DEPARTMENT OF AGRICULTURE

Pesticide Bee Kill Complaint Investigations

Summary of 2021 bee kill investigations

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Background

Under Minnesota Statute <u>18D.201</u>, the Minnesota Department of Agriculture (MDA) is the state agency responsible for investigating bee kills alleged to be caused by pesticides. The MDA's Pesticide and Fertilizer Management Division conducts the investigations.

Under Minnesota Statute <u>18B.055</u>, the MDA must compensate a bee owner for an acute pesticide poisoning resulting in the death of bees or loss of bee colonies owned by the bee owner with some stipulations. Effective July 01, 2014, the Minnesota Legislature appropriated \$150,000 per fiscal year from the pesticide regulatory account to pay compensation claims for bees killed by pesticides. In any fiscal year, a bee owner can only be compensated for claims greater than \$100 or less than \$20,000 for all eligible claims combined.

Effective August 01, 2015, the Minnesota Legislature added a provision requiring a bee owner to be registered with a commonly utilized pesticide registry program, as designated by the commissioner. The Commissioner of Agriculture has designated Beecheck (<u>https://beecheck.org/</u>), a voluntary hive mapping registry administered by FieldWatch, as the required registry program.

Details

For the MDA to respond to an alleged pesticide bee kill, complaints must be reported in writing to the Pesticide and Fertilizer Management Division. A written complaint can be completed and submitted online at the <u>Pesticide and Fertilizer Misuse Complaint webpage</u>.

Upon receipt of a written complaint, the MDA sends a team of pesticide investigators with training in bee handling/colony assessment to the site where the dead bees are located. During the investigation, samples of live/dead bees and other materials are collected to evaluate the presence of pesticides, colony pests, and overall colony health. In addition, the MDA attempts to determine the extent of pesticide use in areas adjacent to hive locations by contacting pesticide dealers, growers, and applicators in the area.

The MDA Laboratory Services Division is a state-certified Federal Insecticide Fungicide Rodenticide Act (FIFRA) laboratory and analyzes MDA samples for pesticide residues. Samples are also analyzed by the United States Department of Agriculture (USDA) Lab in Gastonia, NC.

The Bee Informed Partnership evaluates honey bee samples for *Varroa destructor*, a mite known to vector viruses and reduce bee longevity, and *Nosema spp.*, a fungal pathogen that can infect a bee's gut. The Bee Informed Partnership lab also analyzes honey bees for a set of viruses that cause adverse effects.

Once the Pesticide and Fertilizer Management Division receives analytical results, the division staff confer internally to determine the cause of the bee kill event. Individual investigation summaries are then created reviewing pesticide and colony health findings.

For bee kill investigations, the MDA uses a working definition of "acute pesticide poisoning" to capture acute pesticide effects on honey bee colonies with different populations and developmental stages. The MDA evaluates an "acute pesticide poisoning" based on the presence of non-beekeeper-applied pesticide residues in dead bees, the number of bee frames of bees (a measure of a honey bee colony's living population), and a minimum number of dead bees present in and around the hive at the time of the investigation.

- For a pesticide-related bee kill to be considered an "acute pesticide poisoning," a colony with three or fewer frames of bees present in the hive at the time of investigation must have at least 300 total dead bees in and around the colony.
- Colonies with 3.5 and 9.5 frames of bees at the time of investigation will need between 350 and 950 dead bees to be considered an "acute pesticide poisoning" (50 dead bees for every 0.5 frames of bees).
- If a colony has ten or more frames of bees at the time of investigation, a maximum of 1,000 dead bees must be quantified to be considered an "acute pesticide poisoning."

If it can be demonstrated that pesticides likely led to an "acute pesticide poisoning" and the apiary was registered on BeeCheck before the time of the acute pesticide poisoning, the beekeeper may be eligible for compensation according to <u>Minn. Stat. 18B.055</u>.

The MDA currently does not have a statewide apiary program for pollinator health inspections and does not require the registration of apiaries.

Attachments

- Summaries of the three bee kill investigations and the MDA's findings for 2021
- Pesticide analytes tested for in bee kill investigations

Program Contacts

For pesticide misuse complaint information: Christine Wicks 651-201-6390 **For bee kill compensation information:** Jamison Scholer 651-201-6303

Investigation Summary – Pesticide Bee Kill Complaint

Filmore County

Received date: May 18, 2021

Case File Number: SRK165001384

Date	July 18, 2021	July 19, 2021	July 20, 2021	Sept. 22, 2021	Oct. 18, 2021	Nov. 10, 2021	Nov. 10, 2021	June 27, 2022
Action	Call received	MDA responds	Samples sent to labs for analysis	USDA bee residue results	MDA bee residue	MDA bee residue	USDA bee residue	Case closing letter issued
				received	results	results sent	results sent	
					received	to beekeeper	to beekeeper	

Background of Bee Kill Incident and MDA Response

- The dead bees with protruding proboscises and alive bees disoriented and shaking on the ground in front of the affected hive were first observed on July 14, 2021.
- An apiary of eight honey bee colonies had one colony considered affected and was reported to the MDA on July 18, 2021.
- An MDA Agricultural Chemical Investigator and MDA Pesticide Technical Unit staff entomologists visited the site on July 19, 2021.
- The apiary was located along a patch of trees approximately 130 feet away from the nearest agricultural field border planted with corn, which surrounded the property on three sides.
- No rainfall occurred between the time of the complaint and the time of the on-site investigation.

MDA Colony Health Findings

- The one affected colony was measured for frames of live bees and contained an average of 1.25 frames of live bees/deep, indicating that a minimum threshold of 300 dead bees would be used as guidance to indicate an acute pesticide poisoning.
- Approximately 670 dead bees were collected near the affected hive, with more bees disintegrating on the ground.
- The brood pattern, a crucial indicator of the queen's health and colony productivity, scored 3 out of 5, suggesting a fair egg-laying pattern in the affected colony.
- No disease symptoms were observed in the affected colony.
- 2.88 *Varroa* mites/100 bees were found in the affected colony, approaching the recommended treatment threshold of 3 mites/100 bees.
- No *Nosema* spores/bee were found in the affected colony, which is below the 1.0 million spores/bee threshold at which adverse effects from *Nosema* are thought to be observable in colonies.
- RNA quantification in the affected colony from 12 honey bee viruses was conducted and compared to national prevalence rates. The percentiles, indicated in parenthesis, provide a comparison of viral loads from the sampled hives to the nationally compiled prevalence rates. Lower percentiles signify less pressure from a specific virus.

Black queen cell virus (96/100), Israeli acute paralysis virus (98/100), sacbrood virus (96/100), *Varroa destructor* virus (31/100), and Lake Sinai virus 2 (47/100) were found.

• A nearby, unaffected colony was used to collect live bees. Unaffected live bee samples were also submitted to the MDA laboratory for pesticide residue analysis.

MDA Pesticide Findings

- Pesticide analyses were carried out at the MDA and USDA labs. The MDA laboratory analyzed dead and live bees, and vegetation samples, while the USDA laboratory analyzed comparable samples of dead and live bees.
- The MDA laboratory pesticide residue results found beauvericin, fenpropathrin, and tebufenozide on dead bees and no pesticides on affected or unaffected live bees.
- The USDA laboratory pesticide residue results found trace amounts of metolachlor on dead bees and no pesticides on live bees.
- The beekeeper applied oxalic acid to honey bee colonies for *Varroa* mite control within 12 months of the investigation and provided the product label.
- The Agricultural Chemical Investigator canvassed the area, extending three miles in every direction from the apiary. Using the pesticides identified from analyzed dead bees and considering the pesticide's registered use sites, no potential sources for fenpropathrin or tebufenozide were identified.
- Differences in pesticide residue results between the MDA and USDA laboratories can be due to differences in sample composition, analytical methods, and/or different detection levels between laboratories. Laboratory results are displayed in the table below.

Lab	Active Ingredient (Level of Detection*)	Type of pesticide	Concentration from affected dead bees [*] (% of acute benchmark^)	Concentration from affected live bees* (% of acute benchmark^)	Concentration from unaffected live bees* (% of acute benchmark^)	Adult honey bee acute LD ₅₀ * (EPA ecotoxicity category)
MDA	beauvericin (0.2)	biological¤	1.90 and 3.61 $^{\rm v}$	No Detection	No Detection and No Detection	Not established by EPA (Practically non-toxic)
USDA	beauvericin (not screened for)	biological¤	Not Screened	Not Screened	Not Screened	Not established by EPA (Practically non-toxic)
MDA	fenpropathrin (10)	insecticide	No Detection and 1.72 (≈29%)	No Detection	No Detection and No Detection	Contact: 11.72 ¹ (Highly toxic)
USDA	fenpropathrin (25)	insecticide	No Detection	No Detection	Not Screened	Contact: 11.72 ¹ (Highly toxic)
MDA	metolachlor (250)	herbicide	No Detection	No Detection	No Detection and No Detection	Oral: >664,063 ² (Practically non-toxic)
USDA	metolachlor (25)	herbicide	Trace (<0.009%)	No Detection	Not Screened	Oral: >664,063 ² (Practically non-toxic)
MDA	tebufenozide (0.04)	insect growth regulator	No Detection and 1.19 (≈0.0001%)	No Detection	No Detection and No Detection	Contact: >2,340,000 ³ (Practically non-toxic)
USDA	tebufenozide (1)	insect growth regulator	No Detection	No Detection	Not Screened	Contact: >2,340,000 ³ (Practically non-toxic)

Laboratory Results – Pesticide Residue Analysis

* All values are in μ g/kg (microgram per killogram) which is equivalent to parts per billion (ppb).

⁴ Percentage of benchmark not calculated because the compound is unregulated and naturally occurring.

^ Benchmark = EPA's toxicity value x EPA's acute Level of Concern (LOC) for adult honey bees. The EPA's toxicity value is the acute contact or oral Lethal Dose to 50% of a honey bee population (LD₅₀) in a standardized test (whichever value is lower) and the LOC is 0.4, a pre-determined threshold set for groups of organisms such as bees. Pesticide concentrations determined from the investigation's laboratory results are divided by the benchmark and expressed as a percentage.

* Secondary metabolite produced by *Beauveria bassiana*.

- ¹ Preliminary Comparative Environmental Fate and Ecological Risk Assessment for the Registration Review of Eight Synthetic Pyrethroids and the Pyrethrins. 2016 EPA-HQ-OPP-2010-0422-0021
- ² Metolachlor/S-Metolachlor: Draft Ecological Risk Assessment for Registration Review. 2019. EPA-HQ-OPP-2014-0772-0028.
- ³ Preliminary Environmental Fate and Ecological Risk Assessment for Registration Review of the Insect Growth Regulator, Tebufenozide. 2015. EPA-HQ-OPP-2008-0824-0028.

Investigation Conclusions

- One insecticide, fenpropathrin, was found in dead bees at a concentration of 1.72 ppb, approximately 29% of the acute benchmark used to determine risk to honey bees.
- One insect growth regulator, tebufenozide, was found in dead bees at 1.19 ppb, approximately 0.0001% of the acute benchmark used to determine the risk to honey bees.
- One herbicide, metolachlor, was found at a trace amount in dead bees, less than 0.009% of the acute benchmark used to determine risk to honey bees.
- One biological compound, beauvericin, was found in dead bees. The source of the beauvericin detected in dead bee samples could be from naturally occurring *Beauveria* populations. Nevertheless, beauvericin is a known synergist and has pesticidal properties.
- Several viruses were found at high levels. It is possible that viruses acted as a stressor in the affected honey bees observed.
- It is likely that the quantified pesticide exposure directly contributed to the observed bee mortality.

The investigation concluded that the observed bee mortality was due to acute pesticide poisoning based on the ratio of live to dead bees and the quantity of insecticide residues in dead bees. The minimum threshold of bees killed for an "acute pesticide poisoning" was met (over 300 dead bees). Three pesticides were identified in dead bee samples including fenpropathrin which is considered highly toxic to honey bees. Fenpropathrin was present at approximately 29% of EPA's acute benchmark and may have been lethal to some honey bees. An Agricultural Chemical Investigator canvassed the area surrounding the apiary in every direction. However, no potential use sites were found to match the pesticides identified at higher than trance amounts from the analysis of dead bees. When the case was closed, results were reported to the Environmental Protection Agency (EPA).

Compensation – Because the beekeeper was not registered with BeeCheck before the observed mortality, they were not eligible for compensation.

Investigation Summary – Pesticide Bee Kill Complaint

Chippewa County

Received date: July 22, 2021

Case File Number: MWF160000926

Date	July 22, 2021	July 26, 2021	July 28, 2021	Sept. 22, 2021	Sept. 29, 2021	Oct. 11, 2021	April 13, 2022	Nov. 20, 2023
Action	Call received	MDA responds	Samples sent to labs for analysis	USDA bee residue results received	MDA bee residue results received	MDA/USDA bee residue results to beekeeper	Compensation claim request received from beekeeper	Case closing letter issued

Background of Bee Kill Incident and MDA Response

- An apiary of five honey bee colonies considered affected was reported to the MDA.
- The apiary was allegedly exposed to an aerial pesticide application to a nearby soybean field on the evening of July 21, 2021.
- The apiary was positioned along a patch of forest and near a field of alfalfa planted for bee forage on the bee owner's property.
- Visible symptoms included affected bees with fluttering wings, extended proboscises, and dead bees curled up in the grass.
- An MDA Agricultural Chemical Investigator and MDA Pesticide Technical Unit staff entomologists visited the site on July 26, 2021.

MDA Colony Health Findings

- One colony was measured for frames of live bees and contained an average of nine frames of live bees/deep hive box, indicating that a minimum threshold of 900 dead bees would be used as guidance to indicate an acute pesticide poisoning.
- The number of dead bees collected exceeded 1,000.
- The colony had a brood pattern of 4.5 out of 5, indicating a healthy egg-laying pattern.
- No disease symptoms were observed.
- 2.5 *Varroa* mites/100 bees were found, below the recommended treatment threshold of 3 mites/100 bees.
- 0.05 million *Nosema* spores/bee were found, which is below the 1.0 million *Nosema* spores/bee threshold at which adverse effects from *Nosema* are thought to be observable in colonies.
- RNA quantification from 12 honey bee viruses was conducted and compared to national prevalence rates. The percentiles, indicated in parenthesis, provide a comparison of viral loads from the sampled hives to the nationally compiled prevalence rates. Lower percentiles signify less pressure from a specific virus. Black queen cell virus (43/100), *Nosema ceranae* (35/100), and deformed wing virus (7/100) were found.

MDA Pesticide Findings

- Pesticide analyses were carried out at the MDA and USDA labs. The MDA laboratory analyzed dead and live bees, and vegetation samples, while the USDA laboratory analyzed comparable samples of dead and live bees.
- Pesticide residue results from the MDA laboratory found azoxystrobin, beauvericin, chlorpyrifos, and propiconazole on dead bees and no pesticides on living bees. A vegetation sample consisting of flowering alfalfa collected on the edge of the beekeeper's alfalfa field nearest the hives contained azoxystrobin, chlorpyrifos, propiconazole, and trifloxystrobin.
- The USDA laboratory pesticide residue results found azoxystrobin, chlorpyrifos, propiconazole, and trifloxystrobin, and trace amounts of metolachlor on dead bees and chlorpyrifos and trifloxystrobin on living bees.
- The beekeeper applied oxalic acid to colonies within 12 months of the investigation for *Varroa* mite control.
- Differences in pesticide residue results between the MDA and USDA laboratories can be due to differences in sample composition, analytical methods, and/or different detection levels between laboratories. Laboratory results are displayed in the table below.

Lab	Active Ingredient (Level of Detection*)	Type of pesticide	Concentration from affected dead bees [*] (% of acute benchmark [*])	Concentration from affected live bees [*] (% of acute benchmark^)	Concentration from unaffected live bees* (% of acute benchmark^)	Adult honey bee acute LD ₅₀ * (EPA ecotoxicity category)
MDA	azoxystrobin (NA)	fungicide	9,600 and 16,100 (≈12% and ≈20%)	No Detection	1,800 (≈2%)	Oral: >195,313 ¹ (Practically non-toxic)
USDA	azoxystrobin (2)	fungicide	659 (≈1%)	No Detection	Not Screened	Oral: >195,313 ¹ (Practically non-toxic)
MDA	beauvericin (0.2)	biological¤	1.43 and 1.87 [¥]	No Detection	No Detection	Not established by EPA (Practically non-toxic)
USDA	beauvericin (not screened for)	biological¤	Not Screened	Not Screened	Not Screened	Not established by EPA (Practically non-toxic)
MDA	chlorpyrifos (250)	insecticide	8,050 and 9,300 (4,366% and 5,043%)	No Detection	520 (282%)	Contact: 461 ² (Highly toxic)
USDA	chlorpyrifos (5)	insecticide	855 (464%)	10 (≈5%)	Not Screened	Contact: 461 ² (Highly toxic)
MDA	metolachlor (250)	herbicide	No Detection	No Detection	No Detection	Oral: >664,063 ³ (Practically non-toxic)
USDA	metolachlor (25)	herbicide	Trace (<0.01%)	No Detection	Not Screened	Oral: >664,063 ³ (Practically non-toxic)
MDA	propiconazole (NA)	fungicide	11,200 and 12,400 (≈4%)	No Detection	1,500 (≈0.5%)	Contact: >781,250 ⁴ (Practically non-toxic)
USDA	propiconazole (5)	fungicide	579 (≈0.2%)	No Detection	Not Screened	Contact: >781,250 ⁴ (Practically non-toxic)
MDA	trifloxystrobin (NA)	fungicide	Not Screened	Not Screened	59.8 (≈0.01%)	Contact: >1,562,500 ⁵ (Practically non-toxic)
USDA	trifloxystrobin (1)	fungicide	47 (≈0.01%)	14 (≈0.002%)	NA	Contact: >1,562,500 ⁵ (Practically non-toxic)

Laboratory Results - Pesticide Residue Analysis

* All values are in μ g/kg (microgram per killogram) which is equivalent to parts per billion (ppb).

^v Percentage of benchmark not calculated because the compound is unregulated and naturally occurring. ^ Benchmark = EPA's toxicity value x EPA's acute Level of Concern (LOC) for adult honey bees. The EPA's toxicity value is the acute contact or oral Lethal Dose to 50% of a honey bee population (LD₅₀) in a standardized test (whichever value is lower) and the LOC is 0.4, a pre-determined threshold set for groups of organisms such as bees. Pesticide concentrations determined from the investigation's laboratory results are divided by the benchmark and expressed as a percentage.

^a Secondary metabolite produced by *Beauveria bassiana*.

- ¹ Registration Review: Draft Risk Assessment of the Environmental Fate and Ecological Risk of Azoxystrobin. 2015. EPA-HQ-OPP-2009-0835-0024
- ² Chlorpyrifos: Draft Ecological Risk Assessment for Registration Review. 2020. EPA-HQ-OPP-2008-0850-0940
- ³ Metolachlor/S-Metolachlor: Draft Ecological Risk Assessment for Registration Review. 2019. EPA-HQ-OPP-2014-0772-0028
- ⁴ Propiconazole: Draft Ecological Risk Assessment for Registration Review. 2020. EPA-HQ-OPP-2015-0459-0029
- ⁵ Revised Ecological Risk Assessment for the Registration Review of Trifloxystrobin. 2018. EPA-HQ-OPP-2013-0074-0041

Investigation Conclusions

- One insecticide, chlorpyrifos, was found in dead bees, live bees, and on the vegetation sample. The concentration of chlorpyrifos found on dead bees was extremely high, up to 5,043% of the acute benchmark used to determine risk to honey bees.
- Three fungicides, azoxystrobin, propiconazole, and trifloxystrobin, were found in some samples. Concentrations of propiconazole and trifloxystrobin were found at relatively low levels. The highest detection was azoxystrobin, found in dead bees at levels up to 20% of the acute benchmark used to determine risk to honey bees.
- One herbicide, metolachlor, was found at trace amounts in dead bees.
- One biological compound, beauvericin, was found in dead bees. The source of the beauvericin detected in dead bee samples could be from naturally occurring *Beauveria* populations. Nevertheless, beauvericin is a known synergist and has pesticidal properties.
- Five days and a rainfall event passed between when the beekeeper noticed mortality and when samples were collected. Due to this lapse of time, some amount of pesticide likely degraded before sample collection, and the quantity of pesticide detected is likely lower than what the bees were initially exposed to.
- Affected colonies appeared healthy aside from the large number of dead bees quantified, >1,000 in and around hives. No pests or pathogens were found at concerning levels.
- It is likely that the quantified pesticide exposure directly contributed to the observed bee mortality.

The investigation concluded that the observed bee mortality was due to acute pesticide poisoning based on the ratio of live to dead bees and the quantity of insecticide residues present in dead bees. The minimum threshold of bees killed for an "acute pesticide poisoning" was met (over 1,000 dead bees). Five pesticide residues were found in dead bees, including one insecticide, chlorpyrifos, which is considered highly toxic to honey bees and was present at 5,043% of EPA's acute benchmark. The responsible party was identified and determined not to have applied the pesticide in a manner consistent with the pesticide's product label or labeling. Appropriate enforcement action was taken against the responsible party. When the case was closed, results were reported to the EPA.

Compensation – The beekeeper was registered with BeeCheck and the investigation concluded bee mortality was considered an acute pesticide poisoning. The beekeeper submitted a compensation claim and was compensated at the fair market value for the acute pesticide poisoning of their five hives.

Investigation Summary – Pesticide Bee Kill Complaint

Wright County

Received date: July 30, 2021

Case File Number: SRK165001405

Date	July 30, 2021	August 3, 2021	August 4, 2021	Sept. 22, 2021	Sept. 27, 2021	Sept. 30, 2021	July 27, 2022
Action	Call received	MDA responds	Samples sent to labs for analysis	USDA bee residue results received	MDA bee residue results received	MDA/USDA bee residue results sent to beekeeper	Case closing letter issued

Background of Bee Kill Incident and MDA Response

- An apiary of 28 honey bee colonies considered affected were reported to the MDA in writing on July 30, 2021.
- Conservation reserve lands surrounded the apiary on three sides, and a corn field was located across the road on the remaining side.
- An MDA Agricultural Chemical Investigator visited the site on August 3, 2021.

MDA Colony Health Findings

- No colony health assessments were conducted due to the absence of live bees in the apiary's hive boxes.
- Investigators observed many dead bees, over 1,000, and collected samples for pesticide residue analysis.
- Due to the lack of live bees, pest and disease samples could not be collected for laboratory analysis.

MDA Pesticide Findings

- Pesticide residue analyses were carried out at the MDA laboratory for dead bee and vegetation samples and a USDA laboratory for dead bee samples.
- The MDA laboratory pesticide residue results found amitraz DMPF, beauvericin, chlorpyrifos, and piperonyl butoxide on dead bees. Vegetation samples of unspecified vegetation/grasses were collected near the apiary, where no known pesticides had been applied in 2021, chlorpyrifos was detected on the vegetation sample.
- The USDA laboratory pesticide residue results found amitraz 2,4-DMPF, chlorpyrifos, piperonyl butoxide, and thymol on dead bee samples.
- The beekeeper applied oxalic acid dihydrate to honey bee colonies for *Varroa* mite control within 12 months of the investigation and provided a label print-off for the product used.
- Differences in pesticide residue results between the MDA and USDA laboratories can be due to differences in sample composition, analytical methods, and/or different detection levels between laboratories. Laboratory results are displayed in the table below.

Laboratory Results - Pesticide Residue Analysis

Lab	Active Ingredient (Level of Detection*)	Type of pesticide	Concentration from affected dead bees [*] (% of acute benchmark [^])	Concentration from vegetation [*] (% of acute benchmark^)	Adult honey bee acute LD ₅₀ * (EPA ecotoxicity category)
MDA	amitraz DMPF (0.4)	insecticide/acaricide degradate	272 - 409 (<0.09% - <0.13%)	No Detection	Parent contact: >781,250 ¹ (Practically non-toxic)
USDA	amitraz 2,4- DMPF (25)	insecticide/acaricide degradate	147 - 180 (<0.05% - <0.06%)	Not Screened	Parent contact: >781,250 ¹ (Practically non-toxic)
MDA	beauvericin (0.2)	biological [¤]	2.15 - 43.5 [¥]	No Detection	Not established by EPA (Practically non-toxic)
USDA	beauvericin (not screened for)	biological [¤]	Not Screened	Not Screened	Not established by EPA (Practically non-toxic)
MDA	chlorpyrifos (25)	insecticide	16,000 - 137,000 (≈8,677% - ≈74,295%)	142 (≈77%)	Contact: 461 ² (Highly toxic)
USDA	chlorpyrifos (5)	insecticide	16,600 - 103,000 (≈9,002% - ≈55,857%)	Not Screened	Contact: 461 ² (Highly toxic)
MDA	piperonyl butoxide (NA)	synergist	280 - 2,850 (≈0.4% - ≈3.6%)	No Detection	Contact: >195,312 ³ (Practically non-toxic)
USDA	piperonyl butoxide (3)	synergist	113 – 632 (≈0.14% - ≈0.81%)	Not Screened	Contact: >195,312 ³ (Practically non-toxic)
MDA	thymol (not screened for)	biopesticide	Not Screened	Not Screened	Contact: 400,390 ⁴ (Practically non-toxic)
USDA	thymol (25)	biopesticide	65 and 110 (≈0.04% - ≈0.07%)	Not Screened	Contact: 400,390 ⁴ (Practically non-toxic)

* All values are in μ g/kg (microgram per killogram) which is equivalent to parts per billion (ppb).

^v Percentage of benchmark not calculated because the compound is unregulated and naturally occurring.

^ Benchmark = EPA's toxicity value x EPA's acute Level of Concern (LOC) for adult honey bees. The EPA's toxicity value is the acute contact or oral Lethal Dose to 50% of a honey bee population (LD_{50}) in a standardized test (whichever value is lower) and the LOC is 0.4, a pre-determined threshold set for groups of organisms such as bees. Pesticide concentrations determined from the investigation's laboratory results are divided by the benchmark and expressed as a percentage.

^a Secondary metabolite produced by *Beauveria bassiana*.

¹ Amitraz: Preliminary Ecological Risk Assessment and Endangered Species Assessment for Registration Review of the Conventional Use in Honey Bee Hives. 2018. DP Barcode: 435890

² Chlorpyrifos: Draft Ecological Risk Assessment for Registration Review. 2020. EPA-HQ-OPP-2008-0850-0940

³ Piperonyl Butoxide (PBO): Preliminary Ecological Risk Assessment for Registration Review. 2017. EPA-HQ-OPP-2010-0498-0025

⁴Thymol: Registration Review Draft Risk Assessment. 2019. EPA-HQ-OPP-2010-0002

Investigation Conclusions

- One insecticide, chlorpyrifos, and two insecticide degradants, amitraz DMPF and 2,4-DMPF, were found in dead bees. The concentration of chlorpyrifos found in dead bees was extremely high, up to 74,295% of the acute benchmark used to determine risk to honey bees.
- One biological compound, beauvericin, was found in dead bees. The source of the beauvericin detected in dead bee samples could be from naturally occurring *Beauveria* populations. Nevertheless, beauvericin is a known synergist and has pesticidal properties.
- One pesticide synergist, piperonyl butoxide, was found in dead bees at relatively low levels.
- One biopesticide, thymol, typically applied by beekeepers, was found in dead bees in low levels up to 0.07% of the acute benchmark used to determine risk to honey bees.
- Five days passed between when the beekeeper noticed mortality and when samples were taken. Due to this lapse of time, it is likely that some amount of pesticide degraded, and the quantity of pesticide detected was lower than what the bees were initially exposed to.
- No living bees remained in the apiary to assess for pests or pathogens.
- It is likely that the quantified pesticide exposure directly contributed to the observed bee mortality.

The investigation concluded that this bee kill event met the criteria for acute pesticide poisoning. Investigators quantified the minimum number of dead bees (over 1,000 dead bees), and an insecticide considered highly toxic to honey bees was found well above the acute benchmark for honey bees (74,295% of the acute benchmark for chlorpyrifos).

Compensation – Because the beekeeper was not registered with BeeCheck before the observed mortality they were not eligible for compensation.

MDA and USDA Pesticide Analyte List Used in Bee Kill Investigations

#	Analyte	MDA Limit of Detection*	USDA Limit of Detection*
1	1-Naphthol	Not screened	50
2	2,4 Dimethylphenyl formamide (DMPF)	Not screened	5
3	2, 6-Dichlorobenzamide (BAM)	Not screened	10
4	3-Hydroxycarbofuran	Not screened	10
5	4-Hydroxychlorothalonil	Not screened	10
6	Abamectin	16	100
7	Acephate	16	50
8	Acequinocyl	Not screened	100
9	Acetamiprid	0.2	4
10	Acetochlor	25	15
11	Acrinathrin	Not screened	20
12	Afidopyropen	4	Not screened
13	Alachlor	25	15
14	Aldicarb	16	25
15	Aldicarb sulfone	0.4	15
16	Aldicarb sulfoxide	1.2	25
17	Allethrin	4	Not screened
18	Amitraz DMPF	0.4	Not screened
19	Amitraz DMPMF	0.2	Not screened
20	Ametoctradin	Not screened	10
21	Atrazine	25	4
22	Azinphos methyl	50	50
23	Azoxystrobin	Not screened	10
24	Beauvericin	0.2	Not screened
25	Bendiocarb	0.2	Not screened
26	Bensulide	Not screened	10

#	Analyte	MDA Limit of Detection*	USDA Limit of Detection*
27	Bentazon	Not screened	10
28	Bifenazate	0.2	10
29	Bifenthrin	1.2	10
30	Boscalid	Not screened	10
31	Bromacil	Not screened	20
32	Bromophos-methyl	25	Not screened
33	Bromopropylate	Not screened	20
34	Bromuconazole	Not screened	10
35	Buprofezin	Not screened	10
36	Captan	Not screened	50
37	Carbaryl	0.2	2
38	Carbendazim	Not screened	5
39	Carbofuran	0.2	10
40	Carbofuran, 3-Keto	0.2	Not screened
41	Carbofuran, 3-OH	1.2	Not screened
42	Carfentrazone-ethyl	Not screened	20
43	Chlorantraniliprole	0.4	15
44	Chlorfenopyr	Not screened	20
45	Chlorfenvinphos	25	10
46	Chlorothalonil	25	20
47	Chlorpropham (CIPC)	Not screened	10
48	Chlorpyrifos	25	20
49	Chlorpyrifos methyl	Not screened	20
50	Clofentezine	1.2	6
51	Clomazone	25	Not screened
52	Clothianidin	1.2	15
53	Coumaphos	50	3
54	Coumaphos oxon	Not screened	2
55	Cyantraniliprole	0.4	25
56	Cyazofamid	Not screened	30
57	Cyflufenamid	Not screened	10

#	Analyte	MDA Limit of Detection*	USDA Limit of Detection*
58	Cyflumetofen	Not screened	10
59	Cyfluthrin	5	10
60	Cyhalothrin	5	10
61	Cymiazole	Not screened	10
62	Cymoxanil	Not screened	10
63	Cypermethrin	5	10
64	Cyphenothrin	5	100
65	Cyprodinil	Not screened	10
66	Cyromazine	Not screened	25
67	DCPA	Not Screened	20
68	DDE, p, p'	Not Screened	5
69	DEET	Not Screened	10
70	Deisopropylatrazine	25	Not Screened
71	Deltamethrin/Tralomethrin	5	50
72	Desethylatrazine	25	Not Screened
73	Diazinon	25	15
74	Diazinon oxon	Not Screened	5
75	Dichlorvos (DDVP)	250	15
76	Dicloran	Not Screened	20
77	Dicofol	Not Screened	5
78	Difenoconazole	Not Screened	10
79	Diflubenzuron	Not Screened	5
80	Dimethenamid	25	10
81	Dimethoate	0.2	15
82	Dimethomorph	Not Screened	25
83	Dinotefuran	1.2	10
84	Dinotefuran UF	1.2	Not Screened
85	Diphenamid	Not Screened	3
86	Diphenylamine	Not Screened	20
87	Diuron	Not Screened	6
88	Emamectin Benzoate	4	5

#	Analyte	MDA Limit of Detection*	USDA Limit of Detection*
89	Endosulfan I	Not Screened	20
90	Endosulfan II	Not Screened	20
91	Endosulfan sulfate	Not Screened	20
92	Epoxiconazole	Not Screened	10
93	EPTC	25	Not Screened
94	Esfenvalerate/Fenvalerate	5	10
95	Ethion	Not Screened	15
96	Ethalfluralin	25	Not Screened
97	Ethofumesate	Not Screened	20
98	Ethoxyquin	Not Screened	10
99	Etofenprox	0.2	5
100	Etoxazole	0.2	5
100	Famoxadone	Not Screened	25
101	Fenamidone	Not Screened	30
102	Fenarimol	Not Screened	10
104	Fenazaquin	Not Screened	5
105	Fenbuconazole	Not Screened	15
106	Fenhexamid	Not Screened	10
107	Fenoxaprop-ethyl	Not Screened	15
108	Fenporpathrin	0.4	Not Screened
109	Fenpropathrin	10	10
110	Fenpyroximate	Not Screened	4
111	Fenthion	25	Not Screened
112	Fipronil	25	20
113	Fipronil sulfide	Not Screened	5
114	Fipronil sulfone	Not Screened	5
115	Flonicamid	Not Screened	15
116	Flubendiamide	0.4	Not Screened
117	Fludioxonil	Not Screened	60
118	Flumethrin	10	100

#	Analyte	MDA Limit of Detection*	USDA Limit of Detection*
120	Fluopicolide	Not Screened	10
121	Fluopyram	Not Screened	5
122	Fluoxastrobin	Not Screened	5
123	Flupyradifurone	0.4	25
124	Fluridone	Not Screened	5
125	Flutriafol	Not Screened	10
126	Fluvalinate	5	10
127	Fluxapyroxad	Not Screened	10
128	Fonofos	25	Not Screened
129	Formetanate	Not Screened	25
130	Fpr,etamate HCl	0.4	Not Screened
131	Hexazinone	Not Screened	10
132	Hexythiazox	Not Screened	15
133	Imazalil	Not Screened	20
134	Imidacloprid	0.4	6
135	Imidacloprid desnitro (HCl)	4	Not Screened
136	Imidacloprid olefin	1.6	Not Screened
137	Imidacloprid urea	0.2	Not Screened
138	Imidacloprid, 5-OH	1.6	Not Screened
139	Imidprothrin	5	Not Screened
140	Indoxacarb	Not Screened	30
141	lprodione	Not Screened	20
142	Kresoxim-methyl	Not Screened	10
143	Linuron	Not Screened	15
144	Malathion	25	10
145	Mandipropamide	Not Screened	10
146	Metalaxyl Total	Not Screened	5
147	Metazachlor	25	Not Screened
148	Metconazole	Not Screened	10
149	Methamidophos	1.2	40
150	Methidathion	25	5

#	Analyte	MDA Limit of Detection*	USDA Limit of Detection*
151	Methiocarb	0.2	Not Screened
152	Methiocarb sulfone	0.2	Not Screened
153	Methiocarb sulfoxide	0.2	Not Screened
154	Methomyl	0.2	25
155	Methomyl oxime	1.2	Not Screened
156	Methomyl sulfoxide	16	Not Screened
157	Methyl parathion	25	Not Screened
158	Methoprene	Not Screened	80
159	Methoxyfenozide	Not Screened	5
160	Metofluthrin	5	Not Screened
161	Metolachlor	25	5
162	Metolcarb	1.2	Not Screened
163	Metribuzin	25	10
164	Mevinphos	25	Not Screened
165	MGK-264	Not Screened	25
166	Momfluorothrin	Not Screened	20
167	Monocrotophos	0.2	Not Screened
168	Myclobutanil	Not Screened	15
169	Naled	Not Screened	50
170	Nithiazine	1.2	Not Screened
171	Norflurazon	Not Screened	15
172	Norflurazon desmethyl	Not Screened	25
173	Novaluron	1.2	30
174	Omethoate	0.4	50
175	Oxamyl	0.4	15
176	Oxamyl oxime	1.2	10
177	Oxyfluorfen	Not Screened	10
178	Parathion	Not Screened	10
179	Parathion methyl	Not Screened	10
180	Penconazole	Not Screened	10
181	Pendimethalin	25	10

#	Analyte	MDA Limit of Detection*	USDA Limit of Detection*
182	Penthiopyrad	Not Screened	10
183	Permethrin	10	30
184	Phenothrin	5	30
185	Phophamidon-b	25	Not Screened
186	Phorate	25	10
187	Phosalone	Not Screened	10
188	Phosmet	25	20
189	Phosmet OA	Not Screened	10
190	Picoxystrobin	Not Screened	10
191	Piperonyl butoxide	Not Screened	15
192	pirimiphos-ethyl	25	Not Screened
193	Pirimiphos-ethyl	0.2	Not Screened
194	Prallethrin	10	50
195	Prodiamine	Not Screened	100
196	Profenofos	Not Screened	10
197	Prometon	25	10
198	Prometryn	Not Screened	10
199	Pronamide	Not Screened	10
200	Propachlor	25	25
201	Propamocarb hydrochloride	Not Screened	10
202	Propanil	Not Screened	5
203	Propargite	Not Screened	15
204	Propazine	25	10
205	Propetamphos	Not Screened	20
206	Propiconazole	Not Screened	15
207	Propoxur	4	Not Screened
208	Prothioconazole	Not Screened	125
209	Pymetrozine	Not Screened	30
210	Pyraclostrobin	Not Screened	5
211	Pyridaben	Not Screened	5
212	Pyrofluquinazon	0.2	Not Screened

	Analuta	MDA	USDA
#	Analyte	Limit of Detection*	Limit of Detection*
213	Pyrimethanil	Not Screened	15
214	Pyriproxyfen	Not Screened	5
215	Quinoxyfen	Not Screened	10
216	Quintozene	Not Screened	10
217	Resmethrin	8	30
218	Sethoxydim	Not Screened	10
219	Simazine	25	50
220	Spinetoram	Not Screened	100
221	Spinetoram J	1.2	Not Screened
222	Spinetoram L	20	Not Screened
223	Spinosad	Not Screened	15
224	Spinosad A	1.2	Not Screened
225	Spinosad D	4	Not Screened
226	Spirodiclofen	0.4	10
227	Spiromesifen	1.2	10
228	Spirotetramat	0.4	30
229	Sulfotep	25	Not Screened
230	Sulfoxaflor	0.4	25
231	Tebuconazole	Not Screened	15
232	Tebufenozide	0.04	5
233	Tebuthiuron	Not Screened	15
234	Tefluthrin	5	10
235	Terbufos	25	Not Screened
236	Tetraconazole	Not Screened	15
237	Tetradifon	Not Screened	10
238	Tetramethrin	0.2	30
239	Thiabendazole	Not Screened	5
240	Thiacloprid	0.2	5
241	Thiamethoxam	0.4	10
242	Thiodicarb	0.2	Not Screened
243	ТНЫ	Not Screened	15

#	Analyte	MDA Limit of Detection*	USDA Limit of Detection*
244	Thymol	Not Screened	10
245	Tioxazafen	4.8	Not Screened
246	Tolfenpyrad	0.2	10
247	Triadimefon	Not Screened	10
248	Triadimenol	Not Screened	25
249	Triallate	25	Not Screened
250	Triazophos	Not Screened	10
251	Tribufos	Not Screened	10
252	Trifloxystrobin	Not Screened	10
253	Triflumizole	Not Screened	10
254	Triflumuron	4	Not Screened
255	Trifluralin	25	10
256	Triticonazole	Not Screened	30
257	Vinclozolin	25	10

Detection limits are calculated based on the instrumental minimum detectable amount.

* The detection limit was estimated based on the spike response and all values are in μ g/kg (microgram per killogram) which is equivalent to parts per billion (ppb).