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Pesticides in Honey Bee Colonies: establishing a baseline for real world exposure over seven years in the USA

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Author contribution:

Conceptualization: KR, JS,

JE, DvE

Methodology: KT, ST, KR, EF, JS,

JE, DvE Validation   KT, NS, DvE

Formal analysis KT, NS

Investigation   KR, EF, GK, SM, DL,

HE, RF, Resources   JE, JD

Data Curation KS, KR, NS, EF, GK, SM, SL, HE, RF, DvE

Writing – KS, DvE

Writing - Review & Editing KS, ST, KR, NS, EF,

JP, JE, DvE Visualization   KS, NS, DvE

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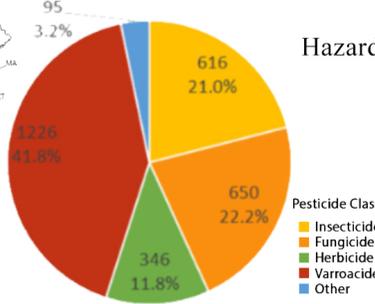HE. RF Funding acquisition JS, JE, DvE

Pollen analyzed for 218 pesticides & metabolites
n = 1,055 apiaries

5 Estimates of Risk

Prevalence: 81.9%
Diversity: = 2.78 residues/sample
Concentration: mean = 600.3 ppb
Significant detects: = 349
Hazard Quotient: 5.4% above threshold

120 different residues
n = 2,933 detects

95
3.2%

616
21.0%

650
22.2%

346
11.8%

1226
41.8%

Pesticide Class
- Insecticide
- Fungicide
- Herbicide
- Varroacide
- Other

Colony Morbidity
Varroa destructor ↑
Nosema spp. ↑
Queen loss ↑
Brood Disease ↑

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Abstract: Honey bees *Apis mellifera* forage in a wide radius around their colony, bringing back contaminated food resources that can function as terrestrial bioindicators of environmental pesticide exposure. Evaluating pesticide exposure risk to pollinators is an ongoing problem. Here we apply five metrics for pesticide exposure risk (prevalence, diversity, concentration, significant pesticide prevalence, and hazard quotient (HQ)) to a nation-wide field study of honey bees, *Apis mellifera* in the United States. We examined samples from 1,055 apiaries over seven years for 218 different pesticide residues and metabolites, determining that bees were exposed to 120 different pesticide products with a mean of 2.78 per sample. Pesticides in pollen were highly prevalent and variable across states. While pesticide diversity increased over time, most detections occurred at levels predicted to be of low risk to colonies. Varroacides contributed most to concentration, followed by fungicides, while insecticides contributed most to diversity

45   above a toxicity threshold. High risk samples contained one of 12 different insecticides/varroacides. Exposures predicted to be low-risk were nevertheless associated with colony morbidity, and low-level fungicide exposures were tied to queen loss, *Nosema* infection, and brood diseases.

Introduction:

50   Honey bees forage in a wide radius of approximately 2 km around their colony during resource plentitude and up to 6 km during dearths, collecting both pollen and nectar(Beekman and Ratnieks, 2000; Visscher and Seeley, 1982). Their critical pollination services are valued at $175 billion worldwide (Gallai et al., 2009) and $17 billion in the US(Calderone, 2012). While foraging in the environment, bees function as living terrestrial bioindicators, picking up traces of 55   heavy metals, pesticides, and other pollutants in the environment (Giglio et al., 2017; Goretti et al., 2020; Smith et al., 2020). Pesticides accumulate in the colony matrix and are often found in the pollen bees consume as their primary protein source (Calatayud-Vernich et al., 2018; de Oliveira et al., 2016). These pesticides play a role in poor bee health (Doublet et al., 2015; Goulson et al., 2015; Sanchez-Bayo and Goka, 2014) and sublethal, chronic pesticide exposures 60   can interact with viruses, parasites, and poor nutrition (Alaux et al., 2010; Poquet et al., 2016; Schmehl et al., 2014; Tosi et al., 2017) leading to decline (Becher et al., 2013; Henry et al., 2012; O'Neal et al., 2018; Steinhauer et al., 2018; vanEngelsdorp et al., 2013). The multiplicity of pesticide residues found in the colony complicate analyses (Johnson et al., 2010; Mullin et al., 2010; Traynor et al., 2016a). While current pesticide risk measures focus on individual 65   contaminants (Carnesecchi et al., 2019), different pesticides have non-additive interactions complicating risk assessments in nature.

Honey bees collect and store pollen from plants. It is consumed primarily by nurse bees, which convert it into proteinaceous glandular secretions fed to developing larvae (Crailsheim, 1992). An individual worker will consume over 100 mg of pollen, predominantly when feeding 70   larvae (2012; Crailsheim et al., 1992). Little is known about the actual risk to honey bee health based on the pesticides found in the pollen bees store in their colonies, which is the main protein source nurse bees consume to rear the next generation (Crailsheim et al., 1992; Schmickl and Crailsheim, 2004). Prior surveys suggest high levels of overall pollen contamination in the United States, though wide scale surveys with samples collected since 2007-2008 (Mullin et al., 75   2010; Traynor et al., 2016a). We hypothesize that the pesticides found in pollen have changed over the years as pesticide use has shifted, and so seek to establish a baseline of pesticide contamination of pollen. Here we report on the overall pesticide exposure risk from pollen in a subset of colonies randomly surveyed for the National Honey Bee Disease Survey (NHBDS) to determine a baseline of pesticide exposure (for details on sampling see the supplemental 80   information section on sample origin and pollen sampling (Traynor et al., 2016b)) and the potential relationships between pesticides and colony morbidity in the United States.

Methods:

Freshly stored pollen can easily be identified in a colony by its bright color and matte 85   appearance, indicating the bees collected it recently and thus it is indicative of the pesticides in pollen in the current environment (see supplemental information pollen sampling), whereas honey can be stored for a long time with no change in appearance. We thus focused our survey on freshly stored pollen. Pollen samples were collected from apiaries (n = 1,055) in 39 US States

and Puerto Rico between 2011 and 2017 (see Table S1 for sampling by state). A subset were
simultaneously inspected for overt disease conditions (n = 151, see SI Apiary Inspection Sheet),
levels of the ectoparasite *Varroa destructor* (n = 1,048), the spore forming fungal gut parasite
Nosema spp. (n = 1,034), and virus presence (n = 1,015). Pesticide contamination of the samples
was analyzed by the USDA Gastonia lab for the presence of 218 different pesticide residues
using a modified QuEChERS method (Lehotay et al., 2005) that was adapted for 3 g instead of
the normal 15 g samples, as amounts greater than 3 g of pollen are hard to obtain from bee
colonies (see supplemental information on multiresidue pesticide analysis for details). To
understand exposure risk under real world field conditions, we calculated five pesticide risk
measures and correlated these with colony morbidity:

1) pesticide prevalence (PP): the percentage of samples positive for any pesticide residue
2) pesticide diversity (PD): the number of different pesticide residues
3) pesticide concentration (in ppb) (PC): the summed concentration of all pesticide residues
4) 50+ diversity (50+D): the number of pesticide residues detected, where that residue contributes ≥ 50 points to a sample's overall Hazard Quotient score; in particular an HQ score of 50 represents 0.5% of a given pesticide's LD_{50} consumed over 10 days
5) HQ score (HQ) (Stoner and Eitzer, 2013; Traynor et al., 2016a): an estimate of an adult worker bee's lifetime consumption risk (pesticide residue in ppb /respective LD_{50} in μg/bee)

Further we summarized exposure patterns by their classification (e.g. fungicides,
herbicides, insecticides, and varroacides) and mode of action (Fungicide Resistance Action
Committee, 2018; Insecticide Resistance Action Committee) and explored potential relationships
between these groups and colony morbidity.

Results:

The five risk estimates provide different insights into the pesticides bees encounter in pollen
(Figs 1 and 2). The continuous risk variables are all correlated (Table 1). Overall 2,933 pesticide
detections were made across the 1,055 samples, representing 120 of the 218 different active
ingredients and metabolites analyzed (Table S2, Table S3). Overall PP was high (Fig. 1A), with
81.9% of all samples contaminated, a stable rate across years (Pearson X^2 = 9.06, n = 1,058, df =
6, p = 0.17). When separated by pesticide class (insecticide, fungicide, herbicide, and
varroacide), insecticide prevalence decreased over time, while herbicides and fungicides
increased (Fig. 2A). Bees were exposed to a diversity of pesticides (Fig. 1B and S1), with up to
21 different residues detected (mean = 2.78 ± 0.09). Varroacides were detected most often,
followed by fungicides, and insecticides (Fig. S2A). The varroacides detected with greatest
frequency are the currently recommended products for *Varroa* control (Amitraz metabolite
DMPF detected in 45.38% and thymol in 20.63% of tested samples). PD increased over years
(r_{1055} = 0.15, p < 0.0001; Fig. 2B), with the highest PD occurring in 2014 and 2016. The five
pesticide risk values varied by state (Fig. 1, Table S3; **PP**, GLM Binomial X^2 = 150.18, df = 39,
p < 0.0001; **PD**, GLM Normal X^2 = 240.56, df = 39, p < 0.0001; **PC**, GLM Poisson X^2 = 100.50,
df = 39, p <0.0001; **50+D**, GLM Normal X^2 = 143.16, df = 39, p < 0.0001; **HQ**, GLM Poisson
X^2 = 220.43, df = 39, p < 0.0001)

Most pesticides were found at concentrations below 1,000 ppb (1 ppm) (Table S2) with
some varroacide and fungicide exceptions and a mean concentration of 600.3 ppb ± 82.0. The

concentration did vary across states (Fig. 1C; GLM Normal X^2 = 56.64, df = 39, p = 0.0336),
with NJ (p < 0.001) and NY (p = 0.047) significantly different (Fig S3). Concentration did not
vary across years (Fig. 2C). Several states had mean concentrations ± SE above our 1,000 ppb
threshold, a rate previously linked to queen losses for fungicide concentrations (Traynor et al.,
2016a) (CA = 1,110.0 ± 228.7; DE = 1,228.1 ± 700.2; IN = 1,306.8 ± 999.7; NJ = 2,941.7 ±
1,475.7; NY = 1,239.3 ± 373.1; WV = 1,146.1 ± 753.8).

Many detected products have low recognized toxicity to bees (LD_{50} in µg/bee > 100) and
so, presumably, pose little risk to bees. To eliminate background noise of low risk exposure, we
calculated the diversity of pesticides detected that each contributed 50 or more HQ points (50+D)
to a sample's overall score (Fig. 1D). Only 11.9% of detections (n = 349) exceeded the 50+
threshold; the majority (92.0%, n = 321) were insecticides, 7.4% (n = 26) were varroacides, one
was a fungicide (THPI), and one was an herbicide (atrazine; Fig. S2B). 50+D differed between
sample years ($F_{6,1051}$ = 6.78, p < 0.001) with more detected in 2012 (0.55 ± 0.06 SE) than any
other year (0.35 ± 0.04 SE or less).

We classified samples as high risk when they had HQ scores ≥ 1,000; an amount
indicating that honey bees consuming this pollen will ingest 10% or more of their LD_{50} over their
nursing lifetime. Overall 5.4% of samples (n = 57) had HQ scores that exceeded 1,000 points
(Fig. 1E, Table S2). Fifteen different insecticides and one varroacide (coumaphos) contributed
substantially to HQ scores (detected in at least 5 samples and adding more than 500 points to at
least one HQ score.). Twelve different products (Table S2) in 52 samples had individual
pesticide residues that exceeded our 1,000 or more point HQ threshold; insecticides: chlorpyrifos
(n = 13), clothianidin (n = 8), bifenthrin (n = 6), carbaryl (n = 6), prallethrin (n = 6),
thiamethoxam (n = 3), cyfluthrin (n = 2), fenpropathrin (n = 2), permethrin (n = 2), pyridaben (n
= 2), imidacloprid (n = 1), and the varroacide coumaphos (n = 1). We analyzed the prevalence
and mean HQ contributed by residues that appear in at least 10% of these 57 samples that exceed
an HQ of 1,000, contributing at least 100 HQ points. These include chlorpyrifos (n = 33, 57.9%,
mean HQ = 877), carbaryl (n = 11, 19.3%, mean HQ = 2,931), bifenthrin (n = 10, 17.5%, mean
HQ = 1,299), clothianidin (n = 8, 14.0%, mean HQ = 2,002), thiamethoxam (n = 8, 14.0%, mean
HQ = 939), and prallethrin (n = 6, 10.5%, mean HQ = 12,750).

Although neonicotinoids were detected only 60 times (2.0% of all pesticide residue
detections) throughout our survey, when they are detected they often contribute substantially to
the HQ (Table S2). An HQ score above 10,000 indicates that a bee will consume the equivalent
of her LD_{50} over her nursing phase; less than 1% of our samples (n = 5) exceeded this threshold
(Table 2). Despite these five extreme risk samples exhibiting high pesticide diversity (mean = 7.4
± 0.93 SE), in each of these samples, one insecticide was the main contributor. This pattern of a
single insecticide as the predominant contributor to exposure risk is common across our 57 high
risk samples (Fig. S3A), except for the five samples from Nebraska where the two
neonicotinoids clothianidin and thiamethoxam are frequently co-detected, potentially because the
former is the insecticidal metabolite of the latter (Nauen et al., 2003). This single residue as the
main contributor to HQ risk contrasts with the diversity of residues found in samples with high
pesticide concentrations where multiple different products co-occur (Fig. S3B).

Our risk measures varied by pesticide class (Fig. 3). Varroacides, applied by beekeepers
directly into colonies to reduce parasite loads, were the main contributor for PP (Fig. 3A), PD
(Fig 3B), and PC (Fig 3C), though PC decreased significantly over time (Fig 2C). Varroacides
were detected 1,226 times (Fig S2A), with at least one varroacide detected in 65.9% of samples

(n = 697), and contributing 0.04 to 1,190 points to the HQ score of samples; the majority (99.51%) of varroacide detections contributed less than 50 points to the HQ score. Thymol, considered practically non-toxic to bees (LD_{50} = 975 μg/bee), contributed little to most HQ scores although it was detected at more than 1,000 ppb in 4.1% of positive samples (n = 43) (Table S2) and at over 10,000 pbb in 0.7% of samples (n = 7). Other varroacides detected at over 1,000 ppb were the amitraz metabolite DMPF (n = 2), coumaphos (n = 4) and fluvalinate (n = 5).

Insecticides were the second most prevalent pesticide residue class, detected 691 times (Fig. S2A), with at least one insecticide detected in 38.1% of samples (n = 404). Insecticide PP was highest in 2012 (47.5% ± 0.04% SE) and lowest in 2017 (26.9% ± 0.05% SE). Insecticide PD varied by year (Fig 2B). Insecticides contributed the majority of the 50+D detections (Figs S2B, 3D), and varied significantly by year. Overall insecticides contributed little to the PC (Fig 3C), though the concentration increased significantly over time (Fig 2C). Six different insecticides were found in 14 different samples at concentrations that exceeded 1,000 ppb (Table S2). Many insecticides are highly toxic to bees, so it is not surprising that as a pesticide class they contributed the most (95.9%) to the HQ score (Fig 3E), adding a mean of 646.15 ± 110.67 SE (range: 0.01 to 29,629.6) points to samples with at least one insecticide detected.

Fungicides were detected 641 times, with at least one detection in 29.5% of samples (n = 312). Fungicide PP increased over years (r_{1055} = 0.13, p < 0.001, Fig. 2A). Fungicide PD also increased (r_{1055} = 0.19, p < 0.001) and varied between years (Fig. 2B). Multiple fungicides were frequently detected; the maximum number of fungicides increased annually from four fungicides in 2011 to ten fungicides in 2017. Altogether 9.4% (n = 30) of the fungicide-positive samples contained five or more fungicides, disproportionately from California (32.5%). After varroacides, fungicides were the most common (22%) (Fig S2A) and largest contributor to the PC (Fig 3C). Fungicide PC didn't change over time (Fig 2C). Eight different fungicides in 25 samples contributed 1,000 or more ppb (Table S2; Fig S4B). Of note is the fungicide tetrahydrophthalimide (THPI) which was detected in 22 samples, contributing a mean of 1,526.6 ± 500.9 SE ppb and 3.14 ± 0.49 SE HQ points to each sample's concentration (range: 1 to 7,060) and HQ score (range: 0.01 to 75.16). THPI is the major metabolite of captan, a widely used fungicide that caused brood mortality in cage trials when integrated into larval food,(Mussen et al., 2004) but not in field trials(Everich et al., 2009). Fungicides were seldom (<1%) found to contribute 50+ points to the HQ score (Fig S2B). HQ_{Fung} scores below 5 were implicated in colony losses in a prior study (Traynor et al., 2016a), hence this risk assessment metric may underestimate the number of significant exposures for products with low toxicity and underestimate potential synergies. Fungicides generally contributed little to a sample's overall HQ score (Fig 2D), so we also calculated which fungicide modes of actions (MOA) were found contributing five or more HQ points (see below).

Herbicides were found in 24% of samples (Fig. S2A). Herbicide PP (r_{1055} = 0.19, p < 0.001) and PD (r_{1055} = 0.20, p < 0.001) increased over the course of the study (Fig. 2A and B). Herbicide PC was low (Fig 3C), and did not change over time (Fig 2C). The herbicides atrazine (n = 1), fluridone (n = 2), metolachlor (n = 2), and propachlor (n = 3) were detected at concentrations above 1,000 ppb (Table S2). Only one detection of atrazine added 50+ points to the HQ; overall herbicides added a mean of 1.29 ± 0.38 SE HQ points to samples with at least one herbicide detection (HQ range: 0.01 to 87.32). No herbicide was found at levels which exceeded our HQ safety threshold of 1,000 points, though our survey didn't test for glyphosate,

previously linked to disruptions of gut microbiota (Motta et al., 2018), as it requires a separate analysis.

225   Pesticides have different modes of action (MOA), and some pesticide MOAs have previously been linked to colony morbidity (Böhme et al., 2018; Douglas et al., 2020; Traynor et al., 2016a), hence we grouped detections by MOA and calculated exposure risk (Table S4; Fig S5). The most frequently detected insecticide and varroacide MOA groups were Group 1 Acetyl Choline Esterases (AChE) and Group 3 Sodium Channel Modulators. These specific MOAs
230   contributed more than 50+ in 324 pollen samples, accounting for 50.1% and 33.9% of 50+ detections in all samples (Table S4). The small contribution fungicides make to HQ scores was mostly made by fungicide products with an MOA defined as M. multisite activity, F. lipid synthesis or transport and C. respiration by the fungicide resistance action committee (Fungicide Resistance Action Committee, 2018) (Table S4, Fig S5).

235   *Varroa destructor* (n = 1048) and *Nosema spp.* (n = 1034) infestation were calculated for most apiaries (Table 3) and correlated with our five measures of risk (Table 1). Overall and varroacide PP, overall and varroacide PD, 50+D , and HQ scores varied across varroa infestation levels (Fig. S5A-F). PP was higher in groups with less than 3 mites, while samples with no mites had more varroacides present than other groups (Fig. S5A, red). Total PD is negatively
240   correlated with *Varroa* infestation (r_{1048} = -0.153, p < 0.0001). Varroacide PD was higher in *Varro*a free colonies than in samples taken from colonies with detectable *Varroa* levels (Fig. S5B), suggesting they were being or had recently been treated for this parasite. Overall PC did not vary with *Varroa* load (Fig. S5C), but varroacide levels were highest in samples with 5 or more mites. When we investigated the major contributors to varroacide PC, we found a
245   surprising inverse relationship between the four most commonly used varroacides, suggesting that amitraz and coumaphos are effective control products, while high levels of thymol and fluvalinate are detected in samples from colonies with high varroa loads (Fig. 4) Interestingly, for both 50+D and total HQ we found the highest scores in the *Varroa* infestation at zero and at 10+ mites per 100 bees (Fig. S5D & E). Elevated HQ levels in the 10+ *Varroa* infestation group
250   suggests that either *Varroa* are more fit in environments of high pesticide exposure (e.g. by perhaps increasing the length of brood development providing *Varroa* with greater fecundity), or that elevated HQ contamination of pollen may reduce a colonies adult bee population (e.g. by shortening the lifespan of adult bees thus increasing the density of *Varroa* per bee), resulting in a greater density of *Varroa* per bee(Gill et al., 2012; Wu et al., 2011).

255   PD, fungicide PD, fungicide PC, 50+ D and fungicide HQ were significantly higher in *Nosema* positive samples (Fig 5), a counterintuitive result as *Nosema* is a fungal spore disease. We suspect that just as antibiotics can wipe out beneficial intestinal flora in the gastrointestinal tract, and allow harmful flora to establish (Pamer, 2016), exposure to fungicides may destroy the beneficial fungi in a colony, permitting fungal diseases like *Nosema* to proliferate as seen in a
260   prior study where fungicide PC increased the probability of *Nosema* infection (Pettis et al., 2013). Apiaries with undetectable *Nosema* exhibited 20.6% lower PD compared to apiaries with *Nosema* (Fig 4B). Samples with *Nosema* had fungicide HQ scores 3x higher than *Nosema* free samples (Fig 3E). *Nosema* spore load was loosely correlated with PD (r_{1032} = 0.086, p = 0.0055), fungicide PD (r_{1032} = 0.084, p = 0.0068) and the fungicide HQ score (r_{1032} = 0.085, p = 0.0065).

265   14.3% of apiaries (n = 151) were simultaneously inspected for overt symptoms of honey bee diseases (Table 3). Brood diseases (American foulbrood, European Foulbrood, Sacbrood, Chalkbrood, and/or Snot Brood) were detected in 29.8% of inspected apiaries (n = 45). The

fungicide HQ was elevated in samples from apiaries positive for brood disease ($X^2 = 3.98$, df =1, p = 0.046) with a mean fungicide HQ = 4.01 ± 1.81 SE in positive samples compared to fungicide HQ = 1.20 ± 0.45 SE in colonies free of brood disease. About one third of apiaries (n = 49) experienced queen issues, where at least 1 of 8 colonies inspected was a drone layer, queenless, or the colony had queen cells (Table 3). Colonies experiencing queen issues had elevated fungicide HQ scores ($X^2 = 3.84$, df = 1, p = 0.049) and an average fungicide HQ score 3x higher (3.82 ± 1.73 SE) than colonies without queen issues (1.18 ± 0.41 SE).

Lastly, we examined how our five exposure risk measures varied with viral prevalence for eight common honey bee viruses (Table 4). Each was associated with either increased or decreased prevelence of at least one of the viruses examined except Acute bee paralysis virus (ABPV) (Table 4).

Discussion:

Our national survey is the first to broadly examine environmental pesticide exposure throughout a wide majority of the United States, establishing an important baseline of pesticide pollution of pollen collected by our most important managed pollinator, *Apis mellifera*. Honey bees forage in a wide radious around their colony, functioning as a terrestrial biomonitor that provides critical insight into the contaminants they encounter and bring back to the nest. Here we link field measurements of colony morbidity to five different ways of summarizing pesticide exposure and risk in pollen, identifying clear areas of concern. Eleven insecticides and one varroacide were the greatest contributors to HQ consumption risk. Because chlorpyrifos, carbaryl, bifenthrin, clothianidin, thiamethoxam, and prallethrin occurred most frequently in samples we considered high risk, our work raises concern about their current use.

Based on standard lethal effects indexes, most of our samples were not considered risky. Many of our 57 high-risk samples had one main contributor, typically an insecticide. Although fungicides contribute little to direct consumption risk, they are often found at high concentrations and were correlated with increased *Nosema* infections, brood disease and queen issues, reaffirming that a reevaluation of their safety is necessary (Fisher et al., 2017; Simon-Delso et al., 2018; Traynor et al., 2016a; Wade et al., 2019). High levels of varroa were associated with high levels of fluvalinate and thymol, two different varroacide products, suggesting that either beekeepers applied them after noticing high varroa levels and then residues dissipated quickly when varroa mites were controlled or that they are less effective control products and varroa infestation levels escalate despite high dose treatments. In contrast, low levels of varroa were associated with high levels of the amitraz metabolite DMPF and the synthetic varroacide coumaphos, potentially suggesting that these two are effective varroa control products that quickly reduce varroa infestations.Our work thus underlines the complexity of pesticide effects, highlighting that they are not limited to lethal exposures. Colony morbidity was often associated with low-risk exposure suggesting the myriad residues detected may result in poorly understood interaction (i.e. additive, antagonistic, synergistic) effects. While our results provide little evidence of poisoning as a major colony loss driver, we stress the mounting evidence that colonies experience sublethal pesticide exposure and unexpected interactions arise when bees are co-exposed to diseases, as well as chemical mixtures (18) and nutritional stress (9). Our results suggest an urgent need for greater understanding of pesticide impacts on bee losses.
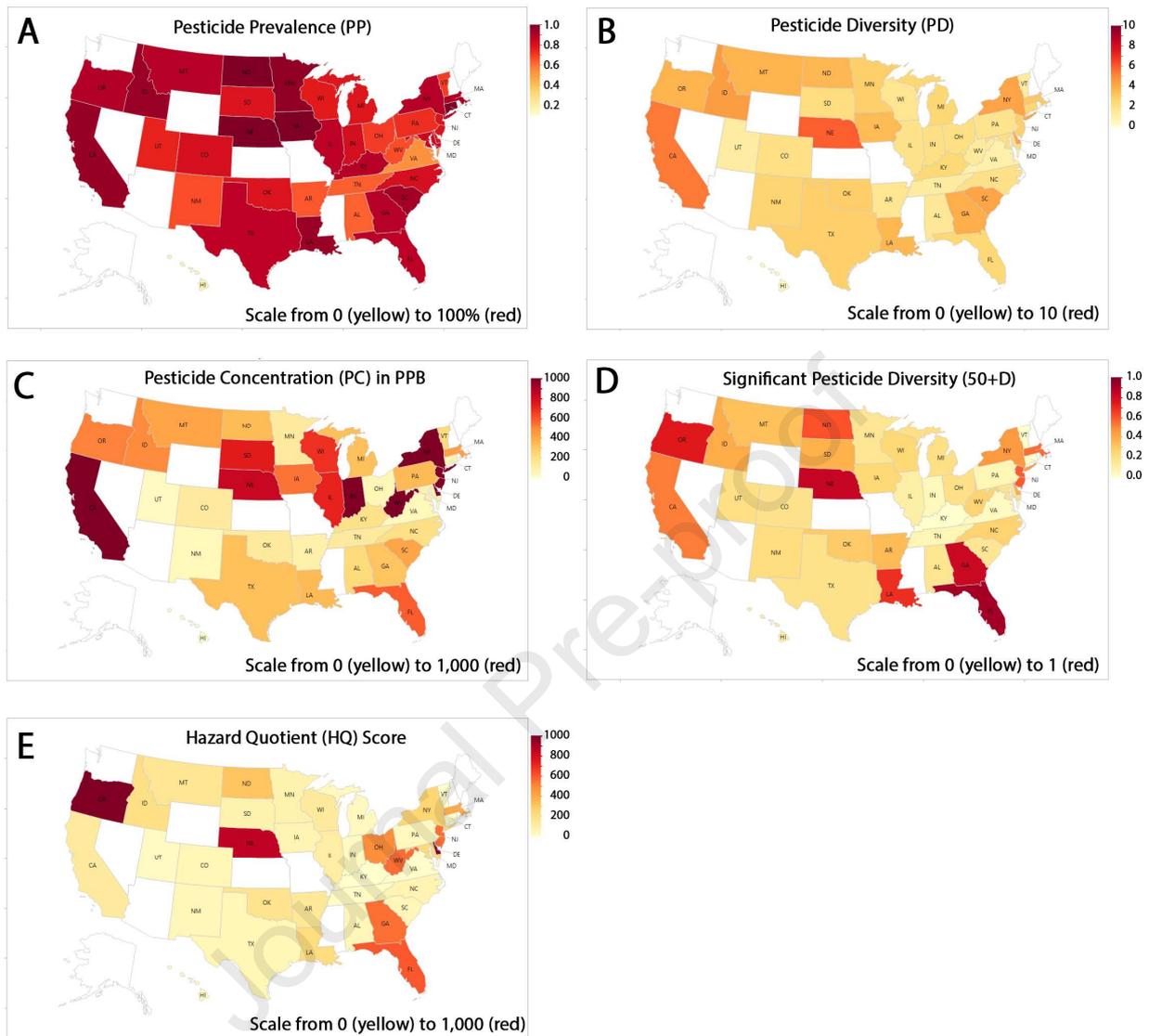
Fig. 1. Quantifying pesticide exposure risk in bee bread samples collected as part of the National Honey Bee Disease Survey. (A) Pesticide prevalence: the percentage of samples in each state with one or more pesticide residues. Heat map shows range of positive, from yellow which indicates no samples 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 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 of xenobiotics, but if increased to 3,000 ppb then all states except NJ are shades of yellow to light orange (see Fig S3); (D) total number of pesticide detections contributing at least 50+ to the hazard quotient, a threshold equivalent to 0.5% of the LD_{50}, 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 risks.

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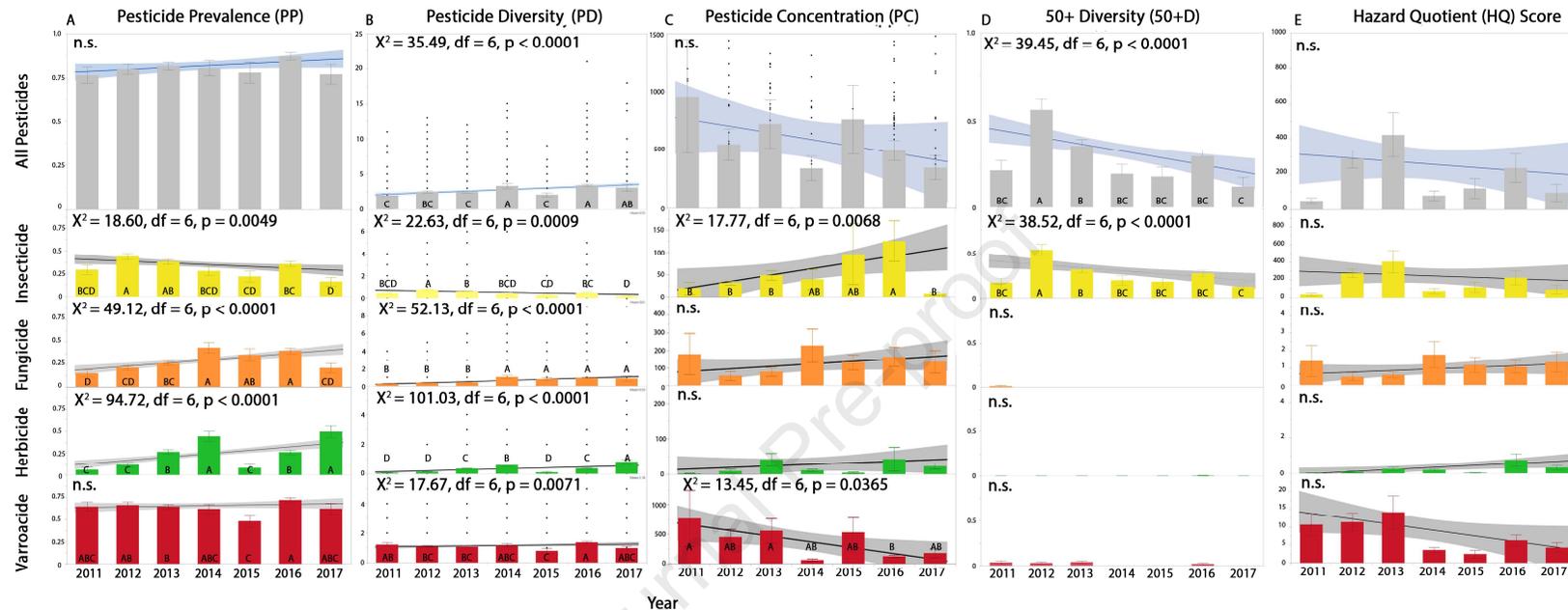
Fig. 2. Pesticide class trends over time. (A) pesticide prevalence of positive detections; (B) pesticide diversity; (C) pesticide concentration, and (D) 50+ detections by class; (E) hazard quotient score). Grey = all pesticide detections, yellow = insecticides, orange = fungicides, green = herbicides, red = varroacides. Differences between years ($\alpha = 0.05$) are marked with different letters. Significant trends over survey years are also indicated by reporting GLM results with binomial distribution for prevalence, normal for diversity and 50+ diversity, and Poisson for concentration and HQ; n.s. indicates that no significant ($p > 0.05$) trends were found. Trend lines show a linear regression with 95% confidence intervals. The first and last survey years covered 6 non-overlapping months (2011 = July – Nov.; 2017 = March – June), so differences from general trends in these years should be viewed cautiously.
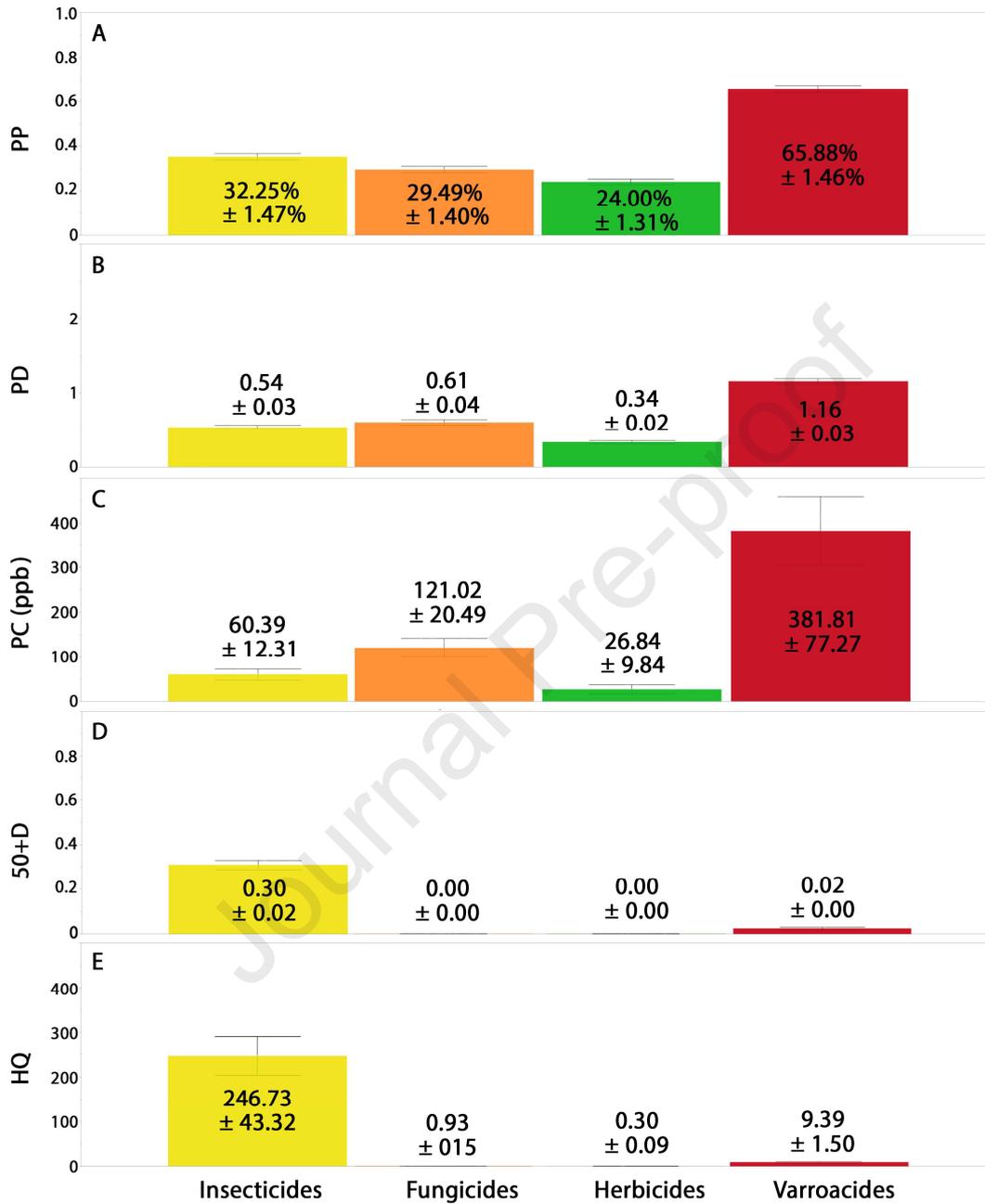
Fig. 3. Exposure risk assessment by pesticide class, mean ± SE reported for all. (A) pesticide prevalence of positive detections; (B) pesticide diversity; (C) pesticide concentration; (D) 50+ detections by class; and (E) HQ score; yellow = insecticides, orange = fungicides, green = herbicides, red = varroacides.
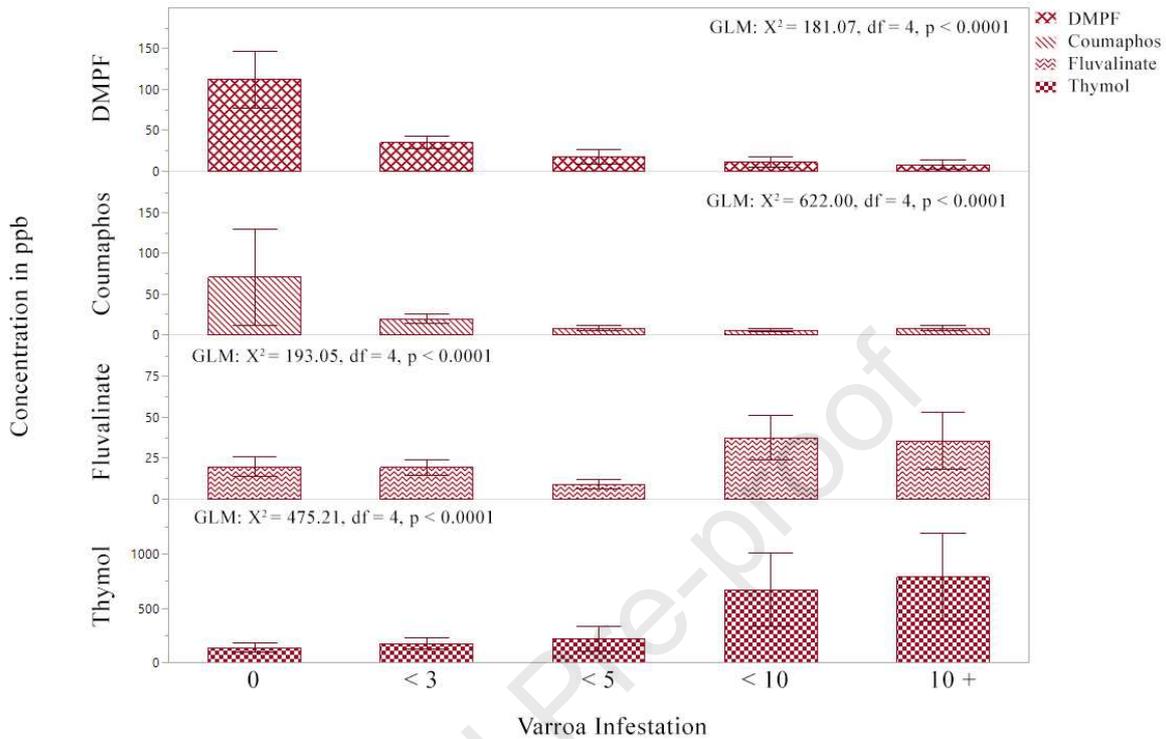
Fig. 4. The concentration of the four most commonly used varroacides by varroa infestation, mean ± SE. The amitraz metabolite DMPF and coumaphos both exhibit high concentrations at samples with no varroa, while fluvalinate and thymol show the inverse relationship with high concentrations appearing in samples with high varroa infestations. This suggests that amitraz and coumaphos may be effective varroa and rapid varroa controls, while fluvalinate and thymol are either ineffective or are applied when beekeepers detect high infestations and dissipate quickly post treatment. Varroa Infestation per 100 adult bees: $0 = 0$, $< 3 = 0$ to 3 varroa, $<5 = 3$ to 5 varroa, $< 10 = 5$ to 10, and 10+ = more than 10. For each reside, we conducted a GLM analysis with exponential distribution.
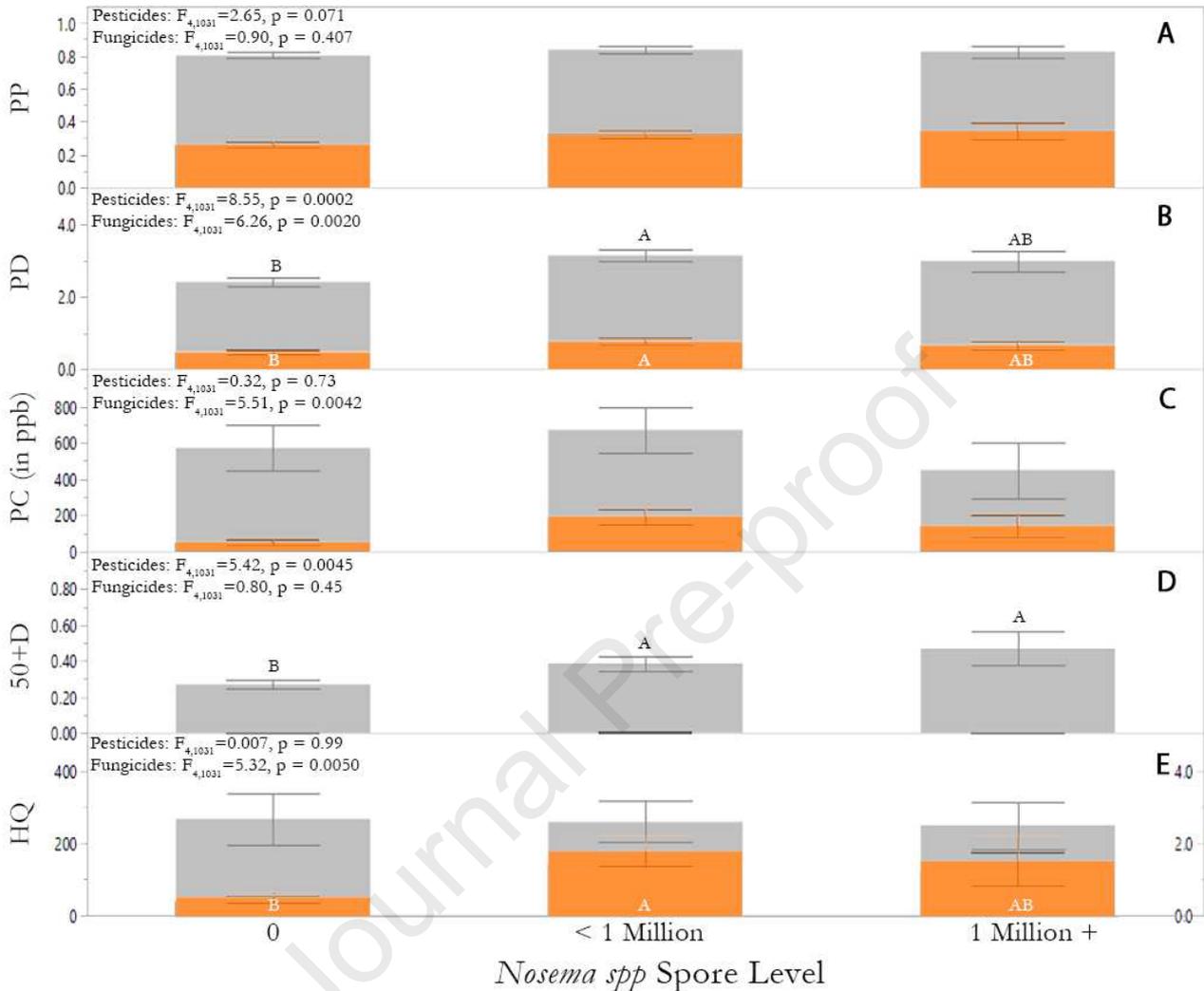
Fig. 5. Exposure risk assessment by *Nosema spp.* spore level, mean ± SE. (A) pesticide prevalence of positive detections; (B) pesticide diversity; (C) pesticide concentration; (D) 50+ detections; and (E) HQ score, with left y-axis for all pesticides and right y-axis for fungicides. Light grey = all pesticides; orange = fungicides. Significant differences (α = 0.05) marked with different letters.

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Table 1. Correlations of different risk measures and parasite loads. We report all significant correlations between our four continues estimates of risk (diversity (PD), concentration (PC), 50+D, and HQ, while excluding prevalence as that is binary). We then also compare our continuous risk measures with parasite (*Varroa* and *Nosema*) loads.

Risk Factor	Compared with	Correlation	Count	Lower 95%	Upper 95%	P value
PD	50+D	0.508	1055	0.46	0.55	1.4×10^{-70}
HQ	50+D	0.340	1055	0.29	0.39	5.9×10^{-30}
PD	HQ	0.218	1055	0.16	0.27	7.3×10^{-13}
PD	PC	0.163	1055	0.10	0.22	1.0×10^{-7}
PC	50+D	0.142	1055	0.08	0.20	3.6×10^{-6}
PC	HQ	0.141	1055	0.08	0.20	4.3×10^{-6}
Parasite Loads						
PD	Varroa	-0.153	1048	-0.21	-0.09	6.1×10^{-7}
Nosema	Varroa	-0.087	1034	-0.15	-0.03	0.005
PD	Nosema	0.086	1034	0.03	0.15	0.006
HQ	Varroa	0.065	1048	0.00	0.12	0.036
50+D	Nosema	0.062	1034	0.00	0.12	0.047

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Table 2: Samples with HQ scores above 10,000 and constituent products

Pesticide residues detected and the amount of points contributed to the HQ score for each of the six samples above 10,000. Residues that contributed more than 1,000 points in bold. For 5 of the 6 samples, one residue was the main contributor to the score. The NJ sample had two residues that contributed substantially to the score: carbaryl and imidacloprid.

Sample ID	95350	93274	93497	96361	96025
State	OR	OR	MD	WV	NJ
Year	2013	2013	2013	2016	2016
HQ Score	19,097.0	29,643.3	11,928.9	11,771.0	16,146.5
Detections	9	6	5	10	7
1	Acephate 1.9	Azoxystrobin 0.2	Atrazine 0.1	DMPF 1.0	1-Naphthol 226.2
2	Azoxystrobin 0.1	Coumaphos 10.9	Cyhalothrin total 10.0	Atrazine 0.01	DMPF 0.9
3	Chlorpyrifos 87.9	Coumaphos oxon 0.2	Fluvalinate 7.4	Azoxystrobin 0.2	Azoxystrobin 0.4
4	Endosulfan II 6.6	Fluvalinate 1.5	**Prallethrin 11,888.9**	Boscalid 0.3	**Carbaryl 14,932.13**
5	Endosulfan sulfate 0.3	**Prallethrin 29,629.6**	Thymol 22.6	Captan 2.5	Chlorantraniliprole 0.81
6	Fluvalinate 0.9	Thymol 1.0		**Carbaryl 11,764.7**	Chlorothalonil 6.1
7	Methamidophos 36.1			Carbendazim 0.8	Imidacloprid 979.9
8	**Prallethrin 18,963.0**			Myclobutanil 0.5	
9	Thymol 0.2			Pyraclostrobin 0.6	
10				Pyriproxyfen 0.4	

Carbaryl is a widely used agricultural insecticide, predominantly used for insect pest control (aphids, ticks, fleas, etc.)

Imidacloprid is a neonicotinoid, frequently applied as seed treatment where bees can contact the planting dust, but also used as a foliar spray, soil drench, and injected directly into trees, i.e. to protect against citrus greening.

Prallethrin is used primarily for mosquito control and in products to kill wasps/hornets

Table 3: Prevalence of Parasites, pathogens, overt brood disease, and queen conditions in inspected apiaries.

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Condition	Sampled Apiaries	Prevalence	Load (mean ±SE)
Parasites & Pathogens			
Varroa	1,048	89%	4.05 ± 2.2 mites per 100 bees
Nosema spore	1,034	43.7%	0.32 ± 0.026 million spores per bee
Viruses			
Acute Bee Paralysis virus (ABPV)	993	23.2%	N/A
Black Queen Cell Virus (BQCV)	368	80.4%	N/A
Chronic Bee Paralysis Virus (CBPV)	992	9.6%	N/A
Deformed Wing Virus (DWV)	1,002	85.2%	N/A
Israeli Acute Paralysis Virus (IAPV)	991	13.7%	N/A
Kashmir Bee Virus (KBV)	912	9.2%	N/A
Lake Sinai Virus II (LSV II)	629	36.1%	N/A
Varroa Destructor Virus (VDV)	282	56.0%	N/A
Overt Brood Disease			
American Foulbrood	151	<1%	1 colony per infected apiary
European Foulbrood	151	5%	1.3 colonies per infected apiary
Sacbrood	151	7.2%	1.4 colony per infected apiary
Chalkbrood	151	13.2%	1.9 colony per infected apiary
Snot Brood and/or Parasitic Mite Syndrome	151	11.9%	2.5 colony per infected apiary
Deformed wing	151	15.2%	1.4 colony per infected apiary
Black Shiny Bees	151	5.3%	2.3 colony per infected apiary
Queen Issue			
Queen Cells	151	16.6%	1.9 colony per infected apiary
Drone Layer	151	4.0%	1 colony per infected apiary
Queenless	151	8.6%	2.2 colony per infected apiary

Table 4: Exposure risk and virus status

For each of our five estimates of exposure risk, we report the mean ± SE for samples that were negative or positive for eight common honey bee viruses.

		Negative		Positive		Significance	
		Mean	SE	Mean	SE	t-test	p
PP	ABPV	81.4%	1.4%	82.6%	2.5%	0.42	0.67
	BQCV	68.1%	5.5%	83.4%	2.2%	2.59	0.0110
	CBPV	81.8%	1.3%	80.0%	4.0%	-0.42	0.67
	DWV	84.4%	2.3%	81.3%	1.0%	-0.94	0.35
	IAPV	80.8%	1.3%	86.8%	2.9%	1.85	0.07
	KBV	81.5%	1.0%	86.9%	4.0%	1.37	0.17
	LSV	79.4%	2.0%	88.1%	2.0%	2.96	0.0030
	VDV	83.9%	3.0%	86.7%	3.0%	0.66	0.51
PD	ABPV	2.81	0.10	2.67	0.17	-0.68	0.49
	BQCV	2.10	0.28	2.68	0.15	1.86	0.07
	CBPV	2.81	0.10	2.53	0.26	-1.00	0.32
	DWV	3.27	0.27	2.68	0.09	-2.05	0.0410
	IAPV	2.73	0.10	3.09	0.26	1.30	0.20
	KBV	2.81	0.10	2.88	0.29	0.22	0.82
	LSV	2.53	0.13	3.57	0.22	3.99	<0.0001
	VDV	2.72	0.24	3.74	0.29	2.67	0.0081
PC	ABPV	527.35	89.26	616.80	137.28	0.55	0.59
	BQCV	414.29	183.02	591.76	156.26	0.74	0.46
	CBPV	540.25	80.33	627.24	221.66	0.37	0.71
	DWV	374.03	78.13	626.43	98.24	2.01	0.0450
	IAPV	511.69	80.47	784.26	219.98	1.16	0.25
	KBV	503.55	57.67	1165.67	677.42	0.97	0.33
	LSV	647.84	165.11	539.15	95.21	-0.57	0.57
	VDV	257.19	57.57	560.72	123.45	2.23	0.0270
50+D	ABPV	0.33	0.02	0.31	0.04	-0.24	0.81
	BQCV	0.31	0.08	0.42	0.04	1.20	0.23
	CBPV	0.34	0.02	0.17	0.04	-3.42	0.0008
	DWV	0.34	0.05	0.32	0.02	-0.50	0.62
	IAPV	0.33	0.02	0.26	0.05	-1.24	0.22
	KBV	0.32	0.02	0.26	0.06	-0.85	0.40
	LSV	0.28	0.03	0.27	0.04	-0.30	0.76
	VDV	0.15	0.04	0.27	0.05	2.05	0.0410
HQ	ABPV	249.42	149.67	315.48	109.22	0.57	0.57
	BQCV	207.98	87.27	244.76	39.09	0.38	0.70
	CBPV	282.96	50.52	91.11	52.79	-2.62	0.0091
	DWV	394.96	205.28	239.62	39.90	-0.74	0.46
	IAPV	240.84	38.09	415.87	235.22	0.73	0.46

KBV	282.48	54.28	141.12	55.46	-1.82	0.07
LSV	339.00	103.62	180.21	58.45	-1.33	0.18
VDV	144.90	96.65	237.10	106.52	0.64	0.52

Viruses abbreviated: 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 II (LSV II), Varroa Destructor Virus (VDV)

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References

2012. White Paper in Support of the Proposed Risk Assessment Process for Bees. Office of Chemical Safety and Pollution Prevention, Washington, DC, p. 275.

Alaux, C., Brunet, J.L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., Brillard, J., Baldy, A., Belzunces, L.P., Le Conte, Y., 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environmental microbiology 12, 774-782.

Becher, M.A., Osborne, J.L., Thorbek, P., Kennedy, P.J., Grimm, V., 2013. REVIEW: Towards a systems approach for understanding honeybee decline: a stocktaking and synthesis of existing models. Journal of Applied Ecology 50, 868-880.

Beekman, M., Ratnieks, F., 2000. Long-range foraging by the honey-bee, Apis mellifera L. Functional Ecology 14, 490-496.

Böhme, F., Bischoff, G., Zebitz, C.P.W., Rosenkranz, P., Wallner, K., 2018. Pesticide residue survey of pollen loads collected by honeybees (Apis mellifera) in daily intervals at three agricultural sites in South Germany. PloS one 13, e0199995.

Calatayud-Vernich, P., Calatayud, F., Simó, E., Picó, Y., 2018. Pesticide residues in honey bees, pollen and beeswax: Assessing beehive exposure. Environmental Pollution 241, 106-114.

Calderone, N.W., 2012. Insect Pollinated Crops, Insect Pollinators and US Agriculture: Trend Analysis of Aggregate Data for the Period 1992–2009. PloS ONE 7, e37235.

Carnesecchi, E., Svendsen, C., Lasagni, S., Grech, A., Quignot, N., Amzal, B., Toma, C., Tosi, S., Rortais, A., Cortinas-Abrahantes, J., Capri, E., Kramer, N., Benfenati, E., Spurgeon, D., Guillot, G., Dorne, J.L.C.M., 2019. Investigating combined toxicity of binary mixtures in bees: Meta-analysis of laboratory tests, modelling, mechanistic basis and implications for risk assessment. Environment International 133, 105256.

Crailsheim, K., 1992. The flow of jelly within a honeybee colony. Journal of Comparative Physiology B 162, 681-689.

Crailsheim, K., Schneider, L.H.W., Hrassnigg, N., Bühlmann, G., Brosch, U., Gmeinbauer, R., Schöffmann, B., 1992. Pollen consumption and utilization in worker honeybees (Apis mellifera carnica): Dependence on individual age and function. Journal of Insect Physiology 38, 409-419.

de Oliveira, R.C., do Nascimento Queiroz, S.C., da Luz, C.F.P., Porto, R.S., Rath, S., 2016. Bee pollen as a bioindicator of environmental pesticide contamination. Chemosphere 163, 525-534.

Doublet, V., Labarussias, M., de Miranda, J.R., Moritz, R.F., Paxton, R.J., 2015. Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. Environmental microbiology 17, 969-983.

Douglas, M.R., Sponsler, D.B., Lonsdorf, E.V., Grozinger, C.M., 2020. County-level analysis reveals a rapidly shifting landscape of insecticide hazard to honey bees (Apis mellifera) on US farmland. Scientific reports 10, 1-11.

Everich, R., Schiller, C., Whitehead, J., Beavers, M., Barrett, K., 2009. Effects of captan on Apis mellifera brood development under field conditions in California almond orchards. Journal of economic entomology 102, 20-29.

Fisher, A., Coleman, C., Hoffmann, C., Fritz, B., Rangel, J., 2017. The synergistic effects of almond protection fungicides on honey bee (Hymenoptera: Apidae) forager survival. Journal of economic entomology 110, 802-808.

Fungicide Resistance Action Committee, 2018. FRAC Code List: Fungicides sorted by mode of action.

450   Gallai, N., Salles, J.-M., Settele, J., Vaissière, B.E., 2009. Economic valuation of the
       vulnerability of world agriculture confronted with pollinator decline. Ecological Economics 68,
       810-821.
       Giglio, A., Ammendola, A., Battistella, S., Naccarato, A., Pallavicini, A., Simeon, E., Tagarelli,
       A., Giulianini, P.G., 2017. Apis mellifera ligustica, Spinola 1806 as bioindicator for detecting
455   environmental contamination: a preliminary study of heavy metal pollution in Trieste, Italy.
       Environmental Science and Pollution Research 24, 659-665.
       Gill, R.J., Ramos-Rodriguez, O., Raine, N.E., 2012. Combined pesticide exposure severely
       affects individual-and colony-level traits in bees. Nature 491, 105-108.
       Goretti, E., Pallottini, M., Rossi, R., La Porta, G., Gardi, T., Goga, B.C., Elia, A., Galletti, M.,
460   Moroni, B., Petroselli, C., 2020. Heavy metal bioaccumulation in honey bee matrix, an indicator
       to assess the contamination level in terrestrial environments. Environmental Pollution 256,
       113388.
       Goulson, D., Nicholls, E., Botías, C., Rotheray, E.L., 2015. Bee declines driven by combined
       stress from parasites, pesticides, and lack of flowers. Science 347, 1255957.
465   Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J.-F., Aupinel, P., Aptel, J.,
       Tchamitchian, S., Decourtye, A., 2012. A Common Pesticide Decreases Foraging Success and
       Survival in Honey Bees. Science 336, 348-350.
       Insecticide Resistance Action Committee, The IRAC mode of action classification online.
       Johnson, R.M., Ellis, M.D., Mullin, C.A., Frazier, M., 2010. Pesticides and honey bee toxicity -
470   USA. Apidologie 41, 312-331.
       Lehotay, S.J., Maštovská, K., Yun, S.J., 2005. Evaluation of two fast and easy methods for
       pesticide residue analysis in fatty food matrixes. Journal of AOAC International 88, 630-638.
       Motta, E.V., Raymann, K., Moran, N.A., 2018. Glyphosate perturbs the gut microbiota of honey
       bees. Proceedings of the National Academy of Sciences 115, 10305-10310.
475   Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., Vanengelsdorp, D., Pettis,
       J.S., 2010. High Levels of Miticides and Agrochemicals in North American Apiaries:
       Implications for Honey Bee Health. PloS one 5, e9754.
       Mussen, E.C., Lopez, J.E., Peng, C.Y., 2004. Effects of selected fungicides on growth and
       development of larval honey bees, Apis mellifera L.(Hymenoptera: Apidae). Environmental
480   entomology 33, 1151-1154.
       Nauen, R., Ebbinghaus-Kintscher, U., Salgado, V.L., Kaussmann, M., 2003. Thiamethoxam is a
       neonicotinoid precursor converted to clothianidin in insects and plants. Pesticide Biochemistry
       and Physiology 76, 55-69.
       O'Neal, S.T., Anderson, T.D., Wu-Smart, J.Y., 2018. Interactions between pesticides and
485   pathogen susceptibility in honey bees. Current Opinion in Insect Science 26, 57-62.
       Pamer, E.G., 2016. Resurrecting the intestinal microbiota to combat antibiotic-resistant
       pathogens. Science 352, 535-538.
       Pettis, J.S., Lichtenberg, E.M., Andree, M., Stitzinger, J., Rose, R., vanEngelsdorp, D., 2013.
       Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut
490   Pathogen Nosema ceranae. PloS one 8, e70182.
       Poquet, Y., Vidau, C., Alaux, C., 2016. Modulation of pesticide response in honeybees.
       Apidologie 47, 412-426.
       Sanchez-Bayo, F., Goka, K., 2014. Pesticide residues and bees–a risk assessment. PloS one 9,
       e94482.

Schmehl, D.R., Teal, P.E., Frazier, J.L., Grozinger, C.M., 2014. Genomic analysis of the interaction between pesticide exposure and nutrition in honey bees (Apis mellifera). Journal of Insect Physiology 71, 177-190.

Schmickl, T., Crailsheim, K., 2004. Inner nest homeostasis in a changing environment with special emphasis on honey bee brood nursing and pollen supply. Apidologie 35, 249-263.

Simon-Delso, N., San Martin, G., Bruneau, E., Hautier, L., 2018. Time-to-death approach to reveal chronic and cumulative toxicity of a fungicide for honeybees not revealed with the standard ten-day test. Scientific reports 8, 7241.

Smith, K.E., Weis, D., Chauvel, C., Moulin, S., 2020. Honey maps the Pb fallout from the 2019 fire at Notre-Dame Cathedral, Paris: a geochemical perspective. Environmental Science & Technology Letters.

Steinhauer, N., Kulhanek, K., Antúnez, K., Human, H., Chantawannakul, P., Chauzat, M.-P., Vanengelsdorp, D., 2018. Drivers of colony losses. Current Opinion in Insect Science 26, 142-148.

Stoner, K.A., Eitzer, B.D., 2013. Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in Connecticut. 8, e77550.

Tosi, S., Nieh, J.C., Sgolastra, F., Cabbri, R., Medrzycki, P., 2017. Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. Proceedings of the Royal Society B: Biological Sciences 284, 20171711.

Traynor, K.S., Pettis, J.S., Tarpy, D.R., Mullin, C.A., Frazier, J.L., Frazier, M., VanEngelsdorp, D., 2016a. In-hive Pesticide Exposome: Assessing risks to migratory honey bees from in-hive pesticide contamination in the Eastern United States. Scientific reports 6, 33207.

Traynor, K.S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G., Madella, S., Evans, J., Lopez, D., 2016b. Multiyear survey targeting disease incidence in US honey bees. Apidologie 47, 325-347.

vanEngelsdorp, D., Tarpy, D.R., Lengerich, E.J., Pettis, J.S., 2013. Idiopathic brood disease syndrome and queen events as precursors of colony mortality in migratory beekeeping operations in the eastern United States. Preventive veterinary medicine 108, 225-233.

Visscher, P.K., Seeley, T.D., 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology 63, 1790-1801.

Wade, A., Lin, C.-H., Kurkul, C., Regan, E.R., Johnson, R.M., 2019. Combined Toxicity of Insecticides and Fungicides Applied to California Almond Orchards to Honey Bee Larvae and Adults. Insects 10, 20.

Wu, J.Y., Anelli, C.M., Sheppard, W.S., 2011. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (Apis mellifera) development and longevity. PloS one 6.

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Highlights

- A US national survey of pesticide residues in bee pollen found only 18% of samples were pesticide free.
- In our 1055 samples we made 2,933 pesticide detections, predominantly at low risk levels.
- However, some low risk residues like fungicides were linked to increased colony morbidity.
- Neonicotinoids were rarely detected (2.0%), but contributed significant risk when found.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: