***June** 2017*

Summary of MDA Pesticide Bee Kill Complaint Investigations in 2016

Background

Under Minnesota Statutes, Chapter 18D.201, the Minnesota Department of Agriculture (MDA) is the state agency responsible for the investigation of bee kills alleged to be caused by pesticides. The MDA's Pesticide & Fertilizer Management Division conducts the investigations.

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 pesticide. In any fiscal year, a bee owner must not be compensated for a claim that is less than $100 or compensated more than $20,000 for all eligible claims.

Effective August 01, 2015, the Minnesota Legislature added a provision that requires 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, https://beecheck.org/, a voluntary hive mapping registry administered by FieldWatch.

Details

In order for the MDA to respond to an alleged pesticide bee kill, complaints must be reported in writing to the Pesticide & Fertilizer Management Division. A written complaint can be completed and submitted online at http://www.mda.state.mn.us/chemicals/pesticides/complaints/misusecomplaints.aspx

Upon receipt of a written complaint, the MDA sends a team of pesticide investigators with training in pesticide investigations and bee handling/colony assessment to the site where the dead bees are located.

Samples of live/dead bees and foliage are taken to determine 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 through contacting pesticide dealers, growers, and applicators in the area.

The MDA Laboratory Services Division (LSD) is a State Federal Insecticide Fungicide Rodenticide Act (FIFRA) laboratory and analyzes MDA samples for pesticide residues. Samples may also be analyzed by the USDA Lab in Gastonia, North Carolina under contract to the MDA.

Additional bee samples are sent to the University of Maryland to be evaluated for the mite, *Varroa destructor*, known to vector viruses and reduce bee longevity and the fungal pathogen, *Nosema* spp. that invades a bee's gut, causing adverse effects. North Carolina State University evaluates samples for a set of bee viruses that cause adverse effects.

Once analytical results are received by the Pesticide & Fertilizer Management Division, the MDA conducts an internal review of all of the evidence collected and attempts to determine the cause of the bee kill as well as provide an underlying assessment of colony health. Individual investigation summaries are created for each bee kill.

For 2016 bee kill investigations, the MDA used a working definition of an "acute pesticide poisoning" intended to capture acute pesticide effects to honey bee colonies at different developmental stages. The MDA evaluates an "acute pesticide poisoning" based on the presence of non-beekeeper applied pesticide residues present in dead bees; the number of 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 3 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 between 3.5 and 9.5 frames of bees quantified 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 .5 frame of bees).

- If a colony has 10 or more frames of bees at the time of investigation, a maximum of a 1,000 dead bees need to be quantified to be considered an "acute pesticide poisoning".

If it can be demonstrated that a non-beekeeper applied pesticides likely caused an "acute pesticide poisoning" and the apiary is in compliance with the pesticide registry program requirements, the MDA considers the following compensation options:

- If the person who applied the pesticide can be identified, and applied the pesticide in a manner inconsistent with the pesticide product's label or labeling, the MDA may issue an enforcement action against the applicator that includes a financial penalty sufficient to compensate the beekeeper;

- If the person who applied the pesticide can be identified, and applied the pesticide in a manner consistent with the pesticide product's label or labeling, then compensation to the beekeeper may be made from the pesticide regulatory account; or

- If an applicator cannot be identified, the MDA may compensate the beekeeper from the pesticide regulatory account.

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

Attachments

- Summaries for the four (4) pesticide bee kill complaints and the MDA's findings for 2016
- Terms and Definitions
- Pesticide Analyte Lists Used in Bee Kill Investigations

Program Contacts

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For bee kill compensation information:
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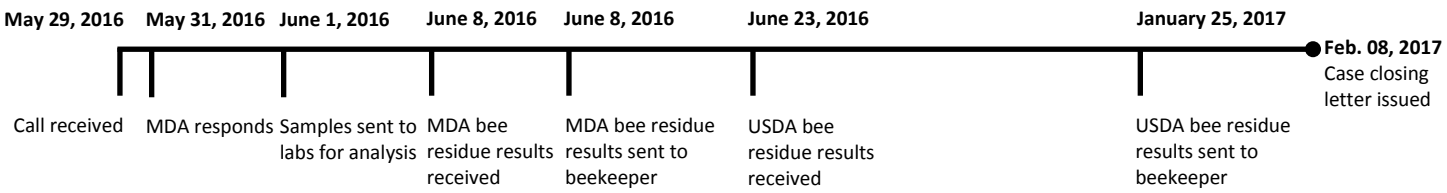
Washington County, Grant **Case File Number: SPE106000302**

Received date: May 29, 2016



Background of Complaint and Minnesota Department of Agriculture (MDA) Response

- In mid-April a hobby beekeeper received and installed two honey bee colonies from packages into hive bodies consisting of a single deep. At the time of installing packages, colonies were fed Pro-Sweet and pollen patties.
- The apiary was located on a multi-acre plot and buffered by a lake and trees.
- At the time of the investigation it was raining.
- On May 29, 2016, the beekeeper reported piles of dead bees outside hive entrances, bees crawling on the ground, and yellow excrement on hive bodies.
- MDA Agricultural Chemical Investigators (ACI) along with an MDA Pesticide Management Unit (PMU) staff entomologist responded on May 31, 2016.
- Investigators examined 2 colonies considered affected by the beekeeper and confirmed dead and crawling bees near hive entrances.
- Using the 2 examined colonies, bees were collected and combined to form composite samples used to evaluate colony stress from *Varroa*, *Nosema*, and common viruses.
- Using the same 2 colonies, frames of bees, brood pattern, and observations of disease were made.
- The MDA obtained composite samples for pesticide residue analysis using the same colonies selected to evaluate hive health. Composite samples included live bees taken from frames consisting of nectar and pollen and dead bees located in front of sampled colonies.

MDA Colony Health Findings

- Colonies contained a mean of 5 or 6 frames of bees, indicating that a 500 or 600 dead bee threshold would be used as guidance to indicate an acute pesticide poisoning.
- Colonies had sustained a minimum population loss between ≈675 and >1,000 dead bees in and around hive entrances.
- Colonies had a brood pattern of 3 to 4 indicating an average to above average laying pattern.
- Colonies had yellow excrement on hive bodies but no other signs of disease were present.
- Results from the *Varroa* sample showed .89 *Varroa* mites/100 bees indicating a level of pressure below the treatment threshold of 3 mites/100 bees.
- Individual bees tested for *Nosema* were found to be free from *Nosema* spores.
- Viral RNA was quantified in colonies sampled and compared to baseline averages. Result indicated colonies were free from the viruses screened.

MDA Pesticide Findings

- The beekeeper did not apply pesticides to their yard or hives in 2016.
- Pesticide analysis was carried out at the MDA and USDA Labs.
- MDA laboratory pesticide results found a single pesticide detection of clothianidin at 2.4 ppb. No pesticides were detected by USDA's lab due to a difference of laboratory analytical methods resulting in a higher limit of detection.
- Investigation follow-up determined that a clothianidin insecticide application had been made to a nearby orchard according to label on May 15, 2016, 14 days prior to the incident being reported. However, the orchard was not in bloom eliminating the trees as a possible exposure point.

Laboratory Results

Laboratory	Active Ingredient (Analytical Lab's Level of Detection [µg/kg])	Quantified sample concentration (µg/kg = parts per billion [ppb])	
		Affected dead bees	Affected live bees
		(% of acute oral benchmark)**	
MDA	Clothianidin[1] (0.4)	2.40 ppb (16.2%)	<LOD*
USDA	Clothianidin[1] (1.0)	<LOQ*	<LOQ*

* If USDA laboratory sample, analyte is not present at the level of quantification (LOQ); if MDA laboratory sample, analyte is not present at the level of detection (LOD).

** Benchmark = EPA's toxicity value x the Level of Concern (LOC). Where 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 is lower, and the LOC is 0.4. Laboratory results are divided by the benchmark and expressed as a percentage.

[1] Registration Review: Problem Formulation for the Environmental Fate and Ecological Risk, Endangered Species, and Drinking Water Exposure Assessment of Clothianidin. DP Barcode: 391491. USEPA. December 13, 2011.
Oral LD_{50} used for calculating percent of acute benchmark.

Investigation Conclusions

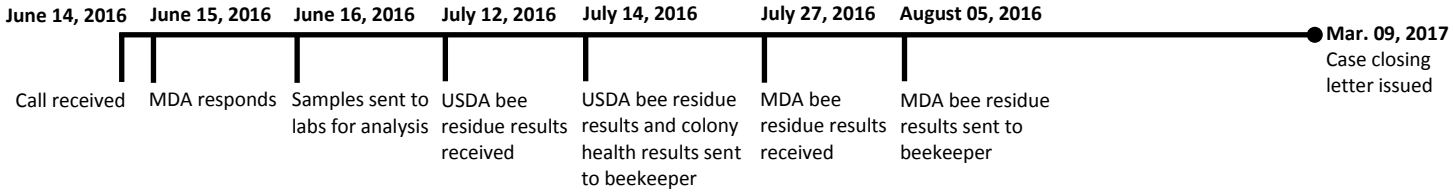
- Clothianidin residues were detected in dead bees at relatively low levels, 2.4 ppb. However, considerable degradation may have occurred due to rain and light exposure before samples were collected. Additional consideration should be given to residues quantified below 10 ppb, as these values are normally associated with a larger margin of error due to the current analytical techniques available to the scientific community.
- Investigators were able to find a producer in the area that had used a clothianidin product 14 days prior to the observed mortality. However, the application was made according to label and occurred after trees had finished blooming.
- Despite the relatively low residues of clothianidin found on/in dead bees, and given that colonies appeared to be in relatively good health (low *Varroa*, *Nosema,* and virus), clothianidin may have acted as a stressor and therefore contributed either directly or indirectly to the observed bee mortality.
- When the case was closed, results were reported to the EPA.

Compensation

Because the beekeeper was not registered with BeeCheck before the incident, the beekeeper did not qualify for compensation. While the beekeeper does not qualify for compensation due to statutory requirements, the incident is considered an acute pesticide poisoning as defined by the MDA.

Location 1: Meeker County
Location 2: Le Sueur County
Received date: June 14, 2016

Case File Number: PTH101071129



Background of Complaint and Minnesota Department of Agriculture (MDA) Response

- On June 14, 2016 MDA received a call from a commercial beekeeper reporting approximately ≥900 of their colonies had significantly diminished colony populations with dead and crawling bees near hive entrances.
- The beekeeper was concerned that applicators in the area were applying Lorsban and Warrior (products containing the active ingredient: chlorpyrifos) to alfalfa.
- Two teams of MDA Agricultural Chemical Investigators, each with an MDA Pesticide Management Unit staff entomologist, responded on June 15, 2016 and visited a total of 5 apiaries representing approximately 20% of affected colonies.
- Land surrounding apiaries varied in its use, ranging from forested land with no visible nearby agricultural activities, to apiaries with adjacent agricultural fields.
- Colonies had been overwintered in Texas and some used to pollinate California's almond crop before being brought back to Minnesota at the end of April, 2016.
- Colony health samples were collected and compiled as composite samples. Samples were evaluated for *Varroa, Nosema,* and common viruses.
- During the collection of colony health samples, frames of bees and brood pattern were quantified and observations of disease noted.

The same colonies selected to evaluate colony health were also used to collect honey bees for pesticide residue analysis. Composite samples included live bees taken from frames consisting of nectar and pollen. Dead bee samples were collected from individual colonies and combined to make composite samples when possible; however in some instances dead bees were collected by apiary. Vegetative samples were collected from 3 apiaries and consisted of vegetation from apiary margins or ditches nearest agricultural fields.

MDA Colony Health Findings

- At the time of the investigation hives consisted of two deep brood boxes with a varying number of honey boxes.
- Investigators confirmed symptoms of crawling and dead bees near hive entrances and low colony populations in colonies evaluated.
- Colonies contained approximately 2.75 to 8 frames of bees, indicating that a 300 to 800 dead bee threshold would be used as guidance to indicate an acute pesticide poisoning.
- Using a graduated cylinder, the number of dead bees per colony was estimated between 270 and 729. For 3 apiaries a total apiary dead bee count was performed and indicated mortality between 270 and >1,000 bees. However, apiaries had long grass and decomposition of bees had likely occurred since the incident began. Heavy rain had also occurred in the days prior to the investigation which likely further contributed to degradation.
- All examined colonies had eggs present and an average brood pattern of 4. Observations indicated the queen had been present within the last 3 days and that the queens laying pattern was above average.

- According to the beekeeper, colonies had been treated with two miticides while in Texas. Oxalic acid was applied in February before almond pollination and Apigaurd (active ingredient: thymol) was applied in April before colonies were brought back to Minnesota.
- At the time of the investigation, there was a thymol treatment present on some colonies. Apigaurd label language indicates the application should be present for a maximum of 6 weeks. Because of the time it takes to apply an application to all colonies (multiple weeks), and lack of application notes, it was unclear the exact length of time the application had been present. Label stipulations and their importance were reviewed with the beekeeper.
- Results from the *Varroa* analysis showed between 0 and .94 *Varroa* mites/100 bees indicating a level of pressure from *Varroa* considered below the treatment threshold of 3 mites/100 bees. Individual bees tested for the presence of *Nosema* spores were found to have between 0 and .65 million spores/bee, indicating a level of pressure from *Nosema* below the level considered to cause damage to colonies, 1.0 million spores/bee.
- No signs of disease were present in any of the colonies evaluated.
- Viral RNA was quantified for colonies sampled and compared to baseline averages. Result indicated virus levels were significantly lower than average for Black Queen Cell Virus and Deformed Wing Virus while Israeli Acute Paralysis Virus was found in 4 samples at higher than average levels.

MDA Pesticide Findings

- MDA evaluated 18 colonies (4 colonies in each of 3 apiaries and 6 colonies in 1 apiary). Combined samples represent bees taken from 2 colonies and included live bees, dead bees (in 3 of the apiaries dead bees were collected as a 'total' apiary count), and foliage samples were collected for 3 of the apiaries. No control samples were collected as all apiaries visited were considered affected.
- Pesticide analysis were carried out at the MDA Lab and the USDA Lab.
- Analytical results found miticide residues of 3 amitraz degradates (amitraz DMPF, amitraz DMPMF, amitraz 2,4-DMA) and thymol in either live or dead bees for all apiaries investigated at concentrations not expected to cause mortality to bees.
- Three apiaries had atrazine (a broadleaf herbicide) present in dead bees.
- Two apiaries had metolachlor (a grass and broadleaf herbicide) present in dead bees.
- One of the four inspected apiaries was found to have desethylatrazine (an amitraz degradate), clothianidin (an insecticide), chlorpyrifos (an insecticide), or carbaryl (an insecticide) present at low levels in at least one live or dead bee sample.
- Analytes of the miticides and herbicides detected are not expected to account for the mortality observed due to the relatively low concentrations detected compared to relatively high LD_{50} values associated with each active ingredient. Similarly, analytes of clothianidin, chlorpyrifos, and carbaryl (all highly toxic to bees) were found at relatively low concentrations, between 0.48 to 4.6 ppb, depending on the insecticide; because of the inconsistent detection of these chemicals between apiaries it is unlikely that they accounted for the majority of observed bee mortality in this incident.
- Fipronil (an insecticide) however was found by MDA's laboratory to be present in every dead bee sample collected at concentrations ranging from 15.5 ppb to 67.5 ppb. USDA's laboratory corroborated the analytes' presence, but was unable to quantify concentrations owing to a higher Limit of Quantification (LOQ) for fipronil.
- Investigators were unable to locate any coop, structure, or field near apiaries investigated where fipronil was used. Given fipronil's limited registered use in agriculture (only available as a seed potato dressing) and the proximity of potato fields to the apiaries under investigation, it is unlikely exposure was related to any legal Minnesota agricultural application of fipronil.

Laboratory Results

Affected Apiary (Sample set #)	Active Ingredient***	Quantified sample concentration (µg/kg = parts per billion [ppb])				
		*MDA Lab Dead bees	*USDA Lab Dead bees	*MDA Lab Live bees	*USDA Lab Live bees	MDA Lab Plant foliage
		(% of acute benchmark)**				
1 (1)	Amitraz (DMPF)[1]	3.9 (<0.001%)	Trace Amounts			
1 (1)	Amitraz (DMPMF)[1]	13 (<0.003%)		2.8 (<0.0007%)		
1 (1)	Atrazine[2]	2.6 (0.007%)				
1 (1)	Fipronil[3]	45 (281%)				
1 (1)	Thymol[4]	Not screened	9,330 (NA)	Not screened	6,940 (NA)	
1 (2)	Amitraz 2,4-DMA[1]	43 (<0.01%)				
1 (2)	Amitraz (DMPF)[1]	7.4 (<0.002%)				
1 (2)	Amitraz (DMPMF)[1]	38 (<0.01%)		3.8 ppb (<0.001%)		
1 (2)	Fipronil[3]	20 (125%)				
1 (2)	Thymol[4]	Not screened	20,500 (NA)	Not screened	9,430 (NA)	
2 (1)	Amitraz 2,4-DMA[1]	57 (<0.01%)				
2 (1)	Amitraz (DMPF)[1]	8.3 (<0.002%)	38.9 (<0.01%)	18 (<0.005%)		
2 (1)	Amitraz (DMPMF)[1]	49 (<0.01%)		15 (<0.004%)		
2 (1)	Fipronil[3]	39 (244%)				
2 (1)	Thymol[4]	Not screened	18,200 (NA)	Not screened	9,780 (NA)	
2 (2)	Amitraz (DMPF)[1]			4.5 (<0.001%)		
2 (2)	Amitraz (DMPMF)[1]			3.0 (<0.0008%)		
2 (2)	Thymol[4]			Not screened	23,000 (NA)	
3 (1)	Chlorpyrifos[5]		3.0 (1.3%)			
3 (1)	Amitraz 2,4-DMA[1]	46 (<0.01%)				
3 (1)	Amitraz (DMPF)[1]	11.5±5.5 (<0.003%)	Trace Amounts	1.8 (<0.0005%)		
3 (1)	Amitraz (DMPMF)[1]	31.5±13.5 (<0.008%)				
3 (1)	Atrazine[2]	3.5±0.6 (0.009%)				
3 (1)	Fipronil[3]	67.5±32.5 (422%)				
3 (1)	Metolachlor[7]	3.8 (<0.01%)				
3 (1)	Thymol[4]	Not screened	17,300 (NA)	Not screened	6,420 (NA)	
3 (2)	Amitraz 2,4-DMA[1]	32.5±10.5 (<0.008%)				
3 (2)	Amitraz (DMPF)[1]	42.1±32.9 (<0.01%)				
3 (2)	Amitraz (DMPMF)[1]	24.5±10.5 (<0.006%)		1.7 ppb (<0.0004%)		
3 (2)	Atrazine[2]	7.5±3.5 (0.02%)				
3 (2)	Chlorpyrifos[5]				4.6 ppb (1.9%)	
3 (2)	Clothianidin[6]	0.28 (1.9%)				
3 (2)	Desethylatrazine[1]	6.1±3.3 (<0.001%)	Not screened		Not screened	
3 (2)	Fipronil[3]	15.5±4.5 (97%)				
3 (2)	Metolachlor[7]	3.8±1.3 (0.001%)				
3 (2)	Thymol[4]	Not screened	7,640 (NA)	Not screened	4,920 (NA)	
3 (3)	Amitraz (DMPMF)[1]			2.8±0.5 (<0.0007%)		
3 (3)	Atrazine[2]			8.9 (0.02%)		
3 (3)	Desethylatrazine[1]			7.8 (<0.002%)	Not screened	
3 (3)	Thymol[4]			Not screened	6,060 (NA)	
4 (1)	Amitraz 2,4-DMA[1]	31 (<0.008%)				
4 (1)	Amitraz (DMPF)[1]	1.6±0.2 (<0.001%)				
4 (1)	Amitraz (DMPMF)[1]	22.5±7.5 (<0.006%)		1.9 (<0.0005%)		
4 (1)	Atrazine[2]	2.7 (0.007%)				
4 (1)	Carbaryl[8]	0.48 (109%)				
4 (1)	Fipronil[3]	21±6.0 (131%)				
4 (1)	Metolachlor[7]	2.0 (<0.0006%)				
4 (1)	Thymol[4]	Not screened	7,310 (NA)	Not screened	6,240 (NA)	
4 (2)	Thymol[4]			Not screened	3,220 (NA)	

***** A composite sample taken from more than one honey bee colony.

****** Benchmark = EPA's toxicity value x the Level of Concern (LOC). Where 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 is lower, and the LOC is 0.4. Laboratory results are divided by the benchmark and expressed as a percentage.

******* See MDA and USDA Pesticide Analyte Lists Used in Bee Kill Investigations pages 16 to 22 for an individual analyte's limits of detection.

[1] Registration Review – Preliminary Problem Formulation for Ecological Risk and Environmental Fate, Endangered Species, and Drinking Water assessment for Amitraz. USEPA. January 7, 2010.

Contact LD_{50} used for calculating percent of acute benchmark.

[2] Atrazine: Finalization of Interim Reregistration Eligibility Decision and Completion of Tolerance Reassessment and Reregistration Eligibility Process. USEPA. April 6, 2006.

Oral LD_{50} used for calculating percent of acute benchmark.

[3] California Environmental Protection Agency. Environmental Fate of Fipronil. March 5, 2007.

Contact LD_{50} used for calculating percent of acute benchmark.

[4] Thymol Summary Document: Registration Review. March 2010.

No LD_{50} available for calculating percent of acute benchmark.

[5] Preliminary Problem Formulation for Ecological Risk and Environmental Fate, Endangered species and Drinking Water Assessments for Chlorpyrifos.

Contact LD_{50} used for calculating percent of acute benchmark.

[6] Registration Review: Problem Formulation for the Environmental Fate and Ecological Risk, Endangered Species, and Drinking Water Exposure Assessment of Clothianidin. DP Barcode: 391491. USEPA. December 13, 2011.

Oral LD_{50} used for calculating percent of acute benchmark.

[7] Registration Review Problem Formulation for Metolachlor and S-Metolachlor. DP Barcode: 420467. USEPA. December 3, 2014.

Contact LD_{50} used for calculating percent of acute benchmark.

[8] Revised EFED Risk Assessment of Carbaryl in Support of the Reregistration Eligibility Decision. DP Barcode: D288451. USEPA. March 18, 2003.

Oral LD_{50} used for calculating percent of acute benchmark.

	Samples not collected
	If USDA laboratory sample, analyte is not present at the level of quantification (LOQ); if MDA laboratory sample, analyte is not present at the level of detection (LOD).
Not screened	Used to denote a difference in laboratory analytical methodologies which limit a laboratories ability to search for a particular pesticide.
NA	Not Available (NA) used when information is not accessible or available.

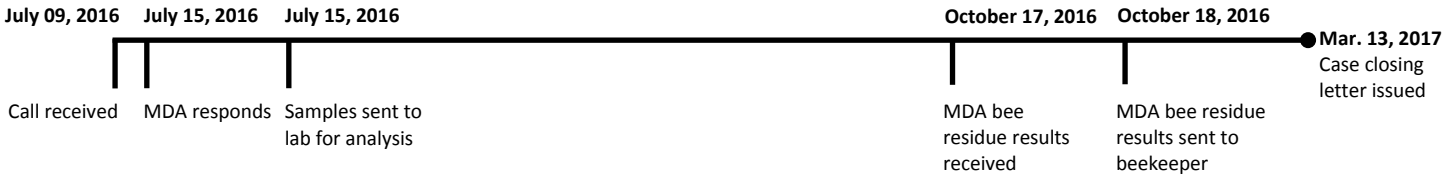
Investigation Conclusions

- Numerous pesticide residues were detected in the pesticide analysis of live and dead bees. Most residues were found at either concentrations (amitraz DMPF, amitraz DMPMF, amitraz 2,4-DMA, desethylatrazine, thymol, atrazine, and metolachlor) or frequencies (clothianidin, chlorpyrifos, and carbaryl) that could not explain the overall observed mortality.
- Fipronil was present in every dead bee sample analyzed by MDA's laboratory. Concentrations ranged from 15.5 ppb to 67.5 ppb. USDA's laboratory corroborated the analytes' presence, but was unable to quantify concentrations owing to a higher Limit of Quantification (LOQ) for fipronil.
- Fipronil's lethal dose to 50% of a population (LD_{50}) is 40 ppb for an acute contact exposure and 16 ppb once EPA's level of concern is applied.
- Given the concentrations of fipronil quantified and its associated LD_{50} value, it is likely that bees exposed to fipronil within the range of concentrations quantified experienced adverse effects from exposure.
- When the case was closed, results were reported to the EPA.

Compensation

Because the beekeeper was not registered with BeeCheck before the incident, the beekeeper did not qualify for compensation. While the beekeeper does not qualify for compensation due to statutory requirements, the incident is considered an acute pesticide poisoning as defined by the MDA.

Stearns County, Clearwater **Case File Number: DCD134000923**
Received date: July 09, 2016



Background of Complaint and Minnesota Department of Agriculture (MDA) Response

- In early May a hobby beekeeper received and installed two 5-frame nucs (small colonies) of Russian honey bees into hive bodies consisting of a single deep brood box. At the time of installation, colonies were fed a 50% sucrose solution.
- The apiary was located in a backyard and buffered from pesticide applications made from the city road by a house while a river provided a large natural buffer on the opposite, northeast side of the apiary.
- On July 09, 2016, the beekeeper reported a 'massive die-off' of honey bees outside of one hive entrance and was concerned that the die-off was related to agricultural spraying occurring in nearby fields the bees were visiting.
- An ACI, along with an MDA Pesticide Management Unit (PMU) staff entomologist responded on July 15, 2016, the day after the next scheduled mosquito treatment.
- Investigators examined 2 colonies (1 unaffected and 1 affected) and confirmed the presence of dead bees in the colony described as affected by the beekeeper.
- Colony health samples were collected for the 2 examined colonies and analyzed for *Varroa*, *Nosema*, and common viruses.
- During the collection of colony health samples, frames of bees and brood pattern were quantified and observations of disease noted.

The same colonies selected to evaluate colony health were also used to collect honey bees for pesticide residue analysis. Individual colony samples included live bees taken from frames consisting of nectar and pollen. Dead bee samples were collected from in front of the affected colony. A vegetation sample was collected from a patch of nearby clover. Vegetation examined by the roadside, nearest the known pesticide application, was determined to be grass with little to no flowering plants.

MDA Colony Health Findings

- At the time of the investigation, each hive consisted of two deep brood boxes and the colony considered unaffected had an additional single shallow hive box for honey collection.
- Dead bees outside hive entrances were observed only at the affected colony where 135 dead bees were collected. However, this likely underrepresents the actual amount of mortality due to a large amount of rain that had been received since the incident began.
- The affected colony contained approximately 4 frames of bees, indicating that a 400 dead bee threshold would be used as guidance to indicate an acute pesticide poisoning. The colony considered unaffected had 7.5 frames of bees.
- Eggs were seen in both affected and unaffected colonies indicating that the queen had been present within the last 3 days. The queen was observed in the affected colony.
- The affected colony had a spotty, below average brood pattern of 2 while the unaffected colony had an average brood pattern of 3.

- Inspection of the affected colony for disease revealed the presence of chalkbrood, brood that had been uncapped, and cells with white sunken larvae. No abnormal smell or roping was observed. No signs of disease were present in the colony considered unaffected.
- Results from the *Varroa* analysis showed 2.1 *Varroa* mites/100 bees indicating a level of pressure nearing the recommended treatment threshold of 3 mites/100 bees.
- Results from *Nosema* analysis showed individual bees had an average of 1.0 million *Nosema* spores/bee. The threshold when *Nosema* is thought to cause damage to colonies is 1.0 million spores/bee.
- Viral RNA was quantified for the colony considered affected and compared to baseline averages. Results indicated virus levels were not significantly different for Black Queen Cell Virus and were significantly higher than average for Israeli Acute Paralysis Virus.

MDA Pesticide Findings

- Three honey bee hives were present at the site. One of the colonies had recently been added from a swarm and two had been installed from nucs in May. The two colonies started from nucs were sampled for purposes of this investigation. One was considered affected while the other was considered unaffected and treated as a control colony.
- Initial follow-up by MDA Agricultural Chemical Investigators (ACI) found that a local company had been contracted by the city of Clearwater to perform weekly mosquito fogging of city streets with a highly toxic bee insecticide, Biomist 4+4 ULV (active ingredient: permethrin).
- The beekeeper applied Hopguard II to hives after colony installation in May for purposes of managing *Varroa* mite loads.
- Pesticide analysis was carried out at the MDA laboratory.

Laboratory Results

Laboratory	Active Ingredient	Quantified sample concentration (µg/kg)		
		Affected dead bees	Affected live bees	Unaffected live bees
MDA	No Detection	No Detection	No Detection	No Detection

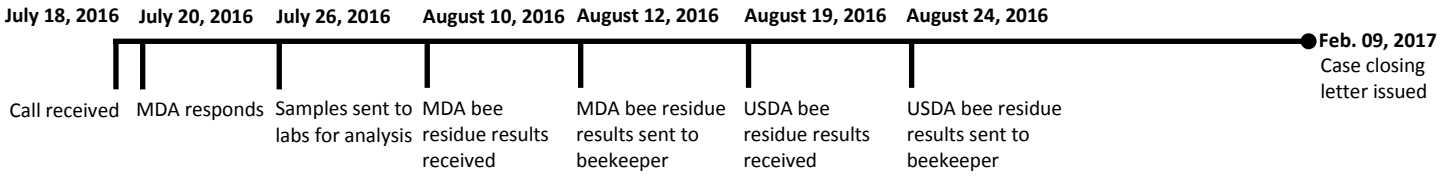
Investigation Conclusions

- It is likely that the colony considered affected was experiencing elevated levels of stress from various stressors as indicated by the quantified *Nosema* load, uncapped pupae, sunken larvae, presence of mid-summer chalkbrood, Israeli Acute Paralysis Virus, and abnormal bee mortality.
- Because no pesticide residues were detected in any of the bee samples (live or dead) it cannot be concluded that pesticide exposure contributed to the observed bee mortality.
- When the case was closed, results were reported to the EPA.

Compensation

Because no pesticide residues were found in the dead bees and because the incident was not considered an acute pesticide poisoning the beekeeper did not qualify for compensation.

Norman County, Twin Valley **Case File Number: MWF148000461**
Received date: July 18, 2016



Background of Complaint and Minnesota Department of Agriculture (MDA) Response

- On July 18, 2016 MDA received a call from a commercial beekeeper who observed a 50% decline in colony populations inside approximately 200 hives and dead honey bees near hive entrances.
- The beekeeper was concerned that spraying for aphid control was occurring in surrounding soybean fields and contributing to the observed population decline.
- MDA Agricultural Chemical Investigators accompanied by a Pesticide Management Unit staff entomologist responded on July 20, 2016 and visited 2 apiaries.
- The apiaries were located near stands of trees acting as windbreaks and providing partial protection to apiaries from direct agricultural activities.
- Colonies had been overwintered in California and moved into Minnesota apiaries May 15, 2016. Upon arriving to Minnesota, colonies were fed supplemental pollen patties and High Fructose Corn Syrup as needed.
- Colony health samples were collected and compiled as composite samples. Samples were evaluated for *Varroa, Nosema,* and common viruses.
- During the collection of colony health samples, frames of bees and brood pattern were quantified, and observations of disease noted.

The same colonies selected to evaluate colony health were also used to collect honey bees for pesticide residue analysis. Composite samples included live bees taken from frames consisting of nectar and pollen. Not enough dead bee were present to be collected for pesticide residue analysis. Vegetative samples were collected near 2 inspected apiaries and consisted of flowering vegetation.

MDA Colony Health Findings

- At the time of the investigation, each hive consisted of two deep brood boxes with between 0 and 4 hive boxes for honey collection.
- Investigators confirmed low colony populations but not enough dead bees were present outside colonies for quantification.
- Colonies had between 2.25 to 4.75 frames of bees indicating that a 300 to 475 dead bee threshold would be used as guidance to indicate an acute pesticide poisoning.
- Colonies evaluated were observed to have eggs present and brood patterns of 3 to 2. Observations indicated that the queen had been present within the last 3 days and that the queens laying pattern was average to below average.
- Colonies had received 2 *Varroa* mite treatments; Apivar strips (active ingredient: amitraz) were applied in March 2016 and a thymol treatment mixed with supplementary syrup was fed to colonies in April 2016.
- Results from the *Varroa* analysis showed between 0 and .29 *Varroa* mites/100 bees indicating a level of pressure from *Varroa* considered below the treatment threshold of 3 mites/100 bees. Individual bees tested for the presence of *Nosema* spores were found to have between 0.1 and 1.15 million spores/bee. Quantification of *Nosema* spores at 1.0 million spores/bee is considered to cause damage to colonies.

- Viral RNA was quantified for the colonies sampled and compared to baseline averages. Results indicated virus levels were not significantly different from averages for Black Queen Cell Virus and were significantly higher than average for Israeli Acute Paralysis Virus.
- One of the inspected colonies was found to have a large amount of chalkbrood present while no signs of disease were found in the other colonies evaluated.

MDA Pesticide Findings

- Investigators visited 2 apiaries located 2 miles apart and evaluated 2 of 40 colonies in each apiary. Like samples were combined to make composite samples. No control samples were collected as both apiaries were considered affected.
- Pesticide analysis were carried out at the MDA lab and the USDA lab.
- Analytical results found miticide residues of amitraz degradates (amitraz DMPF, amitraz DMPMF) and fluvalinate, both pesticides used to treat honey bee colonies for the mite *Varroa destructor*. However, residues quantified are not expected to account for the low populations observed due to the relatively low concentrations detected and relatively high LD_{50} values associated with each active ingredient.

Laboratory Results

Laboratory	Active Ingredient (Analytical Lab's Level of Detection [µg/kg])	Quantified sample concentration (µg/kg = parts per billion [ppb])	
		*Site 1 Affected live bees	*Site 2 Affected live bees
		(% of acute benchmark)**	
MDA	Amitraz DMPF[1] (0.4)	4.27 (<0.001%)	<LOD***
MDA	Amitraz DMPMF[1] (<25)	5.56 (<0.001%)	<LOD***
MDA	Fluvalinate[2] (10)	<LOD***	<LOD***
USDA	Amitraz DMPF[1] (10)	<LOQ***	<LOQ***
USDA	Amitraz DMPMF[1] (50)	<LOQ***	<LOQ***
USDA	Fluvalinate[2] (1.0)	<LOQ***	5.8 (0.7%)

* A composite sample taken from more than one honey bee colony.

** Benchmark = EPA's toxicity value x the Level of Concern (LOC). Where 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 is lower, and the LOC is 0.4. Laboratory results are divided by the benchmark and expressed as a percentage.

*** If USDA laboratory sample, analyte is not present at the level of quantification (LOQ); if MDA laboratory sample, analyte is not present at the level of detection (LOD).

[1] Registration Review – Preliminary Problem Formulation for Ecological Risk and Environmental Fate, Endangered Species, and Drinking water Assessments for Amitraz. DP Barcode: 367920. USEPA January 7, 2010. Contact LD_{50} used for calculating percent of acute benchmark.

[2] Environmental Fate and Ecological Risk Assessment for Tau-Fluvalinate; July 11, 2005. Contact LD_{50} used for calculating percent of acute benchmark.

Investigation Conclusions

- Because there were no non-beekeeper applied pesticide residues found in the live bees and no dead bees to perform pesticide analysis on, it cannot be concluded that pesticide exposure contributed to the observed population decline.
- When the case was closed, results were reported to the EPA.

Compensation

Because no dead bees were available to determine if bee mortality was correlated with pesticide exposure, and because the incident was not considered an acute pesticide poisoning the beekeeper did not qualify for compensation.

Acute Pesticide Kill – Short term exposure to a bee toxic pesticide resulting in bee death. See page 2 for how the MDA evaluates an "acute pesticide poisoning" for purposes of compensation.

Aerial Application – Fixed wing (plane) or helicopter applications of pesticides. Pilots are required to be licensed by the FAA and the MDA.

Analyte – Samples collected during an investigation for pesticide residue analysis undergo a process to identify and measure chemical constituents. The specific chemicals being looked for during this process are considered analytes.

Bee Kill Complaint – The initial information received by the MDA by a complainant who believes that the death of their bees is due to pesticides.

Brood Pattern – The distribution of a queen's egg laying and immature bees in the frames of a hive. A greater concentration of brood, immature bees, indicates less brood disease, a healthier queen, and is correlated with a higher brood pattern score (1 to 5).

Colony – The collection of a queen and all offspring (foragers, nurse bees, and drones) who coinhabit an individual hive.

Commercial Beekeeper – Beekeepers who have 100's to several 1,000 colonies. They are generally migratory and conduct pollination services for hire. Commercial beekeepers generally move their colonies out of Minnesota in winter and bring them back in late April/early May each year.

Compensation – Hive owners may receive monetary reimbursement for "acute pesticide poisonings" under Minnesota state law.

Composite Sample – A sample consisting of like material collected from multiple sources. The analytical output obtained from a composite sample are considered representative results from the multiple, like sample sources.

Enforcement Action – The MDA issues both written warnings and financial penalties to persons who the MDA alleges violated state/federal pesticide law. Financial penalties are referred to as a "Notice of Intent to Sue".

EPA – Environmental Protection Agency. The federal agency that approves the registration of pesticides.

FIFRA – The Federal Insecticide Fungicide Rodenticide Act. Pesticides are only registered by EPA when there will be no unreasonable adverse effects from the legal use of the pesticide according to label instructions.

Frames of bees – An indirect measurement of the number of bees in a colony. Frames of bees can be estimated by the number of frames, in a brood box, that are completely covered with bees on both sides. The estimate is performed by looking at the top and bottom of a brood box and averaging the number of frames covered in bees together before multiplying by an average number of bees known to cover a frame, approximately 2,400.

Hive – The unit in which a honey bee colony lives. Often, beekeepers use a Langstroth hive consisting of stackable hive boxes that are filled with removable frames.

Hobby Beekeeper – Non-migratory beekeepers that may have a single hive or up to dozens of hives who keep bees primarily for honey, pollen and other bee products.

Lethal dose to 50% of a population (LD_{50}) – Amount of pesticide (the Lethal Dose) required to kill one-half (50%) of the test organisms (e.g., bees) in controlled studies. Findings of pesticide residues at less than the LD_{50} may contribute to the death of some, but less than half of the bees that have been exposed to the pesticide.

Level of Concern (LOC) – A value used to assess the risk of an environmental exposure. For honey bees, an EPA LOC of 0.4 is used to compare the acute contact or oral risk from exposure to a pesticide.

Nosema spp. – A microsporidia, fungal, pathogen that infects the gut of honey bees resulting in accelerated behavioral development, alters feeding behavior, and can lead to other adverse effects.

Pesticide – A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest. For compensation, a bee kill resulting from use of a bee toxic pesticide is considered an acute pesticide poisoning. There are General Use pesticides which do not require a license to apply and Restricted Use pesticides which may only be applied by a licensed/certified applicator.

Pesticide Applicator – The person who applies the pesticide. The MDA maintains databases of all licensed applicators in the state.

Pesticide Dealer – A business that has a license to sell pesticides.

Pesticide Label – Any text or images printed directly on, or attached to, the product or its packaging. Pesticide product labels provide critical information about how to safely handle and use pesticide products. Many insecticide products contain directions for use which prohibit the products use when applying in areas where pollinators are present. Pesticide labels are legally enforceable under state law.

Seed Treatments – An insecticide, fungicide, or microbial treatment coating individual plant seeds to protect them against pests or impart other characteristics. A significant number of crop seeds are treated with insecticides and/or fungicides. Seeds treated with pesticides are considered treated articles and exempt from regulation under the Federal Insecticide, Fungicide, and Rodenticide Act, 7 U.S.C. §136-136y.

Treated Articles – An article or substance treated with, or containing, a pesticide to protect the treated article or substance. Articles that fall into this categorization, such as pesticide treated seeds, are exempt from all requirements of FIFRA.

Varroa destructor – A common parasitic mite of the honey bee. High numbers result in reduced vigor of honeybees/colony health. Many beekeepers treat their colonies one to four times per year for varroa mite.

Virus – An agent that causes infectious disease. Honey bees can be infected by a variety of viruses including 7 of which are analyzed for during MDA investigations.

A Note on Public Data

MDA bee kill files contain investigation details. Most information is public with the exception of information that identifies the complainant and information about pesticide applications. All requests to see investigation files must be submitted to the MDA in writing. To request a copy of an MDA investigation, contact the Data Practices and Records Manager at 651-201-6435, or fax at 651-201-6118.

#	Analyte	Limit of Detection (ppb)*
1	3-OH Carbofuran	0.2
2	5-hydroxy Imidacloprid	0.4
3	6-Chloronicotinic acid**	N/A
4	Abamectin	0.4
5	Acephate	0.3
6	Acetamiprid	0.04
7	Acetochlor	5
8	Alachlor	5
9	Aldicarb	0.3
10	Aldicarb Sulfone	0.3
11	Aldicarb Sulfoxide	0.3
12	Allethrin	25
13	Amitraz**	5
14	Amit-Met DMPMF	25
15	Amitraz Metab DMPF	0.4
16	Atrazine	5
17	Azinphos	5
18	Bendiocarb	0.04
19	Bifenazate	0.4
20	Bifenthrin	15
21	Bromophos**	N/A
22	Carbaryl	0.3
23	Carbofuran	0.2
24	Chlorantraniliprole	0.4
25	Chlorfenvinphos	5
26	Chlorpyrifos	5
27	Chlorthalonil	5
28	Clofentezine	0.3
29	Clomazone	5
30	Clothianidin	0.04
31	Coumaphos	2
32	Cyanazine	5
33	Cyfluthrin	15
34	Cyhalothrin	5
35	Cypermethrin	10
36	Cyphenothrin	15
37	Deltamethrin/Traloemthrin	15
38	Desethylatrazine	5
39	Desisopropylatrazine	10
40	Diazinon	10
41	Dichlorvos	5
42	Dimethenamid	50
43	Dinotefuran	0.04
44	Dinotefuran DN 1	0.2
45	Dinotefuran UF**	N/A

#	Analyte	Limit of Detection (ppb)*
46	Emamectin benzoate	0.04
47	EPTC	5.0
48	Esfenvalerate	15
49	Ethafluralin	5.0
50	Etofenprox (NH4)	0.04
51	Etoxazole	0.04
52	Fenpropathrin	15
53	Fenthion	5.0
54	Fipronil	5.0
55	Flubendamide	2.0
56	Flumethrin	15
57	Fluvalinate (tau)	10
58	Fonofos	5.0
59	Formatamate	1.2
60	Imidacloprid	0.04
61	Imidacloprid des nitro olefin	0.04
62	Imidacloprid HCl desnitro	0.05
63	Imidacloprid olefin	1.2
64	Imidacloprid urea	0.04
65	Imiprothrin**	N/A
66	Malathion	30
67	Metazachlor	5.0
68	Methamidophos	20
69	Methidathion	5.0
70	Methiocarb	0.2
71	Methomyl	0.3
72	Methyl Parathion	10
73	Metofluthrin**	N/A
74	Metolachlor	10
75	Metolcarb	0.3
76	Metribuzin	5.0
77	Mevinphos	5.0
78	Monocrotophos	10
79	Nithiazine	0.3
80	Oxamyl	0.3
81	Parallethrin**	N/A
82	Pendimethalin	10
83	Permethrin cis-, trans	5.0
84	Phenothrin	15
85	Phorate	5.0
86	Phosmet	5.0
87	Phosphamidon	20
88	Pirimiphos-me, et	5.0
89	Prometon	5.0
90	Propachlor	5.0

#	Analyte	Limit of Detection (ppb)*
91	Propazine	10
92	Propoxur	0.04
93	Resmethrin	15
94	Simazine	50
95	Spinetoram J	0.4
96	Spinetoram L	2.0
97	Spinosad A	0.3
98	Spinosad D	0.4
99	Spirodiclofen	1.2
100	Spiromesifen	0.4
101	Spirotetramat	0.4
102	Strychnine	5.0
103	Sulfotep**	N/A
104	Tefluthrin	15
105	Terbufos	5.0
106	Tetramethrin	15
107	Thiacloprid	1.2
108	Thiamethoxam	0.04
109	Thiodicarb	0.3
110	Triallate	5.0
111	Triflumuron	0.3
112	Trifluralin	5.0
113	Vinclozolin	10
114	Pyrethrins	50

* Detection limits are calculated based on the instrumental minimum detectable amount. Detection limits could vary depending on the instrument condition and complexities of sample matrix.

**Requires special calibration of equipment but can be looked for upon request

#	Analyte	Limit of Detection (ppb)
1	1-Naphthol	10
2	2,4 Dimethylaniline	50
3	2,4 Dimethylphenyl formamide (DMPF)	10
4	3-Hydroxycarbofuran	10
5	4,4 dibromobenzophenone	4.0
6	Acephate	50
7	Acetamiprid	2.0
8	Acetochlor	50
9	Alachlor	10
10	Aldicarb	4.0
11	Aldicarb sulfone	2.0
12	Aldicarb sulfoxide	20
13	Aldrin	10
14	Allethrin	10
15	Amicarbazone	30
16	Amitraz	4.0
17	Atrazine	6.0
18	Azinphos methyl	6.0
19	Azoxystrobin	2.0
20	Bendiocarb	10
21	Benoxacor	20
22	BHC alpha	4.0
23	Bifenazate	20
24	Bifenthrin	2.0
25	Boscalid	4.0
26	Bromuconazole	20
27	Buprofezin	20
28	Captan	10
29	Carbaryl	30
30	Carbendazim (MBC)	5.0
31	Carbofuran	10
32	Carboxin	4.0
33	Carfentrazone ethyl	1.0
34	Chlorfenopyr	1.0
35	Chlorfenvinphos	6.0
36	Chlorferone	50
37	Chlorothalonil	30
38	Chlorpropham (CIPC)	40
39	Chlorpyrifos	1.0
40	Chlorpyrifos methyl	1.0
41	Clofentezine	100
42	Clothianidin	1.0
43	Coumaphos	5.0
44	Coumaphos oxon	5.0
45	Cyfluthrin	4.0

#	Analyte	Limit of Detection (ppb)
46	Cyhalothrin total	1.0
47	Cypermethrin	4.0
48	Cyphenothrin	20
49	Cyprodinil	1.0
50	DOD p,p'	4.0
51	ODE p,p'	2.0
52	DDT p,p'	4.0
53	Deltamethrin	50
54	Diazinon	5.0
55	Dichlorvos (DDVP)	50
56	Dicloran	1.0
57	Dicofol	1.0
58	Dieldrin	10
59	Difenoconazole	10
60	Diflubenzuron	10
61	Dimethenamid	10
62	Dimethoate	50
63	Dimethomorph	20
64	Dinotefuran	2.0
65	Diphenamid	20
66	Endosulfan I	2.0
67	Endosulfan II	2.0
68	Endosulfan sulfate	2.0
69	Endrin	10
70	Epoxiconazole	1.0
71	Esfenvalerate	2.0
72	Ethion	10
73	Ethofumesate	10
74	Etoxazole	1.0
75	Etridiazole	50
76	Famoxadone	20
77	Fenamidone	10
78	Fenbuconazole	10
79	Fenhexamid	6.0
80	Fenoxaprop-ethyl	20
81	Fenpropathrin	10
82	Fenpyroximate	5.0
83	Fenthion	10
84	Fipronil	10
85	Flonicamid	8.0
86	Flubendiamide	25.0
87	Fludioxonil	20
88	Fluoxastrobin	4.0
89	Fluridone	10
90	Flutolanil	4.0

#	Analyte	Limit of Detection (ppb)
91	Fluvalinate	1.0
92	Heptachlor	4.0
93	Heptachlor epoxide	10
94	Hexachlorobenzene (HCB)	1.0
95	Hexythiazox	30
96	Hydroprene	20
97	Hydroxychlorothalonil	50
98	Imazalil	20
99	Imidacloprid	1.0
100	Imidacloprid 5-hydroxy	25
101	Imidacloprid olefin	10
102	Indoxacarb	3.0
103	Iprodione	50
104	Lindane	4.0
105	Linuron	20
106	Malathion	4.0
107	Metalaxyl	2.0
108	Methamidophos	4.0
109	Methidathion	10
110	Methomyl	10
111	Methoxyfenozide	10
112	Metolachlor	6.0
113	Metribuzin	1.0
114	MGK-264	50
115	MGK-326	10
116	Myclobutanil	15
117	Norflurazon	6.0
118	Oxamyl	5.0
119	Oxyfluorfen	1.0
120	Paradichlorobenzene	10
121	Parathion methyl	2.0
122	Pendimethalin	6.0
123	Permethrin total	10
124	Phenothrin	10
125	Phorate	50
126	Phosalone	10
127	Phosmet	10
128	Piperonyl butoxide	50
129	Pirimiphos methyl	20
130	Prallethrin	4.0
131	Profenofos	10
132	Pronamide	1.0
133	Propachlor	10
134	Propanil	10
135	Propargite	10

#	Analyte	Limit of Detection (ppb)
136	Propazine	20
137	Propetamphos	4.0
138	Propham	20
139	Propiconazole	20
140	Pymetrozine	20
141	Pyraclostrobin	15
142	Pyrethrins	50
143	Pyridaben	10
144	Pyrimethanil	20
145	Pyriproxyfen	10
146	Quinoxyfen	10
147	Quintozene (PCNB)	1.0
148	Resmethrin total	5.0
149	Sethoxydim	2.0
150	Simazine	50
151	Spinosad	50
152	Spirodiclofen	2.0
153	Spiromesifen	10
154	Tebuconazole	8.0
155	Tebufenozide	10
156	Tebuthiuron	2.0
157	Tefluthrin	1.0
158	Tetrachlorvinphos	4.0
159	Tetraconazole	6.0
160	Tetradifon	1.0
161	Tetramethrin	10
162	Thiabendazole	1.0
163	Thiacloprid	1.0
164	Thiamethoxam	1.0
165	THPI	50
166	Thymol	50
167	Triadimefon	2.0
168	Triadimenol	45
169	Tribufos (DEF)	2.0
170	Trifloxystrobin	1.0
171	Triflumizole	50
172	Trifluralin	1.0
173	Triticonazole	10
174	Vinclozolin	1.0