in ug/g, but the concentration values and levels of detection are reported in ng/g.*

^denotes neonicotinoids

*Mean conc. refers to the mean among positive detections only

**mean Hazard Quotient (HQ) calculated as: [(mean conc./1000)/ LD50]

***These columns summarize the number of chemical detections where the HQ value exceeds the EPA level of concern of 0.4. For ease of interpretation, all non-zero values are highlighted in red.



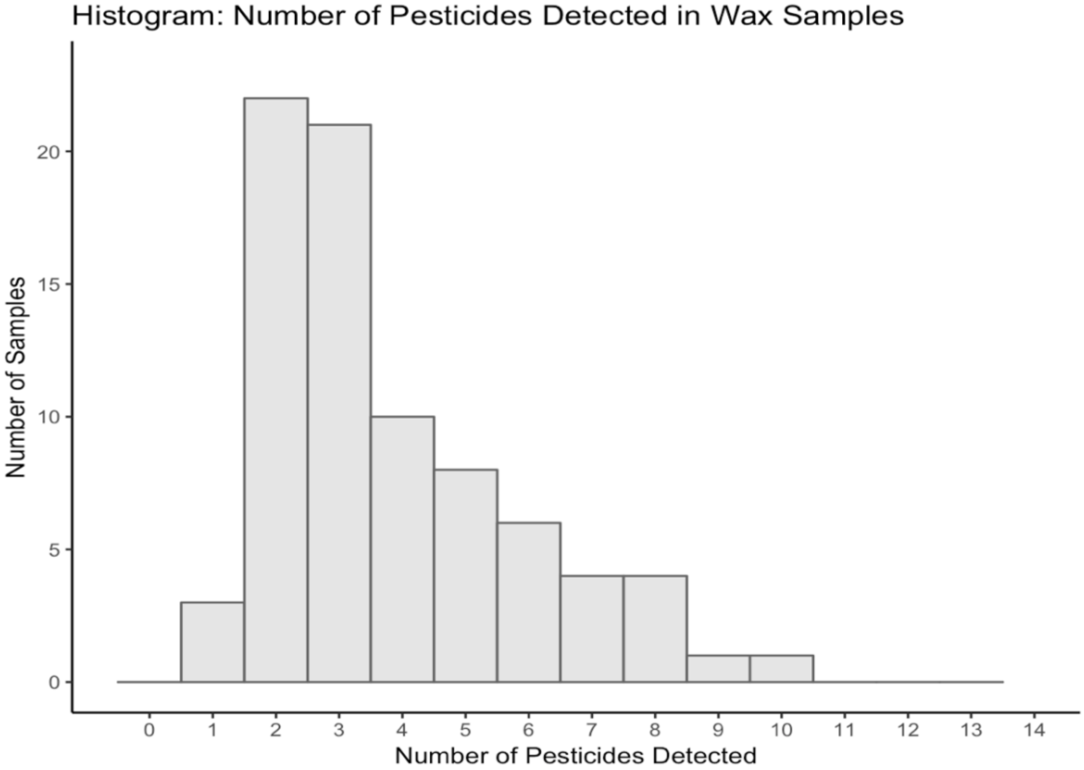


Figure 8. Histograms showing the number of pesticides detected in pollen samples (above, out of 79 total samples) and wax samples (below, out of 80 total samples).

Comparison of chemicals found in wax and pollen. Of the 58 compounds detected, 32 were detected in both wax and pollen, seven were detected only in wax and 19 were detected only in pollen.

Pesticides were found in 96% of pollen samples. Only three contained no pesticides, two of which came from the same apiary (located in the Berkshires). Insecticides and fungicides were the most commonly detected compound types, with insecticides detected most frequently during the early sampling period (June/July), and fungicides detected most frequently in the later sampling period (Aug/Sept). Herbicides were also more frequently detected in pollen than in wax, especially during the earlier sampling period. See **Figures 9 and 10** for a breakdown of detections by chemical class.

All wax samples tested positive for at least one pesticide. Beekeeper-applied miticides and the synergist PBO were the most commonly detected compound types (**Figures 9 and 10**). PBO and the miticide coumaphos were nearly ubiquitous (in 81% and 94% of wax samples, respectively). The next most frequently detected were Fenproximate (which is a beekeeper-applied miticide and farmer-applied insecticide) (41%), the fungicide Azoxystrobin (16%), and the insecticides Imidacloprid (16%), Fipronil sulfone (16%) and Fipronil (16%) (**Table 1**).

Concentration. Overall, the chemicals found in the highest concentrations were fungicides. Of the 12 chemicals found in pollen at an average concentration > 10 ppb, nine were fungicides and three were insecticides. When looking at the top ten chemicals with the highest single concentrations detected, seven were fungicides, two were insecticides, and one was a miticide (**Figure 11**).

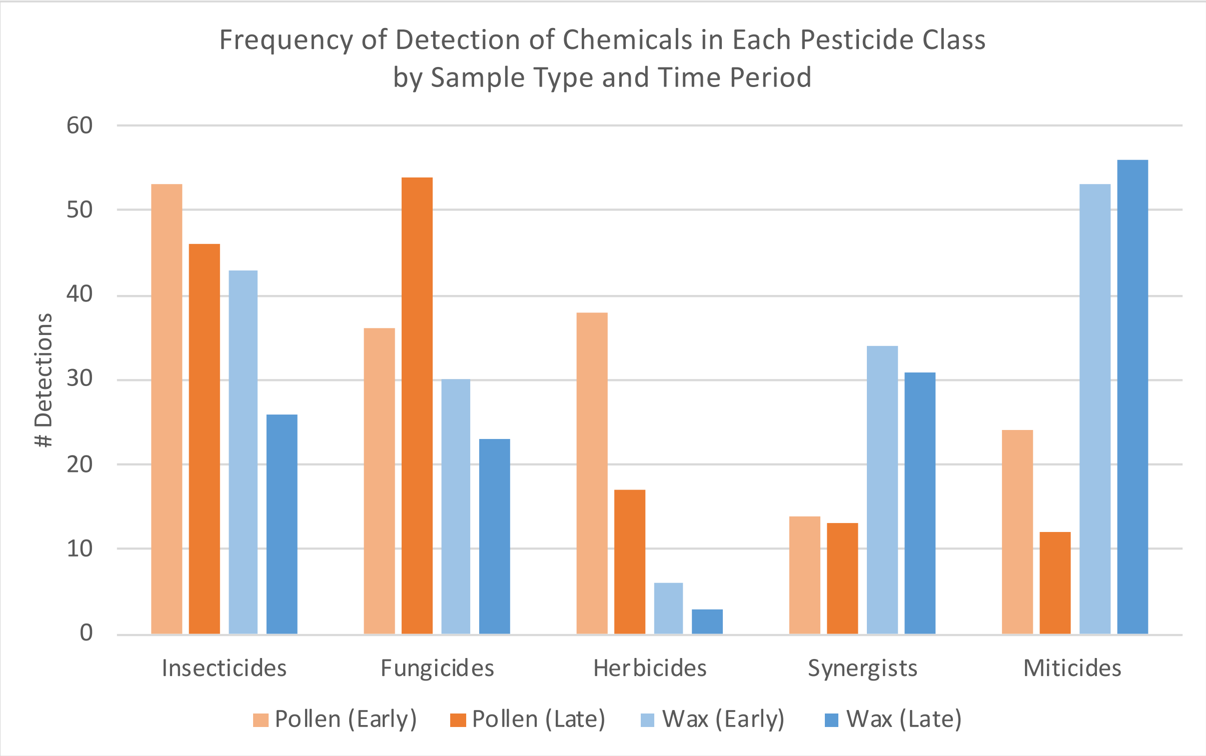


Figure 9. Frequency of detections of chemicals in each pesticide class, by sample type (wax vs. pollen) and sampling period. There were 611 positive pesticide detections across all 159 samples. The number of positive detections exceeds the number of samples because many samples contained more than one pesticide.

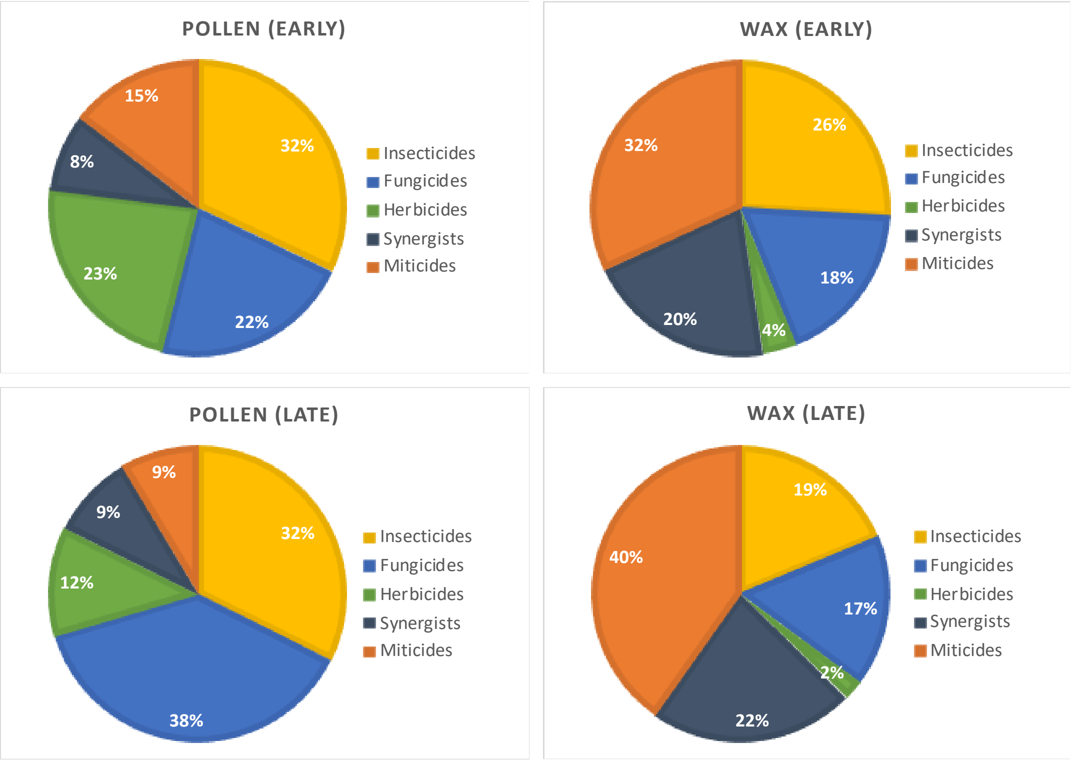


Figure 10. Distribution of positive detections by pesticide class, across sample types (pollen vs. wax) and sampling dates.

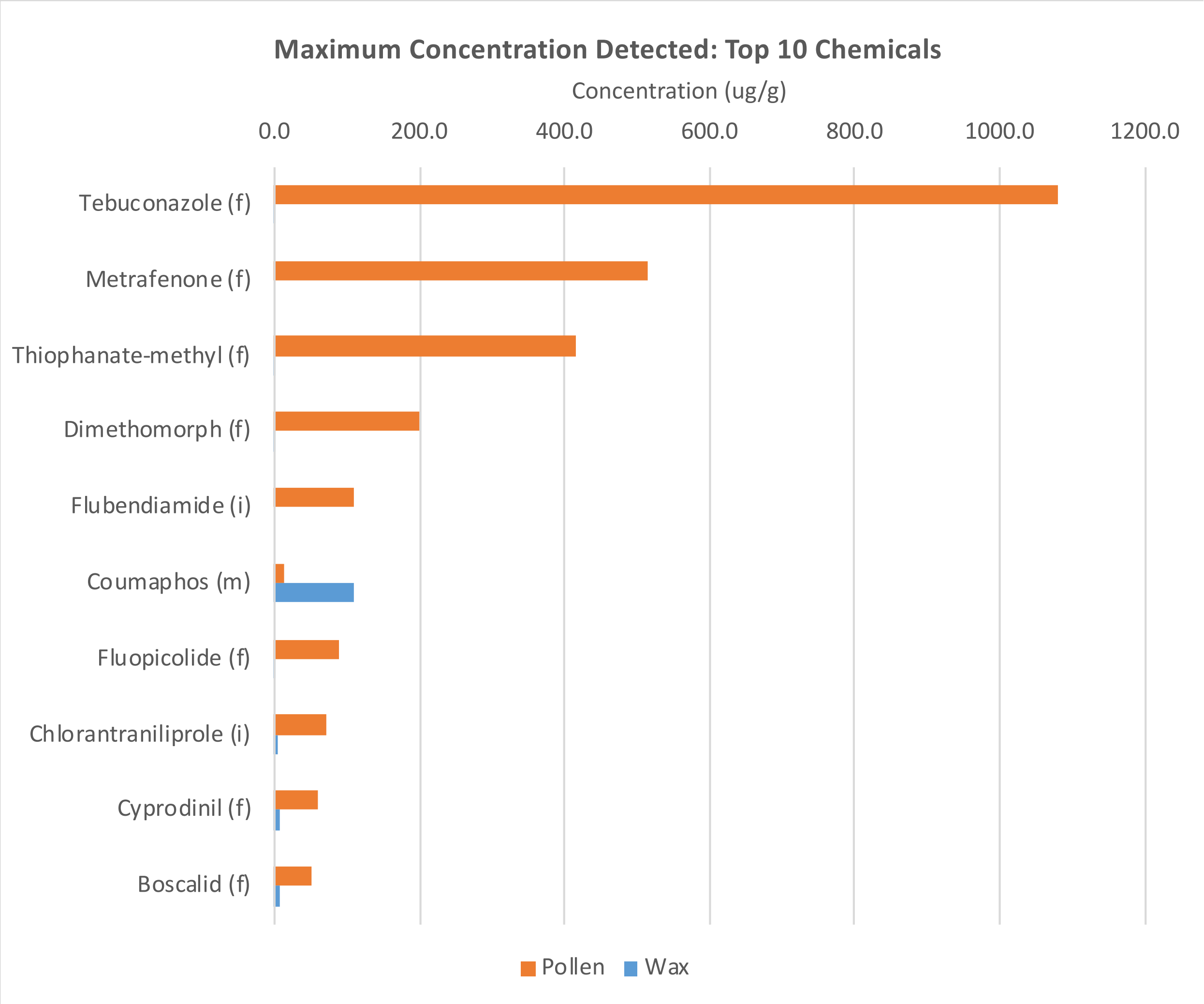


Figure 11. List of the pesticides detected in the top maximum concentrations. f = fungicide, i = insecticide, m = miticide

Hazard Quotient. Seven pesticides were detected in concentrations above the EPA level of concern (HQ > 0.4) using the contact LD_{50} or oral LD_{50}: spinosyn A, etofenprox, fipronil, fipronil sulfone, chlorantraniliprole, imidacloprid and dinotefuran. All of these compounds are insecticides with contact and/or oral LD_{50} values in the highly toxic range (< 2). Two (imidacloprid and dinotefuran) are neonicotinoids of particular concern to beekeepers. The compounds detected above the LOC with the most frequency were fipronil, fipronil sulfone and imidacloprid, and the ones detected in the highest concentrations (well above the LD_{50}) were fipronil and chlorantraniliprole. Overall, these concerning detections occurred more often in pollen than wax. See **Figure 12** for a breakdown of high-risk detections by sampling type (wax/pollen) and timing (early/late). See **Table 2** for a full list of all detections above the LOC by sample type, timing and county. See **Figure 13** for a summary of the mean HQ values for the top 20 most frequently detected chemicals.

In terms of *total* hazard quotient (the sum of all hazard quotients for pesticides detected per sample), 13.8% of wax samples (11 total) and 34% of pollen samples (27 total) had a THQ > 0.4 for oral toxicity. For contact toxicity, 3.8% of wax samples (3 total) and 7.5% of pollen samples (6 total) had a THQ > 0.4.

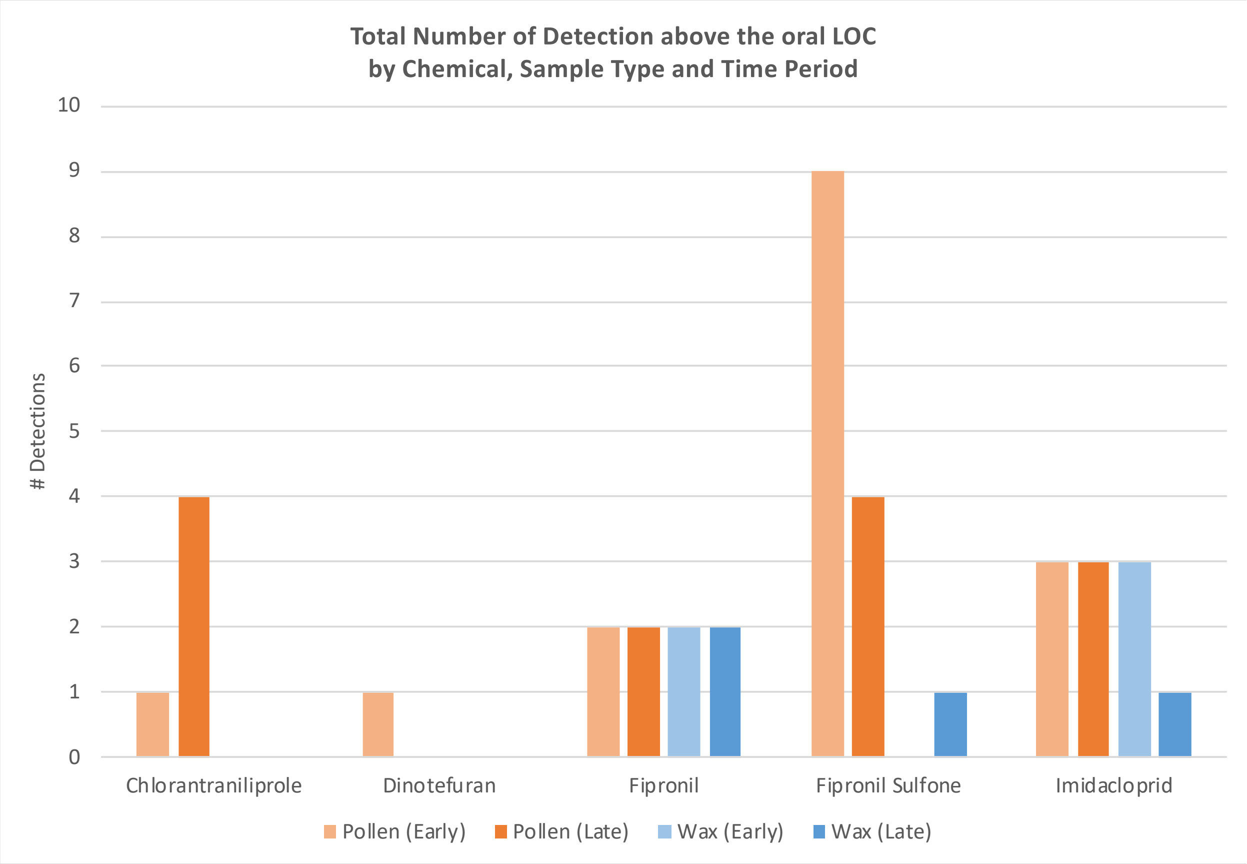


Figure 12. Pesticide detections above the EPA Level of Concern (LOC) for oral exposure, broken down by sample type (wax/pollen) and timing (early/late). Note that most of these detections occur in pollen.

Table 1. Table summarizing pesticides in concentrations above the EPA level of concern (LOC) (i.e., >40% of the LD$_{50}$). We report the subset of instances where HQ > 1 (i.e., concentration > LD$_{50}$) in parentheses as an indication of extremely high detections. Results are presented for contact risk (above) and oral risk (below). Each table is further broken down by sample type and sample time (early/late), with summed totals at the bottom.

*HQ = Concentration(ug/g)/LD50 (ug/g)

CONTACT LD$_{50}$:

Compound	LD$_{50}$	# detections where HQ > 0.4 (# where HQ >1)*	County location of detections where HQ > 0.4 (location of detections where HQ > 1)
Pollen ---			
Early Round			
Spinosyn A	0.003	1 (0)	Suffolk
Fipronil	0.006	2 (0)	Essex, Worcester
Late Round			
Etofenprox	0.015	1 (0)	Bristol
Fipronil	0.006	1 (0)	Berkshire
Wax ---			
Late Round			
Fipronil	0.006	1 (1)	Hampshire (Hampshire)
Fipronil Sulfone	0.006	1 (0)	Hampden
Total --			
Spinosyn A	0.003	1 (0)	Suffolk
Fipronil	0.006	4 (1)	Essex, Worcester, Berkshire, Hampshire (Hampshire)
Fipronil Sulfone	0.006	1 (0)	Hampden
Etofenprox	0.015	1 (0)	Bristol

ORAL LD$_{50}$:

Compound	LD$_{50}$	# detections where HQ > 0.4 (# where HQ >1)*	County location of detections where HQ > 0.4 (location of detections where HQ > 1)
Pollen ---			
Early Round			
Dinotefuran	0.022	1 (0)	Essex
Imidacloprid	0.004	3 (0)	Middlesex, Bristol, Norfolk
Chlorantraniliprole	0.0274	1 (0)	Worcester
Fipronil	0.001	2 (2)	Essex, Worcester (Essex, Worcester)
Fipronil Sulfone	0.001	9 (0)	Hampden [2], Suffolk, Barnstable, Essex, Bristol, Middlesex, Berkshire, Worcester
Late Round			
Imidacloprid	0.004	3 (1)	Worcester, Essex, Bristol (Bristol)
Chlorantraniliprole	0.0274	4 (2)	Plymouth, Worcester, Hampden, Norfolk (Hampden, Norfolk)
Fipronil	0.001	2 (2)	Berkshire, Hampshire (Berkshire, Hampshire)
Fipronil Sulfone	0.001	4 (0)	Barnstable, Essex, Franklin, Worcester
Wax ---			
Round 1			
Imidacloprid	0.004	3 (1)	Bristol [2], Worcester (Bristol [1])
Fipronil	0.001	2 (1)	Franklin, Brisol (Bristol)
Round 2			
Imidacloprid	0.004	1 (0)	Worcester
Fipronil	0.001	2 (1)	Hampden, Hampshire (Hampshire)
Fipronil Sulfone	0.001	1 (1)	Hampden (Hampden)
Total --			
Dinotefuran	0.022	1 (0)	Essex
Imidacloprid	0.004	10 (2)	Middlesex, Bristol [4], Norfolk, Worcester [3], Essex, (Bristol [2])
Chlorantraniliprole	0.0274	5 (2)	Worcester [2], Plymouth, Hampden, Norfolk (Hampden, Norfolk)
Fipronil	0.001	8 (6)	Franklin, Hampden, Bristol, Berkshire, Hampshire [2], Essex, Worcester (Bristol, Berkshire, Hampshire [2], Essex, Worcester)
Fipronil Sulfone	0.001	14 (1)	Hampden [3], Suffolk, Barnstable [2], Essex [2], Bristol, Middlesex, Berkshire, Worcester [2], Franklin (Hampden [1])

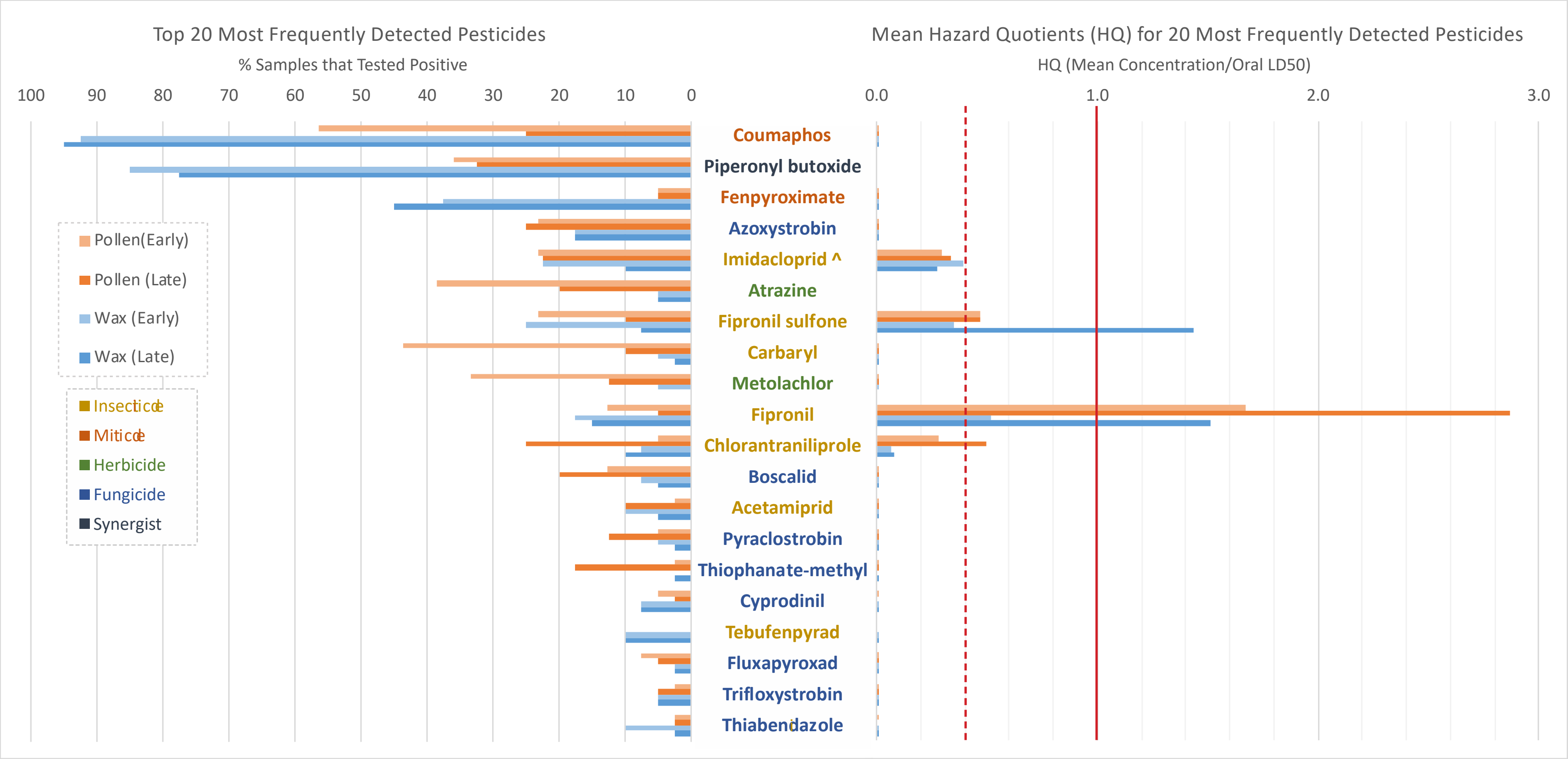


Figure 13. Left: Top 20 most frequently detected pesticides across all samples. **Right:** Pesticide hazard quotient for 20 most frequently detected pesticides. The EPA level of concern (0.4) is indicated with the dashed red line. The concentration level equal to the LD50 (HQ = 1.0) is indicated with the solid red line. Mean HQ = Mean Concentration (ug/g))/Contact LD50 (ug/g). ^ Indicates neonicotinoids.

Geographic Trends. Figure 14 shows all pesticide detections with individual *oral* HQ values over the level of concern (0.4). (We also mapped high *contact* HQ values, but there were too few data points to look for meaningful patterns). Sample type is indicated by shape, and pesticide type is indicated by color. Note that there are no striking trends, except that high-risk imidacloprid detections were more common in the east, high-risk fipronil detections were more common in the west, and high-risk chlorantraniliprole detections were more common towards the south.

For a summary of pesticide detections by county see **Table 3**. For a list of all pesticide detections by county, see **Appendix C**.

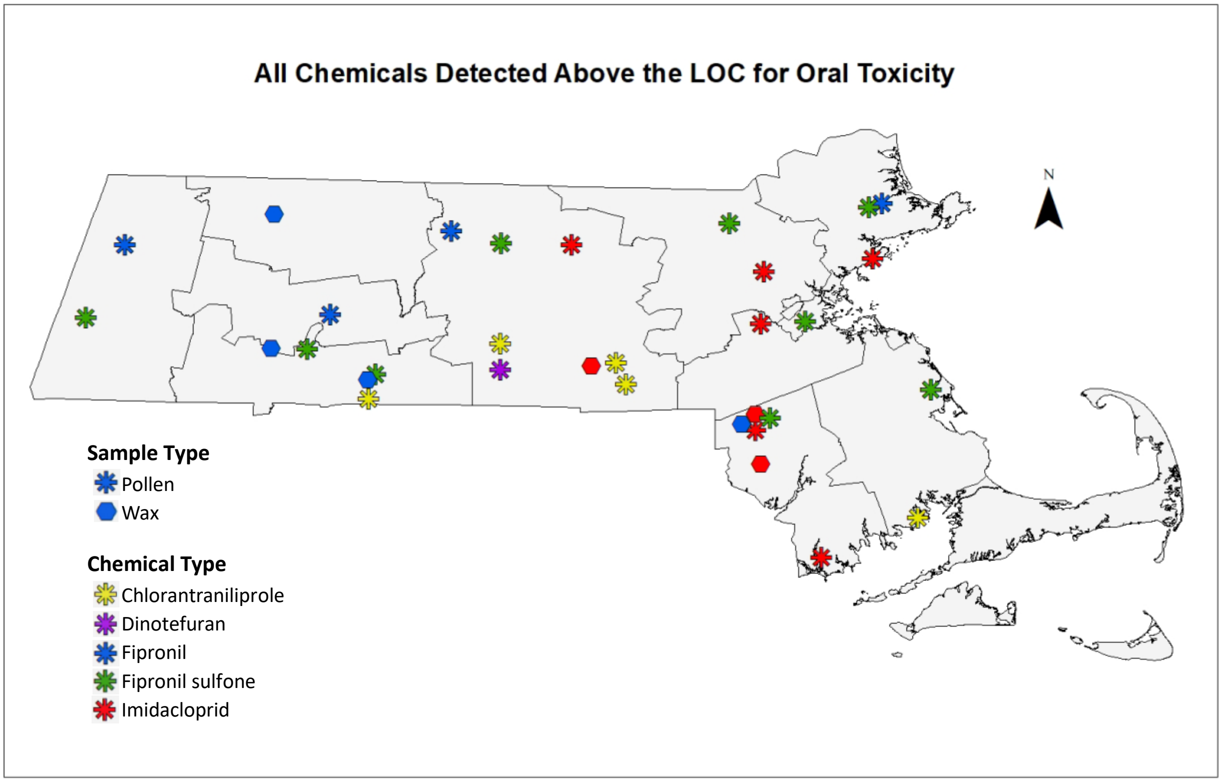


Figure 14. Location of samples with individual pesticides detected in concentrations above the EPA LOC (HQ > 0.4) for oral toxicity. Shape indicates wax or pollen; color indicates pesticide type.

Figure 15 shows the location of samples with a *total* oral hazard quotient greater than the EPA level of concern (0.4). This total hazard quotient is the sum of the HQ values for all chemicals detected in a sample. There were no striking geographic trends in THQ.

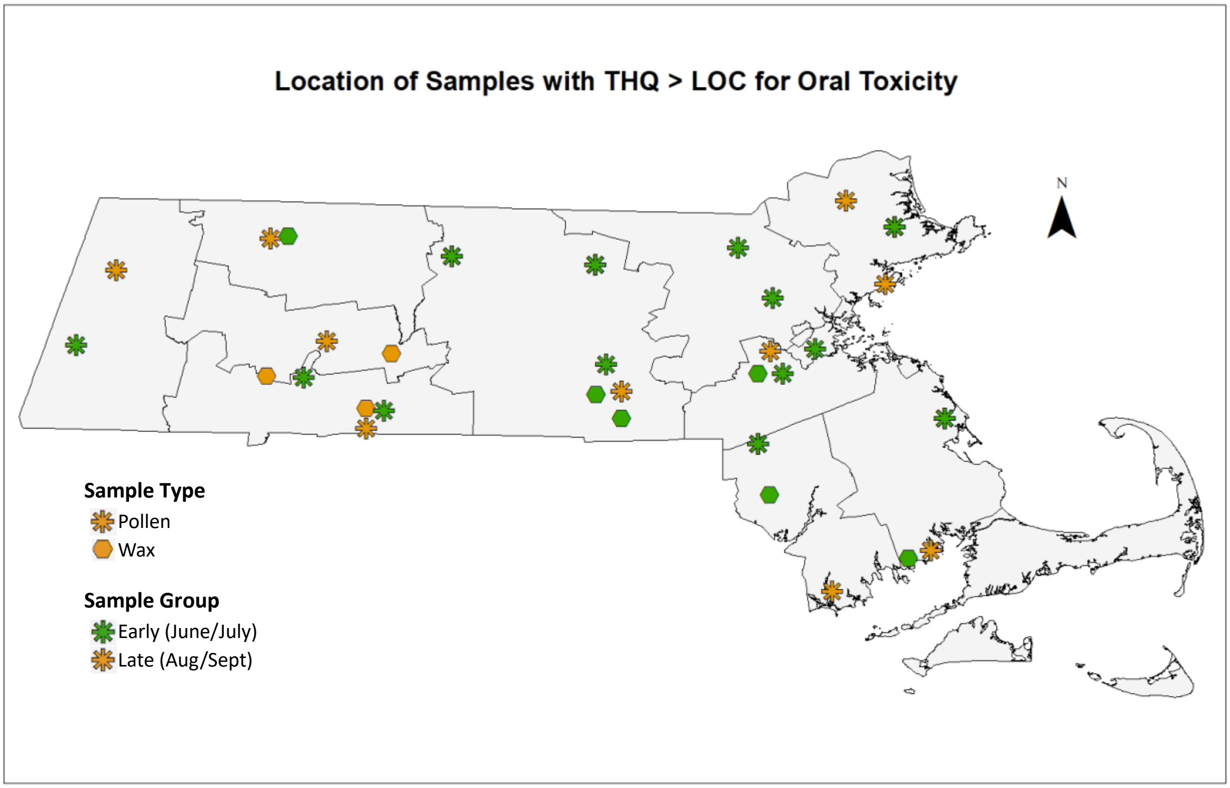


Figure 15. Location of samples with a total hazard quotient above the EPA LOC (THQ > 0.4) for oral toxicity. Shape indicates wax or pollen; color indicates sample period (early or late).

Neonicotinoids. 52 samples tested positive for at least one neonicotinoid insecticide (33% of all samples). Overall, 27 out of 40 apiaries had at least one neonicotinoid detected in wax or pollen (68%). Five out of the six neonicotinoids tested were found in at least one sample: imidacloprid (31), acetamiprid (11), clothianidin (5), thiamethoxam (4) and dinotefuran (1). Thiacloprid was not found in any samples. Imidacloprid, the most common neonicotinoid, was detected more frequently in pollen than in wax. See **Figure 16** for a breakdown of the most commonly detected neonicotinoids by sample type. See **Table 3** for a breakdown of neonicotinoid detection by county.

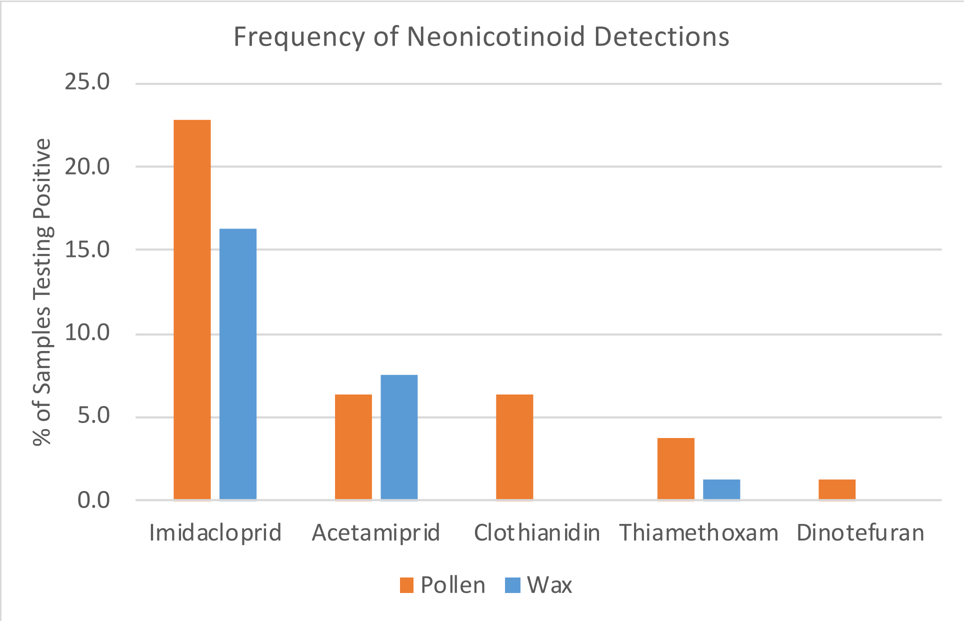


Figure 16. Frequency of neonicotinoid detection by sample type.

County	# Apiaries	Total # Pesticides Detected (all pesticides)	Mean # Pesticides per Sample	Neonicotinoid Frequency (% samples positive)									
				Imidacloprid		**Clothianidin**		**Acetamiprid**		**Thiamethoxam**		**Dinotefuran**	
				pollen	*wax*	*pollen*	*wax*	*pollen*	*wax*	*pollen*	*wax*	*pollen*	*wax*
Berkshire	3	19	3.0	0%	17%	0%	0%	0%	17%	0%	0%	0%	0%
Bristol	3	22	4.9	67%	67%	0%	0%	17%	0%	0%	0%	0%	0%
Essex	3	18	3.3	33%	33%	0%	0%	0%	0%	0%	0%	17%	0%
Hampden	6	33	4.1	25%	0%	8%	0%	17%	17%	8%	8%	0%	0%
Hampshire/Franklin	6	27	4.1	17%	8%	17%	0%	8%	0%	17%	0%	0%	0%
Middlesex	5	13	2.4	10%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Norfolk/Suffolk	3	22	3.8	67%	50%	17%	0%	0%	17%	0%	0%	0%	0%
Plymouth/Barnstable	4	16	4.1	0%	0%	13%	0%	0%	0%	0%	0%	0%	0%
Worcester	7	30	4.5	14%	14%	0%	0%	7%	14%	0%	0%	0%	0%

Table 2. Pesticide distribution by county, including neonicotinoid detection frequency. On the left are the total number of pesticides detected in that county, and the mean number of pesticides per sample (including all pesticides, not just neonicotinoids). The right-hand side of the table summarizes the frequency of detections for all neonicotinoids found.

Correlation Analysis. We first assessed relationships *among* pesticide detections in pollen and wax (across both sampling periods). The presence of coumaphos and PBO were positively correlated in pollen and wax and across sampling dates – meaning that hives with coumaphos-positive wax often had coumaphos-positive pollen, and hives with early coumaphos detections often had late coumaphos detections (**Figure 17**).

We then assessed correlations between pesticides and diseases within each sampling period. For the early sampling period (June/July), we again saw positive correlations between coumaphos and PBO in pollen and wax. We also saw a slight negative correlation between coumaphos in wax and *Varroa* mite levels. There was a positive relationship between fipronil in the wax and DWV, and imidacloprid in wax and *Nosema* and *Varroa* (**Figure 18**).

For the second sampling period (Aug/Sept), we again saw positive correlations between coumaphos and PBO. We also saw a positive relationship between coumaphos in wax and VDV, coumaphos in pollen and DWV, and imidacloprid in pollen and VDV (**Figure 19**).

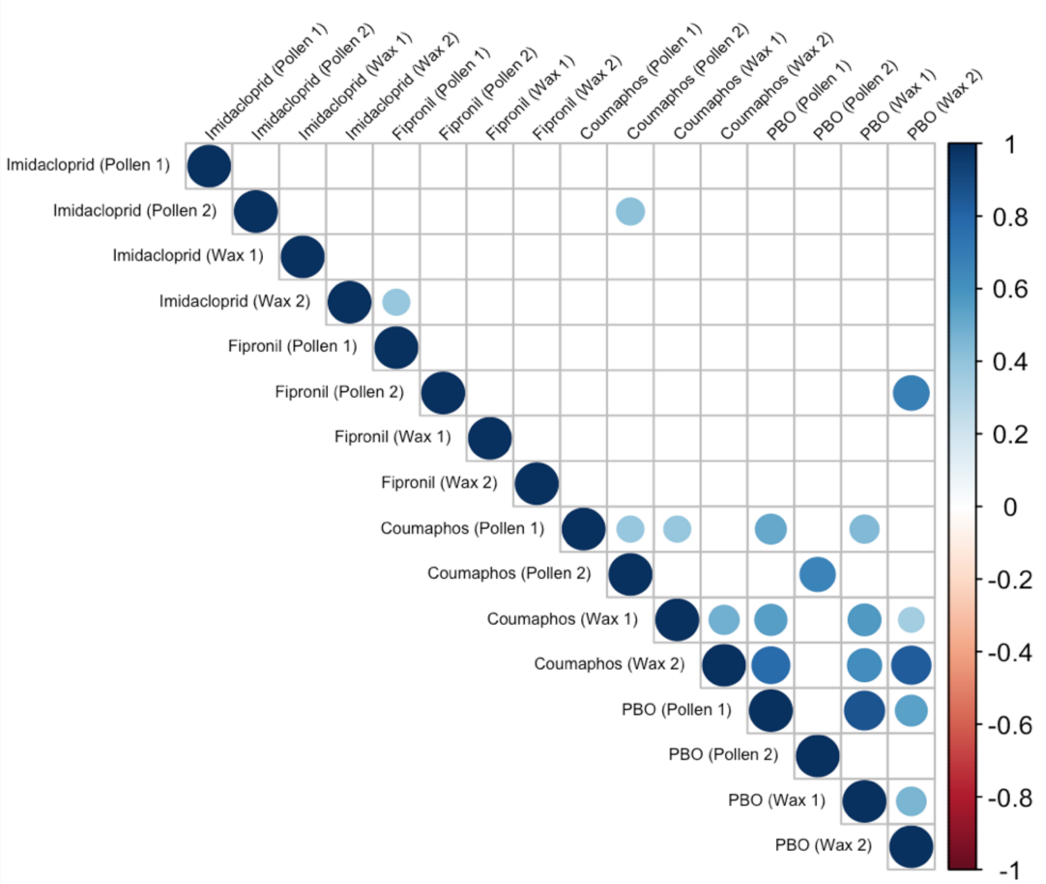


Figure 17. Correlation table showing relationships among pesticides for both sampling periods. Wax/Pollen 1 indicates samples from the early sampling round; Wax/Pollen 2 indicates samples from the late sampling round. Only significant correlations (P < 0.05) are shown. Positive correlations are represented by blue circles; negative correlations are represented by red circles. The size of the circle and the intensity of the color correspond with the strength of the correlation (larger, darker circles denote stronger relationships).

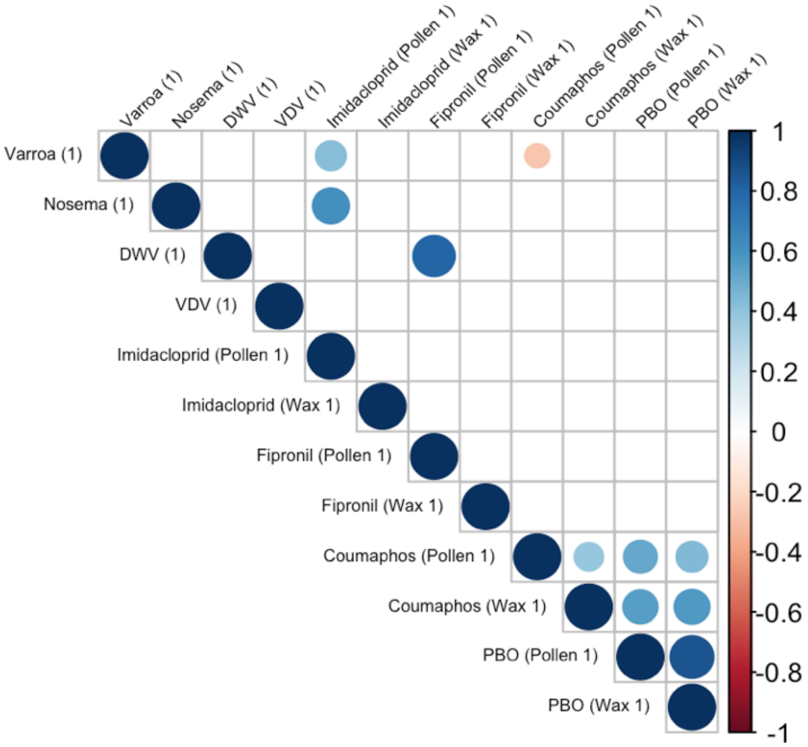


Figure 18. Correlation table showing relationships between pesticides and disease for the June/July sampling period.

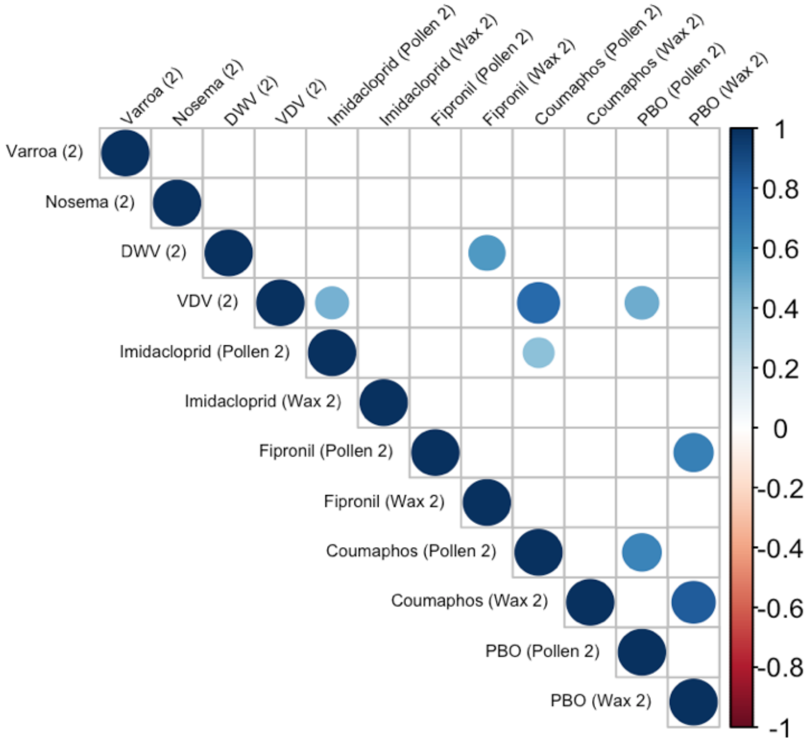


Figure 19. Correlation table showing relationships between pesticides and disease for the Aug/Sept sampling period.

4. DISCUSSION

There are several important take-aways from this study:

1. A number of samples had a total hazard quotient above the EPA level of concern: 34% of pollen samples and 13.8% of wax samples had total hazard quotients (sum of hazard quotients for individual pesticides) above the EPA level of concern for acute oral toxicity; and 7.5% and 3.8% respectively, for acute contact toxicity. This differs from the results of an analogous NY State study (Mullen et al. 2016), where researchers tested wax samples from around the state and found that all total hazard quotients were well below the EPA threshold for contact toxicity. Interestingly, Mullen et al. 2016 found more pesticides per sample on average than we did (5.5 compared to 3.81).

It is important to note that this analysis assumes pesticides interact additively. However, research suggests that certain compounds interact synergistically, so that their cumulative harm is greater than the sum of individual toxicity. Some fungicides, for instance, can dramatically increase the toxicity of pyrethroid and neonicotinoid insecticides (Pilling and Jepson 1993, Schmuck Stadler and Schmidt 2003). Future analysis could explore the potential synergies in this data set.

2. The most concerning pesticides were imidacloprid, fipronil and fipronil sulfone. These compounds were detected with high frequency, and often in concentrations above the level of concern. Chlorantraniliprole and dinotefuran were also detected above the level of concern, though not as frequently. High imidacloprid detections were more common on the eastern side of the state, and high fipronil concentrations were more common on the western side, but further analysis is needed to determine if these trends are significant, and whether there is any correlation with land use.

3. About a third of samples tested positive for neonicotinoids, and imidacloprid was by far the most commonly detected neonicotinoid. The frequency of neonicotinoid detection in our study was lower than a previous Massachusetts study (Lu et al. 2016) which gathered pollen from 62 hives in 10 counties and found neonicotinoids in 73% of samples. (In that study, imidacloprid was also the most common, detected in 57% of samples). However, we found neonicotinoids far more frequently than studies from other states: a 2018 study out of Virginia detected imidacloprid in 5.95% of beebread samples (Gooley et al. 2018), and a 2018 study in Maine did not detect any neonicotinoids in pollen samples (Drummond et al. 2018).

4. Coumaphos and piperonyl butoxide were ubiquitous in wax samples, and common in pollen samples. This is relatively consistent with the Mullen et al. 2016, who found PBO in 99% of wax samples, and coumaphos in 51%. Similarly, in a national study, Mullin et al. (2010) found coumaphos in nearly all wax samples, and the vast majority of beebread samples. Stoner and Eitzer (2013) found coumaphos in nearly half of Connecticut pollen samples, even though none of the participating beekeepers had used coumaphos in several years.

Coumaphos (tradename: Checkmite+) is a miticide that was once commonly used but is now rarely applied because of mite resistance. However, it is highly persistent in wax. The fact that several studies have detected coumaphos in pollen as well as wax suggests that it may be volatilizing in hives and redepositing on pollen.

PBO is used to magnify the toxicity of insecticides, most commonly pyrethrins, pyrethroids and carbamates. It works by interfering with insects' ability to produce enzymes that detoxify pesticides, so that the insecticides remain in the insects' body for longer (Mullen 2016). It has a very low toxicity for honey bees.

5. Fungicides were detected in the highest concentrations in pollen but pose a relatively low toxicity risk. This is consistent with other studies. McArt et al. (2017) found that fungicides accounted for 94% of total pesticide residues in beebread from apple-pollinating hives, but insecticides accounted for 98% of toxicity risk.

6. We found a consistent relationship between the presence of coumaphos and PBO in both wax and pollen. There were inconsistent relationships between pesticide residues and disease. Coumaphos and PBO were positively correlated in pollen or wax across both sampling dates. This supports the hypothesis that coumaphos enters pollen from nearby wax. But why are coumaphos (an out-of-date beekeeper-applied miticide) and PBO (a farmer-applied pesticide synergist) correlated? It's possible that we're detecting the difference between older vs. newer wax samples: perhaps older samples were simply more exposed to both chemicals. In future research, it would be better to record whether wax samples are from new or old comb, or to only collect comb of similar age.

Our analysis found some inconsistent relationships between pesticides and disease; in the first but not the second round of sampling, fipronil was correlated with higher DWV levels, and imidacloprid was correlated with higher *Nosema* and *Varroa* levels. In the second but not the first round, coumaphos and imidacloprid were both associated with elevated virus levels. Since these results were so inconsistent, they are hardly conclusive. Instead they suggest nuanced relationships between pesticides and disease that could be explored with further analysis.

Study limitations. Finally, it is important to point out several caveats about this study. First, the two sampling periods were fairly close together. In future research, it would be better separate the sampling into two tighter clusters (though this is logistically more difficult and would require an earlier start-date). Second, we intentionally collected both new and old wax to get a diverse sample, but in future it would be better to collect only old or new wax, so that wax-age is not a confounding factor in the pesticide analysis. Third, it would be more effective to collect wax only once (rather than twice), since wax composition captures longer-term pesticide exposure (rather than pollen, which captures a time-sensitive snapshot). Fourth, we tested *Varroa* in both the field and the lab. These numbers did not always match up, and it is possible that some mites were lost during field testing. Next time, it would be better take two separate samples: one for the field and one for the lab. Finally, it would be helpful to formally collect information on mite management as part of the survey, since it could contextualize mite levels.

In the future, it would be interesting to explore potential synergies among pesticides in this data, dig deeper into correlations between pesticides and diseases, and assess spatial relationships between pesticides and land-use.

References

"Bee Informed Partnership - APHIS Survey State Reports." Accessed September, 2019. https://bip2.beeinformed.org/state_reports/.

Decourtye, Axel, and James Devillers. 2010. "Ecotoxicity of Neonicotinoid Insecticides to Bees." In *Insect Nicotinic Acetylcholine Receptors*, edited by Steeve Hervé Thany, 85–95. Advances in Experimental Medicine and Biology. New York, NY: Springer New York. https://doi.org/10.1007/978-1-4419-6445-8_8.

Drummond, Francis A., Elissa S. Ballman, Brian D. Eitzer, Brianne Du Clos, and James Dill. 2018. "Exposure of Honey Bee (*Apis mellifera* L.) Colonies to Pesticides in Pollen, A Statewide Assessment in Maine." *Environmental Entomology* 47 (2): 378–87. https://doi.org/10.1093/ee/nvy023.

"ECOTOX | Home." n.d. Accessed September 27, 2019. https://cfpub.epa.gov/ecotox/.

Gooley, Zuyi C., Aaron C. Gooley, and Richard D. Fell. 2018. "Relationship of Landscape Type on Neonicotinoid Insecticide Exposure Risks to Honey Bee Colonies: A Statewide Survey." *Journal of Economic Entomology* 111 (6): 2505–12. https://doi.org/10.1093/jee/toy284.

Lu, Chensheng (Alex), Chi-Hsuan Chang, Lin Tao, and Mei Chen. 2015. "Distributions of Neonicotinoid Insecticides in the Commonwealth of Massachusetts: A Temporal and Spatial Variation Analysis for Pollen and Honey Samples." *Environmental Chemistry* 13 (1): 4–11. https://doi.org/10.1071/EN15064.

McArt, Scott H., Ashley A. Fersch, Nelson J. Milano, Lauren L. Truitt, and Katalin Böröczky. 2017. "High Pesticide Risk to Honey Bees despite Low Focal Crop Pollen Collection during Pollination of a Mass Blooming Crop." *Scientific Reports* 7 (April): 46554. https://doi.org/10.1038/srep46554.

Mullen, Emma, Mary Kate Wheeler, Scott McArt, and Paul Cappy. 2017. "2016 NYS Beekeeper Tech Team Report: Pesticide Residues." Cornell University, NYS Beekeper Tech Team.

Mullin, Christopher A., Maryann Frazier, James L. Frazier, Sara Ashcraft, Roger Simonds, Dennis vanEngelsdorp, and Jeffery S. Pettis. 2010. "High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health." *PLOS ONE* 5 (3): e9754. https://doi.org/10.1371/journal.pone.0009754.

National Pesticide Information Center. http://npic.orst.edu. Oregon State University and US EPA. (Accessed September 2019)

Ostiguy, Nancy, Frank A. Drummond, Kate Aronstein, Brian Eitzer, James D. Ellis, Marla Spivak, and Walter S. Sheppard. 2019. "Honey Bee Exposure to Pesticides: A Four-Year Nationwide Study." *Insects* 10 (1). https://doi.org/10.3390/insects10010013.

Pilling, Edward D., and Paul C. Jepson. 1993. "Synergism between EBI Fungicides and a Pyrethroid Insecticide in the Honeybee (Apis Mellifera)." *Pesticide Science* 39 (4): 293–97. https://doi.org/10.1002/ps.2780390407.

Sanchez-Bayo, Francisco, and Koichi Goka. 2014. "Pesticide Residues and Bees – A Risk Assessment." *PLOS ONE* 9 (4): e94482. https://doi.org/10.1371/journal.pone.0094482.

Schmuck, Richard, Teodoro Stadler, and Hans-Werner Schmidt. 2003. "Field Relevance of a Synergistic Effect Observed in the Laboratory between an EBI Fungicide and a Chloronicotinyl Insecticide in the Honeybee (*Apis mellifera* L, Hymenoptera)." *Pest Management Science* 59 (3): 279–86. https://doi.org/10.1002/ps.626.

Spivak, Marla, Eric Mader, Mace Vaughan, and Ned H. Euliss. 2011. "The Plight of the Bees." *Environmental Science & Technology* 45 (1): 34–38. https://doi.org/10.1021/es101468w.

Stoner, Kimberly A., and Brian D. Eitzer. 2013. "Using a Hazard Quotient to Evaluate Pesticide Residues Detected in Pollen Trapped from Honey Bees (*Apis mellifera*) in Connecticut." *PLOS ONE* 8 (10): e77550. https://doi.org/10.1371/journal.pone.0077550.

Suchail, S., D. Guez, and L. P. Belzunces. 2001. "Discrepancy between Acute and Chronic Toxicity Induced by Imidacloprid and Its Metabolites in *Apis mellifera*." *Environmental Toxicology and Chemistry* 20 (11): 2482–86.

Sun, Dali, Li Li, Hongwu Liang, Wei Li, Ran Ji, Yijun Wu, and Chenglan Liu. 2012. "The Dissipation of Ethofenprox in Cabbage and Soil under Open Conditions." *Environmental Monitoring and Assessment* 184 (9): 5743–47. https://doi.org/10.1007/s10661-011-2377-z.

USEPA. 2004. "Pesticide Fact Sheet: Dinotefuran". Office of Prevention, Pesticides and Toxic Substances (7501C). Accessed October 2019. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-044312_01-Sep-04.pdf

USEPA. 2008. "Pesticide Fact Sheet: Chlorantraniliprole". Office of Prevention, Pesticides and Toxic Substances (7505P). Accessed October 2019. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-090100_01-Apr-08.pdf

USEPA. 2012. "White Paper in Support of the Proposed Risk Assessment Process for Bees." Office of Chemical Safety and Pollution Prevention Office of Pesticide Programs Environmental Fate and Effects Division, Environmental Protection Agency, Washington DC; Environmental Assessment Directorate, Pest Management Regulatory Agency, Health

Canada, Ottawa, CN; California Department of Pesticide Regulation. https://www.regulations.gov/document?D=EPA-HQ-OPP-2012-0543-0004.

USEPA. 2012*a*. Ecological Effects Test Guidelines OCSPP 850.3030 Honey Bee Toxicity of Residues on Foliage. EPA 712-C-018. January 2012.

USEPA 2014. "Guidance for Assessing Pesticide Risks to Bees." Office of Chemical Safety and Pollution Prevention Office of Pesticide Programs Environmental Fate and Effects Division, Environmental Protection Agency, Washington DC; Environmental Assessment Directorate, Pest Management Regulatory Agency, Health Canada, Ottawa, CN; California Department of Pesticide Regulation. https://www.epa.gov/sites/production/files/2014-06/documents/pollinator_risk_assessment_guidance_06_19_14.pdf.

Zhu, Yu Cheng, Jianxiu Yao, John Adamczyk, and Randall Luttrell. 2017. "Feeding Toxicity and Impact of Imidacloprid Formulation and Mixtures with Six Representative Pesticides at Residue Concentrations on Honey Bee Physiology (Apis Mellifera)." *PLoS ONE* 12 (6). https://doi.org/10.1371/journal.pone.0178421.

Appendix A. Full list of compounds tested, including level of detection (LOD) for wax and pollen (LOD-w and LOD-p, respectively)

*^indicates neonicotinoids, *indicates beekeeper-applied miticides*

Full List of Compounds Tested (266 Total)							
Insecticides + Acaracides							
Pesticide Name	*Chemical Class*	*LOD-w*	*LOD-p*	*Name*	*Chemical Class*	*LOD-w*	*LOD-p*
3-Hydroxy-carbofuran	*Carbamate*	0.21	0.28	Formothion	*Organophosphate*	2.63	3.50
Acephate	*Organophosphate*	8.75	11.67	Fosthiazate	*Organophosphate*	0.07	0.09
^Acetamiprid	*Neonicotinoid*	0.04	0.05	Furathiocarb	*Carbamate*	0.09	0.12
Aldicarb sulfone	*Carbamate*	1.75	2.33	Halofenozide	*Diacylhydrazine*	0.18	0.23
Aminocarb	*Carbamate*	0.18	0.23	Heptenophos	*Organophosphate*	0.21	0.28
*Amitraz	*Amidine*	0.04	0.05	Hexaflumuron	*Benzoylurea*	0.70	0.93
Aramite	*Alkylbenzene*	0.18	0.23	Hexythiazox	*Hexythiazox*	0.35	0.47
Avermectin B1a	*Avermectin*	1.75	2.33	^Imidacloprid	*Neonicotinoid*	0.18	0.23
Azamethiphos	*Organophosphate*	0.09	0.12	Indoxacarb	*Oxadiazine*	1.05	1.40
Azinphos-methyl	*Organophosphate*	0.88	1.17	Isocarbophos	*Organophosphate*	0.18	0.23
Bendiocarb	*Carbamate*	0.18	0.23	Isoprocarb	*Carbamate*	0.11	0.14
Benzoximate	*Organochlorine*	0.18	0.23	Malaoxon	*Organophosphate*	0.09	0.12
Bifenazate	*Carboxylic ester*	0.14	0.19	Methamidophos	*Organophosphate*	0.53	0.70
Buprofezin		0.04	0.05	Methiocarb	*Carbamate*	0.07	0.09
Butocarboxim sulfoxide	*Carbamate*	1.23	1.63	Methiocarb-sulfone	*Carbamate*	0.53	0.70
Butoxycarboxim	*Carbamate*	1.23	1.63	Methiocarb-sulfoxide	*Carbamate*	0.04	0.05
Carbaryl	*Carbamate*	0.07	0.09	Methomyl	*Carbamate*	0.18	0.23
Carbofuran	*Carbamate*	0.04	0.05	Methoxyfenozide	*Diacylhydrazine*	0.14	0.19
Chlorantraniliprole	*Anthranilic diamide*	0.88	1.17	Metolcarb	*Carbamate*	0.14	0.19
Chlorfenvinphos	*Organophosphate*	0.18	0.23	Mevinphos	*Organophosphate*	0.35	0.47
Chlorfluazuron	*Benzoylurea*	0.88	1.17	Mexacarbate	*Carbamate*	0.04	0.05
Chlorpyrifos	*Organophosphate*	0.35	0.47	Monocrotophos	*Organophosphate*	0.35	0.47
^Clothianidin	*Neonicotinoid*	0.18	0.23	Omethoate	*Organophosphate*	0.53	0.70
*Coumaphos	*Organophosphate*	0.18	0.23	Oxamyl	*Carbamate*	0.21	0.28
Crotoxyphos	*Organophosphate*	0.09	0.12	Phenthoate	*Organophosphate*	0.09	0.12
Cyromazine	*Cyromazine*	0.88	1.17	Phoxim	*Organophosphate*	0.14	0.19
Demeton-S-methylsulfone	*Organophosphate*	0.18	0.23	Pirimicarb	*Carbamate*	0.03	0.04
Desmethyl-pirimicarb	*Carbamate*	0.18	0.23	Pirimiphos-methyl	*Organophosphate*	0.07	0.09
Dicrotophos	*Organophosphate*	0.70	0.93	Profenophos	*Organophosphate*	0.11	0.14
Diethofencarb	*Carbamate*	0.18	0.23	Promecarb	*Carbamate*	0.18	0.23
Diflubenzuron	*Benzoylurea*	0.88	1.17	Propetamphos	*Organophosphate*	1.05	1.40
Dimethoate	*Organophosphate*	0.14	0.19	Propoxur	*Carbamate*	0.04	0.05
^Dinotefuran	*Neonicotinoid*	0.53	0.70	Pymetrozine	*Azomethine*	0.53	0.70
Ethiofencarb	*Carbamate*	0.09	0.12	Resmethrin	*Pyrethroid*	0.35	0.47
Ethiofencarb-sulfone	*Carbamate*	0.70	0.93	Rotenone	*Rotenone*	0.70	0.93
Ethiofencarb-sulfoxide	*Carbamate*	0.09	0.12	Schradan	*Organophosphate*	0.04	0.05
Ethiprole	*Phenylpyrazole*	0.53	0.70	Spinosyn A	*Spinosyn*	0.18	0.23
Etofenprox	*Pyrethroid*	0.09	0.12	Spiromesifen	*Tetronic acid derivative*	0.53	0.70
Etoxazole	*Organofluorine*	0.04	0.05	Spirotetramat	*Tetramic acid derivative*	0.18	0.23
Etrimfos	*Organophosphate*	0.18	0.23	Sulfotep	*Organophosphate*	0.11	0.14
Fenamiphos	*Organophosphate*	0.05	0.07	Sulprofos	*Organophosphate*	0.88	1.17
Fenazaquin	*Quinazoline*	0.09	0.12	Tebufenozide	*Diacylhydrazine*	0.05	0.07
Fenobucarb	*Carbamate*	1.40	1.87	Tebufenpyrad	*Pyrazole*	0.14	0.19
Fenoxycarb	*Carbamate*	5.25	7.00	Teflubenzuron	*Benzoylurea*	0.88	1.17
*Fenpyroximate	*Pyrazole*	0.07	0.09	Tetramethrin	*Pyrethroid*	0.53	0.70
Fensulfothion	*Organophosphate*	0.18	0.23	^Thiacloprid	*Neonicotinoid*	0.04	0.05
Fenthion-sulfoxide	*Organophosphate*	0.11	0.14	^Thiamethoxam	*Neonicotinoid*	0.14	0.19
Fipronil	*Phenylpyrazoles*	0.18	0.23	Tolfenpyrad	*Pyrazole*	0.18	0.23
Fipronil sulfone	*Phenylpyrazoles*	0.35	0.47	Triazophos	*Organophosphate*	0.04	0.05
Flubendiamide	*Anthranilic diamide*	0.53	0.70	Trichlorfon	*Organophosphate*	0.53	0.70
Flufenoxuron	*Benzoylurea*	0.53	0.70	Vamidothion	*Organophosphate*	0.04	0.05
Formetanate hydrochloride	*Carbamate*	0.09	0.12				

Herbicides							
Name	*Chemical Class*	*LOD-w*	*LOD-p*	*Name*	*Chemical Class*	*LOD-w*	*LOD-p*
2,4-D	*Phenoxy-carboxylic acid*	5.25	7.00	Methoprotryne	*Triazine*	0.04	0.05
Alachlor	*Chloroacetamide*	0.70	0.93	Metobromuron	*Urea*	0.18	0.23
Anilofos	*Organophosphate*	0.53	0.70	Metolachlor	*Chloroacetamide*	0.11	0.14
Atrazine	*Triazine*	0.09	0.12	Metosulam	*Triazolopyrimidines*	0.18	0.23
Bentazon	*Benzothiadiazinone*	0.53	0.70	Metoxuron	*Urea*	0.18	0.23
Benzoylprop-ethyl	*Benzamide*	0.18	0.23	Monolinuron	*Urea*	0.18	0.23
Bromacil	*Uracil*	0.18	0.23	Napropamide	*Acetamide*	0.04	0.05
Bromoxynil	*Nitrile*	0.88	1.17	Neburon	*Urea*	0.05	0.07
Butachlor	*Chloroacetamide*	0.26	0.35	Phenmedipham	*Phenyl-carbamate*	3.50	4.67
Butafenacil	*Pyrimidinedione*	0.09	0.12	Piperophos	*Organothiophosphate*	0.04	0.05
Carbetamide	*Carbanilate*	0.14	0.19	Prometon	*Triazine*	0.04	0.05
Carfentrazone-ethyl	*Triazolinone*	0.88	1.17	Prometryn	*Triazine*	0.04	0.05
Chloridazon	*Pyridazinone*	0.18	0.23	Propyzamide	*Benzamide*	0.70	0.93
Chlorotoluron	*Urea*	0.18	0.23	Prosulfocarb	*Thiocarbamate*	0.05	0.07
Chloroxuron	*Urea*	0.21	0.28	Pyroxsulam	*Triazolopyrimidines*	0.00	0.00
Clethodim	*Cyclohexanedione*	0.35	0.47	Quizalofop-P	*Aryloxyphenoxypropionate*	0.88	1.17
Clomazone	*Oxazolidinone*	0.05	0.07	Quizalofop-P-ethyl	*Aryloxyphenoxypropionate*	0.04	0.05
Cumyluron	*Urea*	0.07	0.09	Sethoxydim	*Cyclohexanedione*	0.14	0.19
Cyanazine	*Triazine*	0.26	0.35	Simetryn	*Triazine*	0.07	0.09
Cycloate	*Thiocarbamate*	0.70	0.93	Tebuthiuron	*Urea*	0.02	0.02
Cycluron	*Urea*	0.26	0.35	Tepraloxydim	*Cyclohexanedione*	0.88	1.17
Desmedipham	*Phenyl-carbamate*	3.50	4.67	Terbumeton	*Triazine*	0.04	0.05
Desmetryn	*Triazine*	0.04	0.05	Terbuthylazine	*Triazine*	0.02	0.02
Dimefuron	*Urea*	0.35	0.47	Terbutryn	*Triazine*	0.14	0.19
Dimethametryn	*Triazine*	0.02	0.02	Thidiazuron	*Urea*	0.88	1.17
Dimethenamid	*Chloroacetamide*	0.04	0.05	Thiobencarb	*Thiocarbamate*	0.11	0.14
Dithiopyr	*Pyridine*	1.23	1.63	Tralkoxydim	*Cyclohexanedione*	0.18	0.23
Diuron	*Urea*	0.18	0.23	Trietazine	*Triazine*	0.18	0.23
DNOC	*Dinitrophenols*	1.05	1.40				
Esprocarb	*Thiocarbamate*	0.05	0.07				
Ethofumesate	*Benzofurane*	0.88	1.17				
Fenuron	*Urea*	0.18	0.23				
Florasulam	*Triazolopyrimidines*	0.88	1.17				
Fluazifop	*Aryloxyphenoxypropionates*	0.88	1.17				
Flufenacet	*Oxyacetamide*	0.09	0.12				
Flumetsulam	*Triazolopyrimidines*	0.35	0.47				
Fluometuron	*Urea*	0.18	0.23				
Fluorochloridone	*Pyrrolidine*	1.40	1.87				
Haloxyfop	*Aryloxyphenoxypropionate*	0.88	1.17				
Haloxyfop-methyl	*Aryloxyphenoxypropionate*	0.07	0.09				
Hexazinone	*Triazine*	0.04	0.05				
Imazaquin	*Imidazolinone*	0.14	0.19				
Imazethapyr	*Imidazolinone*	0.14	0.19				
Ioxynil	*Nitrile*	0.35	0.47				
Isoproturon	*Urea*	0.18	0.23				
Isoxaben	*Benzamide*	0.04	0.05				
Lenacil	*Uracil*	0.05	0.07				
MCPA	*Phenoxy-carboxylic acid*	1.75	2.33				
Mefenacet	*Oxyacetamide*	0.04	0.05				
Metamitron	*Triazinone*	0.53	0.70				
Metazachlor	*Chloroacetamide*	0.04	0.05				
Methabenzthiazuron	*Urea*	0.02	0.02				

Fungicides							
Name	*Chemical Class*	*LOD-w*	*LOD-p*	*Name*	*Chemical Class*	*LOD-w*	*LOD-p*
Azaconazole	*Triazole*	0.18	0.23	Prochloraz	*Imidazole*	0.11	0.14
Azoxystrobin	*Methoxy-acrylate*	0.03	0.04	Propamocarb	*Carbamate*	0.14	0.19
Benodanil	*Phenyl-benzamide*	0.09	0.12	Propiconazole	*Triazole*	0.53	0.70
Boscalid	*Pyridine-carboxamide*	0.18	0.23	Pyraclostrobin	*Methoxy-carbamate*	0.04	0.05
Bromuconazole	*Triazole*	1.05	1.40	Pyrimethanil	*Anilino-pyrimidine*	0.18	0.23
Bupirimate	*Hydroxy-(2-amino-)pyrimidine*	0.11	0.14	Quinoxyfen	*Aryloxyquinoline*	0.11	0.14
Carpropamid	*Cyclopropane-carboxamide*	0.18	0.23	Simeconazole	*Triazole*	0.18	0.23
Cyazofamid	*Cyano-imidazole*	0.26	0.35	Spiroxamine	*Morpholine*	0.04	0.05
Cyflufenamid	*Phenyl-acetamide*	0.07	0.09	Tebuconazole	*Triazole*	0.35	0.47
Cyprodinil	*Anilino-pyrimidine*	0.18	0.23	Tetraconazole	*Triazole*	0.18	0.23
Diclobutrazol	*Triazole*	0.53	0.70	Thiabendazole	*Benzamidazole*	0.05	0.07
Difenoconazole	*Triazole*	0.18	0.23	Thiophanate-methyl	*Thiophanate*	0.53	0.70
Dimethomorph	*Cinnamic acid amide*	0.18	0.23	Triadimefon	*Triazole*	0.18	0.23
Dimoxystrobin	*Oximino-acetamide*	0.04	0.05	Triadimenol	*Triazole*	0.18	0.23
Diniconazole	*Triazole*	0.88	1.17	Tricyclazole	*Triazolobenzothiazole*	0.09	0.12
Dodemorph	*Morpholine*	0.18	0.23	Tridemorph	*Morpholine*	0.70	0.93
Epoxiconazole	*Triazole*	0.18	0.23	Trifloxystrobin	*Oximino-acetate*	0.04	0.05
Etaconazole	*Triazole*	0.35	0.47	Triflumizole	*Imidazole*	0.04	0.05
Ethirimol	*Pyrimidine*	0.14	0.19	Zoxamide	*Toluamide*	0.07	0.09
Fenamidone	*Imidazolinone*	0.18	0.23				
Fenarimol	*Pyrimidine*	0.88	1.17				
Fenbuconazole	*Triazole*	0.35	0.47				
Fenhexamid	*Hydroxyanilide*	0.88	1.17				
Fenoxanil	*Propionamide*	0.14	0.19				
Fluazinam	*2,6-dinitro-aniline*	0.18	0.23				
Fluopicolide	*Pyridinylmethyl-benzamide*	0.09	0.12				
Fluopyram	*Pyridinyl-ethylbenzamide*	0.05	0.07				
Fluoxastrobin	*Dihydro-dioxazine*	0.07	0.09				
Fluquinoconazole	*Triazole*	1.75	2.33				
Flusilazole	*Triazole*	0.11	0.14				
Flutriafol	*Triazole*	0.35	0.47				
Fluxapyroxad	*Pyrazole-carboxamide*	0.07	0.09				
Fuberidazole	*Benzimidazole*	0.04	0.05				
Griseofulvin	*Benzofuran*	0.18	0.23				
Hexaconazole	*Triazole*	0.35	0.47				
Imazalil	*Imidazole*	0.18	0.23				
Imibenconazole	*Triazole*	0.35	0.47				
Iprovalicarb	*Valinamide carbamate*	0.18	0.23				
Isoprothiolane	*Dithiolane*	0.04	0.05				
Kresoxim-methyl	*Oximino-acetate*	0.14	0.19				
Mandipropamid	*Mandelic acid amide*	0.18	0.23				
Mepronil	*Phenyl-benzamide*	0.04	0.05				
Metconazole	*Triazole*	1.75	2.33				
Metrafenone	*Benzophenone*	0.18	0.23				
Myclobutanil	*Triazole*	0.35	0.47				
Nuarimol	*Pyrimidine*	0.53	0.70				
Ofurace	*Butyrolactone*	0.11	0.14				
Oxadixyl	*Oxazolidinone*	0.09	0.12				
Penconazole	*Triazole*	0.18	0.23				
Pencycuron	*Phenylurea*	0.09	0.12				
Penthiopyrad	*Pyrazole-carboxamide*	0.04	0.05				
Picoxystrobin	*Methoxy-acrylate*	0.05	0.07				

Plant Growth Regulators

Name	*Chemical Class*	*LOD-w*	*LOD-p*
Ancymidol	*Pyrimidine*	0.53	0.70
Chlormequat	*Quaternary ammonium*	0.35	0.47
Forchlorfenuron	*Urea*	0.14	0.19
Mepiquat	*Quaternary ammonium*	0.11	0.14
Paclobutrazol	*Triazole*	0.35	0.47

Herbicide Safeners

Name	*Chemical Class*	*LOD-w*	*LOD-p*
Benoxacor	*Benzoxazine*	0.88	1.17
Isoxadifen-ethyl	*Isoxazoline*	0.11	0.14

Food Preservatives

Name	*Chemical Class*	*LOD-w*	*LOD-p*
Ethoxyquin	*Quinoline*	0.53	0.70

Rodenticides

Name	*Chemical Class*	*LOD-w*	*LOD-p*
Brodifacoum	*4-Hydroxycoumarin*	0.53	0.70
Difenacoum	*4-Hydroxycoumarin*	0.11	0.14

Synergists

Name	*Chemical Class*	*LOD-w*	*LOD-p*
Piperonyl butoxide	*Benzodioxole*	0.04	0.05

Appendix B. Description of the top 10 most commonly detected pesticides, and all pesticides detected > LOC. *text from Appendix 2 in Mullen et al. 2017.

Summary	Description
Coumaphos (miticide) - *Found in 93.8% of wax samples, and 40.5% of pollen samples*	*This insecticde/miticide was first registered with the EPA in 1958 as a treatement for livestock, primarily cattle. Coumaphos is the active ingredient in CheckMite+, which was approved in NYS in 1999 as a treatment for *Varroa* mites, and later for small hive beetles. Today, coumaphos is rarely used as a treatment for *Varroa* due to widespread resistance. It is highly persistent in wax, and does not break down when wax is melted. Studies show that coumaphos can harm the development and reproductive abilty of queens and drones.
Piperonyl butoxide (synergist) - *Found in 81.3% of wax samples, and 34.2% of pollen samples*	*As a pesticide synergist, piperonyl butoxide (PBO) has little or no direct effect on insects by itself. Rather, it is used in combination with insecticides to magnify their toxicity. It is most commonly used with pyrethrins, pyrethroids, and carbamates. PBO inhibits natural enzymes that insects produce in their bodies to detoxify other pesticides. Without these enzymes, insecticides remain in the insects' bodies for a longer period of time. Unlike adjuvants, pesticide synergists are included in a pesticide product's active ingredient label.
Fenproximate (insecticide/miticide) - *Found in 41.3% of wax samples and 5.1% of pollen samples*	*This insecticide is used to control spider mites and other plant-infesting mites, leafhoppers, mealybugs, whiteflies and psylla. In some areas outside of NYS, this pesticide is used to kill the *Varroa* mite. Fenproximate is applied to greenhouse vegetables, ornamental plants, nursery crops and non-bearing fruit trees to inhibit feeding and reproduction of target insects.
Azoxystrobin (fungicide) - *Found in 17.5% of wax samples and 24.1% of pollen samples*	*This broad spectrum fungicide is widely used to control many different fungal diseases in agriculture, especially in grain, vegetable and fruit crops. It is also used on commercial and residential turf, athletic fields and golf courses
Imidacloprid (insecticide) - *Found in 16.3% of wax samples and 22.8% of pollen samples*	Imidacloprid is the most widely used agricultural insecticide in the world (Zhu et al. 2017). It interferes with the invertebrate nervous system, and is used to control sucking and mining pests like beetles, fleas, aphids, stink bugs, termites, locusts and thrips. It is highly toxic to honey bees (Suchail et al. 2001)
Atrazine (herbicide) - *Found in 5% of wax samples and 29.1% of pollen samples*	*Atrazine is the second most widely used herbicide in the US, after glyphosate. It is commonly used to control boadleaf weeds in corn, and it is also approved for use on turf grass, including golf course, recreational fields, and residential and commercial lawns.
Fipronil sulfone (insecticide) - *Found in 16.3% of wax samples and 16.5% of pollen samples*	Fipronil (also Fipronil sulfide or Fipronil sulfone) is an insecticide that is used to control ants, beetles, cockroaches, fleas, ticks, termites, thips, rootworms and other insects. It is used in agriculture, as well as home and lawn car. It is highly toxic to honey bees (National Pesticide Information Center).
Fipronil (insecticide) - Found in 16.3% of wax samples and 8.9% of pollen samples	
Carbaryl (insecticide) - *Found in 3.8% of wax samples and 26.6% of pollen samples*	Carbaryl is used to control a wide variety of garden pests including fire ants, ticks and mosquitos, moths, beetles, cockroaches. It is also used on agricultural fields and rangeland. It is highly toxic to honey bees (National Pesticide Information Center).
Metolachlor (herbicide) - *Found in 2.5% of wax samples and 22.8% of pollen samples*	*Farmers and pesticide applicators commoly use this herbicide to control weeds in various cropping systems, including field corn and soy. Metolchlor inhibits protein synthesis in plants, and is highly effective against grasses.
Chlorantraniliprole (insecticide) - *Found in 8.8% of wax samples and 15.2% of pollen samples*	An insecticide used to control a broad array of pests, including moths, beetles and caterpillers. Used in agriculture, turf, residential and public landscaped areas (EPA Pesticide Fact Sheet).
Etofenprox (insecticide) - *Found in 1.3% of wax samples and 1.3% of pollen samples*	Etofenprox is a systemic pyrethroid insecticide that is used to control sucking insects (like leafhoppers aphids and thrips) on a variety of agricultural crops including fruit, vegetables, rice and cotton (Sun et al. 2011).
Spinosyn A (insecticide) - *Found in 1.3% of wax samples and 5.1% of pollen samples*	A bacteria-derived insecticide used to control a wide variety of invertebrates (National Pesticide Information Center).
Dinotefuran (insecticide) - *Found in 0% of wax samples and 1.3% of pollen samples*	A neonicotinoid insecticide used to control a wide variety of insects on leafy crops, turf, and residential/commercial lawns, including aphids, thrips, leafhoppers, leafminers and white grubs (EPA Pesticide Fact Sheet).

Appendix C. Compounds detected in each county. Counties with only one participating apiary (Franklin, Suffolk, Barnstable) were combined with neighboring counties. The mean concentration refers to the mean among **positive samples**. Note that the total number of wax or pollen samples per county is the number of apiaries multiplied by two (for two sampling rounds). A slash (/) in the LD_{50} column indicates that the information was not available in Sanchez-Bayo & Goka (2014), the Tomlin Pesticide Manual, the ECOTOX database of the U.S. Environment Protection Agency (http://cfpub.epa.gov/ecotox/) or the AgriTox Database of the French government (http://www.agritox.anses.fr/index.php). Note that the mean concentration and LD_{50} have different units, so the mean oral HQ is calculated as: mean HQ = mean conc./1,000/LD_{50}. ^denotes neonicotinoids.

Berkshire County (3 apiaries x 2 sampling rounds)

Pesticide	Pesticide Class	Oral LD50 (ug/g)	Pollen			Wax		
			# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ	# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ
Acetamiprid ^	Insecticide	14	0			1	0.04	0.00
Azoxystrobin	Fungicide	25	2	0.04	0.00	0		
Boscalid	Fungicide	166	2	21.43	0.00	0		
Carbaryl	Insecticide	0.15	2	1.33	0.01	0		
Chlorantraniliprole	Insecticide	0.027	1	1.17	0.04	0		
Coumaphos	Miticide	4.6	0			4	2.27	0.00
Cyprodinil	Fungicide	100	1	2.31	0.00	0		
Difenoconazole	Fungicide	177	1	0.76	0.00	0		
Fenbuconazole	Fungicide	/	1	10.18		0		
Fenpyroximate	Miticide	118.5	0			4	0.42	0.00
Fipronil	Insecticide	0.001	1	4.31	4.31	0		
Fipronil sulfone	Insecticide	0.001	1	0.47	0.47	0		
Imidacloprid ^	Insecticide	0.004	0			1	0.18	0.04
Metolachlor	Herbicide	110	2	0.14	0.00	0		
Metrafenone	Fungicide	114	1	0.79	0.00	0		
Piperonyl butoxide	Synergist	/	2	1.11		6	1.27	
Pyraclostrobin	Fungicide	73	1	3.91	0.00	0		
Tebuconazole	Fungicide	83	1	1080.69	0.01	0		
Trifloxystrobin	Fungicide	200	0			1	0.25	0.00

Bristol County (3 apiaries x 2 sampling rounds)

Pesticide	Pesticide Class	Oral LD50 (ug/g)	Pollen			Wax		
			# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ	# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ
Acetamiprid ^	Insecticide	14	1	3.90	0.00	0		
Atrazine	Herbicide	/	1	0.67		0		
Azoxystrobin	Fungicide	25	5	0.12	0.00	2	0.14	0.00
Boscalid	Fungicide	166	1	0.23	0.00	1	0.75	0.00
Carbaryl	Insecticide	0.15	3	0.50	0.00	0		
Coumaphos	Miticide	4.6	3	5.28	0.00	6	2.83	0.00
Etofenprox	Insecticide	0.024	1	8.14	0.34	0		
Fenamidone	Fungicide	159	1	1.04	0.00	0		
Fenbuconazole	Fungicide	/	1	11.49		0		
Fenhexamid	Fungicide	1.7	0			1	4.15	0.00
Fenpyroximate	Miticide	118.5	0			2	0.25	0.00
Fipronil	Insecticide	0.001	0			2	1.19	1.19
Fipronil sulfone	Insecticide	0.001	1	0.47	0.47	1	0.35	0.35
Flubendiamide	Insecticide	200	1	109.35	0.00	0		
Fluopicolide	Fungicide	241	0			1	0.43	0.00
Imidacloprid ^	Insecticide	0.004	4	1.99	0.50	4	2.46	0.61
Methomyl	Insecticide	0.24	2	5.24	0.02	0		
Metolachlor	Herbicide	110	1	1.13	0.00	1	0.11	0.00
Metosulam	Herbicide	/	0			1	0.18	
Piperonyl butoxide	Synergist	/	2	1.75		6	1.45	
Prometryn	Herbicide	/	0			1	0.04	
Quinoxyfen	Fungicide	316	1	4.54	0.00	1	0.43	0.00

Essex County (3 apiaries x 2 sampling rounds)

Pesticide	Pesticide Class	Oral LD50 (ug/g)	Pollen			Wax		
			# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ	# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ
Atrazine	Herbicide	/	1	0.12		0		
Azoxystrobin	Fungicide	25	0			1	0.09	0.00
Carbaryl	Insecticide	0.15	2	0.27	0.00	0		
Chlorantraniliprole	Insecticide	0.0274	1	3.60	0.13	0		
Chlorpyrifos	Insecticide	0.051	1	0.47	0.01	0		
Coumaphos	Miticide	4.6	4	1.85	0.00	6	0.90	0.00
Cyazofamid	Fungicide	/	1	1.36		0		
Dimethomorph	Fungicide	32	1	198.67	0.01	0		
Dinotefuran ^	Insecticide	0.022	1	10.38	0.47	0		
Fenpyroximate	Miticide	118.5	0			1	0.42	0.00
Fipronil	Insecticide	0.001	1	4.37	4.37	1	0.18	0.18
Fipronil sulfone	Insecticide	0.001	2	0.47	0.47	0		
Fluopicolide	Fungicide	241	1	88.34	0.00	0		
Imidacloprid ^	Insecticide	0.004	2	1.48	0.37	2	0.35	0.09
Metolachlor	Herbicide	110	1	0.76	0.00	0		
Metrafenone	Fungicide	114	1	513.07	0.00	0		
Napropamide	Herbicide	113.5	1	1.56	0.00	0		
Piperonyl butoxide	Synergist	/	1	0.70		6	0.38	

Hampden County (6 apiaries x 2 sampling rounds)

Pesticide	**Pesticide Class**	**Oral LD50 (ug/g)**	**Pollen**			**Wax**		
			# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ	# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ
Acetamiprid ^	Insecticide	14	2	3.94	0.00	2	0.07	0.00
Amitraz	Miticide	/	0			1	0.04	
Atrazine	Herbicide	/	6	0.41		0		
Azoxystrobin	Fungicide	25	4	0.04	0.00	2	0.30	0.00
Boscalid	Fungicide	166	3	11.37	0.00	0		
Carbaryl	Insecticide	0.15	2	0.41	0.00	0		
Chlorantraniliprole	Insecticide	0.0274	1	34.36	1.25	1	0.88	0.03
Chlorpyrifos	Insecticide	0.051	1	2.06	0.04	0		
Clomazone	Herbicide	85.3	1	0.07	0.00	0		
Clothianidin ^	Insecticide	0.004	1	0.23	0.06	0		
Coumaphos	Miticide	4.6	3	0.23	0.00	12	3.85	0.00
Cyprodinil	Fungicide	100	1	1.68	0.00	2	0.63	0.00
Difenoconazole	Fungicide	177	1	1.36	0.00	0		
Dimethomorph	Fungicide	32	0			1	0.18	0.00
Dithiopyr	Herbicide	/	3	3.81		0		
Fenpyroximate	Miticide	118.5	0			5	0.31	0.00
Fipronil	Insecticide	0.001	0			1	0.77	0.77
Fipronil sulfone	Insecticide	0.001	2	0.47	0.47	2	1.98	1.98
Fluopicolide	Fungicide	241	1	2.52	0.00	0		
Fluopyram	Fungicide	102.3	1	2.21	0.00	1	0.17	0.00
Fluxapyroxad	Fungicide	110.9	1	0.34	0.00	0		
Imidacloprid ^	Insecticide	0.004	3	0.23	0.06	0		
Methiocarb-sulfoxide	Insecticide	0.47	1	0.17	0.00	0		
Metolachlor	Herbicide	110	7	0.91	0.00	0		
Metrafenone	Fungicide	114	1	0.72	0.00	0		
Penthiopyrad	Fungicide	385	1	0.05	0.00	0		
Piperonyl butoxide	Synergist	/	2	0.98		7	1.64	
Pyraclostrobin	Fungicide	73	2	1.42	0.00	0		
Pyrimethanil	Fungicide	100	2	2.20	0.00	0		
Tebufenpyrad	Insecticide	1.8	0			3	0.51	0.00
Thiamethoxam ^	Insecticide	0.005	1	0.19	0.04	1	0.14	0.03
Triadimenol	Fungicide	/	1	0.23		0		
Trifloxystrobin	Fungicide	200	1	0.26	0.00	1	0.38	0.00

Hampshire + Franklin Counties (6 apiaries x 2 sampling rounds)

Pesticide	**Pesticide Class**	**Oral LD50 (ug/g)**	**Pollen**			**Wax**		
			# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ	# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ
Acetamiprid ^	Insecticide	14	1	0.94	0.00	0		
Atrazine	Herbicide	/	4	1.02		4	0.19	
Azoxystrobin	Fungicide	25	2	0.11	0.00	3	0.08	0.00
Boscalid	Fungicide	166	1	13.24	0.00	0		
Carbaryl	Insecticide	0.15	1	5.59	0.04	2	0.65	0.00
Clomazone	Herbicide	85.3	3	0.19	0.00	0		
Clothianidin ^	Insecticide	0.004	2	0.64	0.16	0		
Coumaphos	Miticide	4.6	4	3.04	0.00	11	25.97	0.01
Cyazofamid	Fungicide	/	1	1.40		0		
Difenoconazole	Fungicide	177	1	0.23	0.00	0		
Fenbuconazole	Fungicide	/	1	2.19		0		
Fenpyroximate	Miticide	118.5	2	2.73	0.00	6	9.69	0.00
Fipronil	Insecticide	0.001	2	0.83	0.83	3	2.78	2.78
Fipronil sulfone	Insecticide	0.001	1	0.47	0.47	3	0.35	0.35
Fluopyram	Fungicide	102.3	1	2.09	0.00	0		
Imidacloprid ^	Insecticide	0.004	2	0.48	0.12	1	0.18	0.04
Methoxyfenozide	Insecticide	100	0			1	0.47	0.00
Metolachlor	Herbicide	110	5	2.03	0.00	1	0.11	0.00
Penthiopyrad	Fungicide	385	0			2	0.09	0.00
Piperonyl butoxide	Synergist	/	4	3.11		8	9.34	
Pyraclostrobin	Fungicide	73	1	3.44	0.00	1	0.29	0.00
Tebuconazole	Fungicide	83	0			1	0.35	0.00
Tebufenpyrad	Insecticide	1.8	0			3	2.25	0.00
Terbumeton	Herbicide	/	0			1	0.04	
Thiamethoxam ^	Insecticide	0.005	2	0.19	0.04	0		
Thiophanate-methyl	Fungicide	100	3	0.70	0.00	0		
Trifloxystrobin	Fungicide	200	2	25.05	0.00	2	0.15	0.00

Middlesex County (5 apiaries x 2 sampling rounds)

Pesticide	Pesticide Class	Oral LD50 (ug/g)	Pollen			Wax		
			# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ	# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ
Atrazine	Herbicide	/	2	0.12		0		
Boscalid	Fungicide	166	1	0.23	0.00	0		
Carbaryl	Insecticide	0.15	1	0.84	0.01	0		
Chlorantraniliprole	Insecticide	0.0274	1	3.78	0.14	0		
Coumaphos	Miticide	4.6	4	2.14	0.00	10	2.12	0.00
Fenpyroximate	Miticide	118.5	0			5	1.17	0.00
Fipronil	Insecticide	0.001	1	0.23	0.23	1	0.18	0.18
Fipronil sulfone	Insecticide	0.001	1	0.47	0.47	0		
Imidacloprid ^	Insecticide	0.004	1	3.62	0.91	0		
Metolachlor	Herbicide	110	1	0.14	0.00	0		
Piperonyl butoxide	Synergist	/	4	0.39		9	1.51	
Spinosyn A	Insecticide	0.057	1	1.00	0.02	1	0.79	0.01
Tebufenpyrad	Insecticide	1.8	0			1	0.51	0.00

Norfolk + Suffolk Counties (3 apiaries x 2 sampling rounds)

Pesticide	Pesticide Class	Oral LD50 (ug/g)	Pollen			Wax		
			# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ	# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ
Acetamiprid ^	Insecticide	14	0			1	0.11	0.00
Atrazine	Herbicide	/	2	0.41		0		
Azoxystrobin	Fungicide	25	2	0.13	0.00	0		
Boscalid	Fungicide	166	2	3.87	0.00	0		
Carbaryl	Insecticide	0.15	4	2.68	0.02	0		
Chlorantraniliprole	Insecticide	0.0274	1	71.35	2.60	0		
Clothianidin ^	Insecticide	0.004	1	0.23	0.06	0		
Coumaphos	Miticide	4.6	1	1.88	0.00	6	4.33	0.00
Cyflufenamid	Fungicide	100	1	0.45	0.00	0		
Fenpyroximate	Miticide	118.5	0			4	0.43	0.00
Fipronil	Insecticide	0.001	0			1	0.18	0.18
Fipronil sulfone	Insecticide	0.001	1	0.47	0.47	2	0.35	0.35
Fluxapyroxad	Fungicide	110.9	1	4.93	0.00	1	1.44	0.00
Imibenconazole	Fungicide	125	0			1	0.35	0.00
Imidacloprid ^	Insecticide	0.004	4	0.88	0.22	3	0.55	0.14
Neburon	Herbicide	/	1	3.20		0		
Piperonyl butoxide	Synergist	/	3	0.71		7	1.79	
Propiconazole	Fungicide	77	1	0.70	0.00	0		
Pyraclostrobin	Fungicide	73	1	1.75	0.00	0		
Spinosyn A	Insecticide	0.057	3	0.59	0.01	0		
Thiabendazole	Fungicide	34	1	0.07	0.00	3	1.14	0.00
Thiophanate-methyl	Fungicide	100	2	207.44	0.00	0		

Plymouth + Barnstable Counties (4 apiaries x 2 sampling rounds)

Pesticide	Pesticide Class	Oral LD50 (ug/g)	Pollen			Wax		
			# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ	# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ
Atrazine	Herbicide	/	2	0.12		0		
Azoxystrobin	Fungicide	25	2	0.70	0.00	5	0.68	0.00
Carbaryl	Insecticide	0.15	1	0.72	0.00	0		
Chlorantraniliprole	Insecticide	0.0274	1	13.92	0.51	4	1.93	0.07
Clothianidin ^	Insecticide	0.004	1	0.23	0.06	0		
Coumaphos	Miticide	4.6	3	1.36	0.00	6	2.97	0.00
Cyprodinil	Fungicide	100	0			1	0.70	0.00
Fenbuconazole	Fungicide	/	0			1	0.35	
Fenpyroximate	Miticide	118.5	0			2	0.44	0.00
Fipronil	Insecticide	0.001	0			2	0.18	0.18
Fipronil sulfone	Insecticide	0.001	2	0.47	0.47	1	0.35	0.35
Methoxyfenozide	Insecticide	100	1	1.06	0.00	0		
Penthiopyrad	Fungicide	385	0			1	0.04	0.00
Piperonyl butoxide	Synergist	/	5	0.96		6	0.98	
Tebuthiuron	Herbicide	/	1	0.36		0		
Thiabendazole	Fungicide	34	0			1	1.16	0.00

Worcester County (7 apiaries x 2 sampling rounds)								
Pesticide	**Pesticide Class**	**Oral LD50 (ug/g)**	**Pollen**			**Wax**		
			# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ	# Samples Positive	Mean Conc. (ng/g)	Mean Oral HQ
Acetamiprid ^	Insecticide	14	1	0.21	0.00	2	0.04	0.00
Atrazine	Herbicide	/	5	0.40		0		
Azoxystrobin	Fungicide	25	2	0.12	0.00	1	0.03	0.00
Bifenazate	Insecticide	141	2	1.34	0.00	1	0.84	0.00
Boscalid	Fungicide	166	3	23.78	0.00	4	2.17	0.00
Carbaryl	Insecticide	0.15	5	0.93	0.01	1	1.03	0.01
Chlorantraniliprole	Insecticide	0.0274	6	7.34	0.27	2	2.39	0.09
Clomazone	Herbicide	85.3	1	0.07	0.00	0		
Coumaphos	Miticide	4.6	10	2.79	0.00	14	6.37	0.00
Cyazofamid	Fungicide	/	1	0.35		0		
Cyprodinil	Fungicide	100	1	59.76	0.00	3	3.09	0.00
Dithiopyr	Herbicide	/	1	1.63		0		
Etofenprox	Insecticide	0.024	0			1	1.49	0.06
Fenhexamid	Fungicide	1.7	1	12.36	0.01	2	2.59	0.00
Fenpyroximate	Miticide	118.5	2	0.96	0.00	4	9.22	0.00
Fipronil	Insecticide	0.001	2	1.76	1.76	2	0.18	0.18
Fipronil sulfone	Insecticide	0.001	2	0.47	0.47	4	0.35	0.35
Fluopyram	Fungicide	102.3	2	0.44	0.00	0		
Fluxapyroxad	Fungicide	110.9	3	1.23	0.00	1	0.25	0.00
Hexythiazox	Insecticide	/	0			1	0.35	
Imidacloprid ^	Insecticide	0.004	2	1.49	0.37	2	2.98	0.74
Metolachlor	Herbicide	110	1	0.14	0.00	0		
Napropamide	Herbicide	113.5	1	2.79	0.00	0		
Piperonyl butoxide	Synergist	/	4	4.56		10	7.32	
Pyraclostrobin	Fungicide	73	2	5.20	0.00	2	1.23	0.00
Pyrimethanil	Fungicide	100	1	1.09	0.00	0		
Tebufenpyrad	Insecticide	1.8	0			1	2.36	0.00
Tebuthiuron	Herbicide	/	1	0.29		0		
Thiabendazole	Fungicide	34	0			1	0.05	0.00
Thiophanate-methyl	Fungicide	100	3	1.21	0.00	1	0.53	0.00