WPI

ARE SYSTEMIC PESTICIDES POISONING OUR POLLINATORS?

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Abstract

Bees play a critical role in the agricultural industry through the vital pollination services they provide to crop plants. In recent decades, there has been a sharp decline in bee populations all over the world, including many native North American bumblebee species; thus posing significant risk to our food supply and economy. In this study, we tested the hypothesis that exposure to systemic neonicotinoid pesticide residues in floral nectar is a significant contributing factor in bee declines. In a series of controlled laboratory experiments, we chronically fed individual queen, worker, and male bumblebees (*Bombus impatiens*) environmentally realistic doses (1, 5, 7 and 10ppb) of clothianidin, a common systemic neonicotinoid pesticide, and monitored their survival over several days. We found that consumption at a dose of 10ppb significantly increased mortality rate for all bee castes. Alarmingly, this dose killed 50% of queen, worker and male bees after 5, 4 and 1 exposures, respectively. Our results are the first to show that clothianidin can have detrimental effects on bumblebee health at field realistic doses, particularly in males. These results implicate pesticide exposure in the decline in bumblebee populations, filling an important gap in pollinator decline research.

Table of Contents

Introduction

Insect pollinators have tremendous ecological, social, and economic importance because they provide a vital ecosystem service to most flowering plant species and agricultural crops on the planet. Insect pollinators also play an integral role in the maintenance of biodiversity by providing food, shelter and other resources to animals through the plants that they pollinate. The total loss of insect pollinators would therefore have catastrophic consequences for life on our planet. In recent years, research has demonstrated dramatic declines in many native insect pollinator species in North America, including several species of bumblebee [8, 14, 22, 24, 6] and the migratory monarch butterfly [5]. These population declines have prompted a major effort to identify environmental stressors that negatively affect pollinator health.

Although the exact cause of pollinator declines remains unknown, one significant contributing factor is thought to be the extensive use of systemic '*neonicotinoid*' pesticides in agricultural and urban habitats that are extensively utilized by insect pollinators, including farms, roadsides, parks, golf courses and ornamental gardens. Neonicotinoids are synthetic neurotoxins that act as a nicotinic acetylcholine receptor agonists in the insect central nervous system [19]. Chemical variants in this pesticide family are thought to represent a major threat to pollinators because they can persist in environment for long periods of time post-application [12-13] and being systemic, readily translocate from soil into flowering plants rendering them toxic to individuals that feed on them [9, 4, 15-18]. Adult insect pollinators are thus unintentionally exposed to neonicotinoids when they consume contaminated floral nectar and pollen. Here, we examined how consumption of the widely used neonicotinoid pesticide Clothianidin impacts bumblebee health.

About Bumblebees

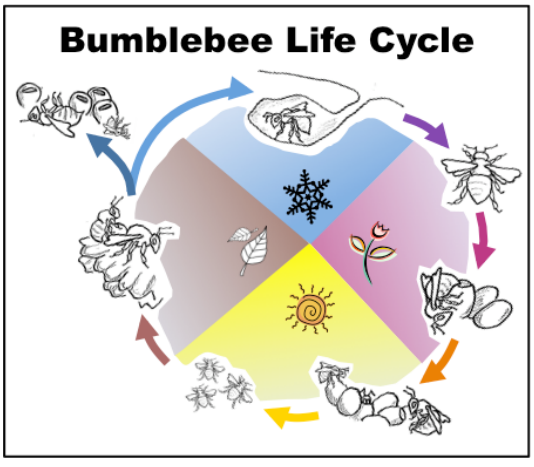

Figure 1: Bumblebee Lifecycle [21]

Bumblebees are social insects that create in-ground nests. The size of these colonies can range according to the nest's capacity. The colony lifecycle is shown in Figure 1. In the spring, an over-wintered queen who had mated prior to entering hibernation establishes a colony, preferably below ground [16]. The colony is comprised of female worker bees that either perform duties around the nest or forage for food (floral nectar and pollen). Towards the end of the colony's lifecycle, the queen lays unfertilized haploid male and virgin queen eggs. These males will leave the colony to reproduce with the newly emerged queens,

maintaining genetic diversity. These queens enter hibernation for the winter to hopefully form their own colonies in the spring while the remainder of the colony dies [3].

The species of bumblebee that we used in this study was *Bombus impatiens*. This species is native to Canada and regions east of the Rocky Mountains in the Continental United States. It has been introduced to other regions, such as California, as well as Mexico. There are several factors that make bumblebees more valuable than the more widely studied honeybee: bumblebees "buzz pollinate" flowers, allowing them access to pollen unavailable to other species; they can work at cooler temperatures; will visit non-nectar producing crops, such as tomatoes; and can transfer greater amounts of pollen in a single visit [12-13, 23, 20).

About the systemic neonicotinoid pesticide Clothianidin

The commercial systemic neonicotinoid pesticide Clothianidin ($C_6N_5H_8SO_2Cl$) is widely used in both agricultural and urban habitats. The United Nations suggested an international tolerance level for Clothianidin at 50ppb [1].

Clothianidin underwent join-review under the North American Free Trade Agreement (NAFTA) in 2003 as an insecticide and fungicide for use in corn, canola and rapeseed treatment. In 2009, Bayer registered new uses in vegetable and cereal seeds, as well as potato seed piece treatment [10]. Clothianidin was identified as an alternative to organophosphate and carbamate pesticides, which are highly acutely toxic to humans, wildlife and bees [13].

Initial registration was granted following this NAFTA joint-review, as it appeared to meet the risk/benefit and safety standards required by the US government. However, the registration appears to pose Clothianidin as a lesser of two evils, causing less harm to wildlife when compared to older alternatives, but still harmful to bees [1]. The EPA issues an Environmental Hazards statement for products under their jurisdiction, including Clothianidin. Here, eight LD_{50}/LC_{50} studies are taken into account when looking at basic acute toxicity, focusing on mammals, birds, freshwater fish and honeybees [27]. In the registration documents for Clothianidin, it states: "Clothianidin is highly toxic to honey bees on an acute contact basis (LD_{50}>0.0439µg/bee)" [28].

More specifically, a "Bee Hazard" warning is required by the US government for most products. This warning is alleged to cover all pollinating species, however different species have differing metabolisms and should not be generalized. It was recommended to include detailed "Directions for Use" with products outlining steps that can be taken to protect pollinators, such as applying pesticides at night for continuously blooming crops, or avoiding application while pollinating insects are actively in the area. However, Clothianidin is a systemic pesticide and can persist in plants for several years, negating these efforts [27].

The European Food Safety Authority (EFTA) issued a press release regarding neonicotinoids, including Clothianidin, in January 2013 implying that the use of neonicotinoids was unacceptable, as it harms a vital player in the agricultural system, bees. They advised the use of these pesticides on crops that are not considered attractive to bees. The EFTA has identified gaps in previous research, including the fact that only honeybees are considered despite a large portion of pollination being performed by other species. They also asked Bayer to meet with Parliament officials to explain a lack of research regarding how long these pesticides can persist in the environment. The EFTA is being commended by the public for taking a significant step towards protecting pollinators, and inspired a "stop use order" and petition against the EPA, both of which were denied due to insufficient evidence [7].

The EPA is currently accepting independent scientific peer reviews on the risks pesticides pose to our pollinating species [26].

Our study will provide important information on the effects of Clothianidin on bumblebee health, a neglected species in toxicity studies. In particular, our work will provide insight into how chronic oral exposure, as opposed to acute oral exposure, impacts bumblebee survival, which is currently unknown.

Materials and Methods

Bee Preparation Protocol

To ensure genetic diversity, *Bombus impatiens* were chosen from various colonies for each trial, obtained from Biobest Biological Systems Canada (Leamington, ON, Canada). Bees were removed from their colony either manually or caught by net from a flight cage, chilled in a 4°C fridge for 15mins to 2 hours, marked by a small dot of acrylic paint on the thorax, and weighed down to the millionth gram (0.000g). Each bee was individually housed in plastic cups approximately 4" in diameter. The plastic cups were outfitted with feeders created from an inverted cap from a 1.5mL or 0.5mL (depending on the volume being fed) conical tube and a pollen supply, which was refilled *ad lib*. These cups were then arranged on trays to be placed into a Percival chamber. The Percival chamber was kept on a 12-hour light cycle (12pm, day – 12am, night) with 50% humidity at 24°C. To protect bees from environmental changes due to the fan within the Percival chamber, foam was placed between the fan and the bees, allowing for airflow to continue, but keeping the breeze off of the bees. Feedings fell between 2-4 hours after initiation of the light cycle daily under red light.

Acclimation Period Protocol

Bees underwent an acclimation period prior to the start of all trials. After weighing, bees would be placed in their individual housing and into the Percival. They were fed a 30% honey solution daily with their allotted volumes. The goal of this period was to experience zero deaths within 48 hours prior to beginning the trial to ensure appropriate bee health. Therefore, this period, ranging from 2-4 days, would persist until all bees appeared fit for the trial. In the event of numerous deaths bringing the population below the required 50 bees/trial, newly collected bees were inserted into the acclimation period, delaying the start of the trial by a minimum of 48 hours.

The average weights of workers and males were between approximately 0.204g ± 0.045g and 0.130g ± 0.040g, respectively, while the average queen bee weighed 0.536g ± 0.091g. Due to this considerable difference in weight, the queens were fed 150µl solution per day in a single serving, while workers and males were fed 75µl. These volumes were determined to be small enough that bees consumed the entire solution, but large enough that the high activity level of the bees were maintained throughout the entire trial. In the event of a bee not eating, a blank piece of pink tape was placed on their lid to warn against their use in the trial following the acclimation period. Similarly to mortalities, newly collected bees replaced non-eating bees.

Drug Preparation Protocol

Following the acclimation period, surviving bees would be sorted into groups for drug administration. Concentrations of drugs were picked representing

environmentally realistic doses found in fields where the pesticide has persisted in the plants or in non-target fields, as opposed to the higher concentrations found post-application in target fields. These groups consisted of: Controls (0ppb, 30% honey), 1ppb, 5ppb, 7ppb and 10ppb. All drug solutions were mixed in a 30% honey solution, which was prepared fresh for each trial. Figure 2 provides a summary of housing and dosing information.

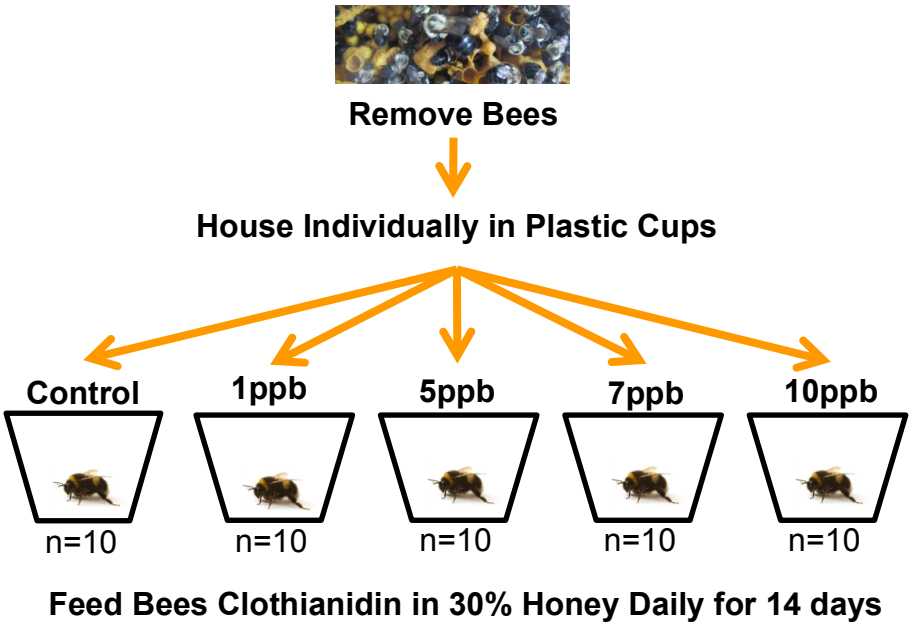

Figure 2: Schematic of individual housing [21]

The drug was prepared between 0 and 4 days prior to initiating each trial, during the acclimation period. For the 30% honey solution, organic clover honey (Great Value brand by Walmart) was diluted in dH2O using heat and stir bars. Clothianidin ((*E*)-1-(2-Chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine) was procured from Sigma Aldrich (33589), and the DMSO from Fisher Scientific. Clothianidin drug solutions were prepared by creating a stock solution of 0.005g Clothianidin in 1mL DMSO for a $5x10^6$ ng/mL (ppb) solution. Serial dilutions were performed to acquire the desired concentrations of $5x10^4$, $5x10^2$ and $5x10^1$ ng/mL in DMSO. Solutions were vortexed prior to each dilution to produce an even dispersal of drug in DMSO. Feeding solutions at 1, 5, 7 and 10ppb were created from these Clothianidin + DMSO solutions in the 30% honey mentioned above and placed on a shaker for even dispersal. Between daily feedings, the feeding solutions were refrigerated at all times to avoid bacterial growth. The stock solutions and powder-form drug were kept in dark conditions, as it may be unstable with prolonged exposure to light. For drug preparation calculations, please see Appendix I.

Trial Protocol

Bees were sorted according to pre-acclimation weight, aiming for an equal distribution of weight between groups as well as genetic diversity mentioned

earlier. Bees that appeared to be outliers according to weight were avoided for use in trials. To safeguard from any mortality due to environmental factors, the individuals from each group were randomized on the trays and rotated throughout the Percival chamber daily. Concentrations were labeled on the cups and lids, with both numbers and colors, to prevent human error.

Feedings were logged manually and electronically, noting mortalities (■), self-induced starvation (/) and normal eating (X). In the event of a bee not eating, their plastic cup was marked with the date and subsequent checkmarks for every day of starvation on a piece of pink tape. If the bee resumed eating, the date was strikethrough. Feeding Logs can be found in Appendix II.

Mortalities were removed from the trays and placed in vials marked with the date, trial number, drug concentration of the bee, and number of bees. Multiple bees could be placed in a single vial if they were from the same concentration and date of death. Vials were stored in Ziploc bags according to trial number in the refrigerator until dissected. At the conclusion of the trial, live bees were euthanized via freezing and placed in vials, specifically noting that they were alive upon trial completion. One representative trial was chosen from each bee caste for analysis. Bee dissections took place on no specific timetable. The bees were separated under a dissecting scope into three portions: head, thorax and abdomen. To accomplish this, all limbs, antennae, proboscis and wings were removed, and excess pollen was brushed off the body surface. Each bee was placed in a cryogenic tube to be stored at -80°C for later quantitative analysis, with the goal of assessing the concentrations of drug present in their tissues.

Results and Discussion

Adaptation and opportunity drove the timeline of this study. It was necessary to perform trials around the natural lifecycle of the colonies. Therefore, the number of trials per bee caste is not equal, but will become equal over time before publication.

Female Trial 1

Female Trial 1 began on 1 January 2013. An acclimation period was performed prior to the start of this trial beginning on 27 December 2012. Starting with this trial, drug concentrations were: Control, 1, 5, 7 and 10ppb for an appropriate range of environmentally realistic doses. Mortality at 50% in the 10ppb group can be observed in Figure 3 below at 2 exposures. This trial was performed by Melissa Mobley and was deemed successful.

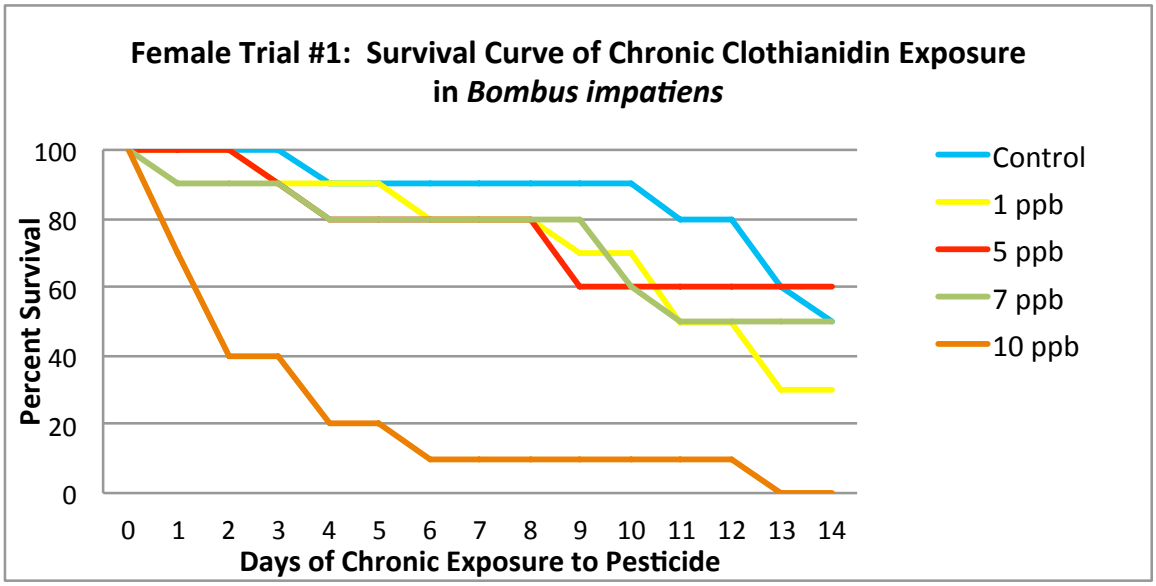

Figure 3: Female Trial 1 survival curve in *Bombus impatiens*

Male Trial 1

We began Male Trial 1 on 15 January 2013, collecting 58 bees to be able to afford a few deaths during the acclimation period. The bees were fed 75µl of freshly prepared 30% honey solution under red light for three days. Previous iterations of these trials did not have an acclimation period, and the difference in bee behavior was noted with the addition of the acclimation period. Bees appeared eager and knowledgeable in how to attain food. The drug solutions were prepared on 18 January 2013 for Day 1 on the following day. Concentration groups of 10 bees/group for 1, 5, 7 and 10ppb were arranged according to pre-acclimation weight. Any unused bees over the 50 bees/trial limit were placed into the male chamber for future use, as is the case for all later trials mentioned below. At this

point in time, bees were not randomized throughout the trays, but were placed with the remainder of their group.

Day 2 showed massive deaths across 1, 5, 7 and 10ppb. All 10 bees in the 1ppb group died, with 5 deaths in 5ppb, 7 in 7ppb and 9 in 10ppb. Possible explanations were discussed, including: male metabolism, absence of pollen, activity levels of bees and human error in drug preparation. Environmental factors were not considered, as the control group did not suffer any fatalities. After performing several male trials after Male Trial 1, the most likely cause of these deaths was human error. Mortalities can be seen in the Survival Curve for Male Trial 1 in Figure 4 below. This trial was continued for the extent of the 14 days despite the radical deaths.

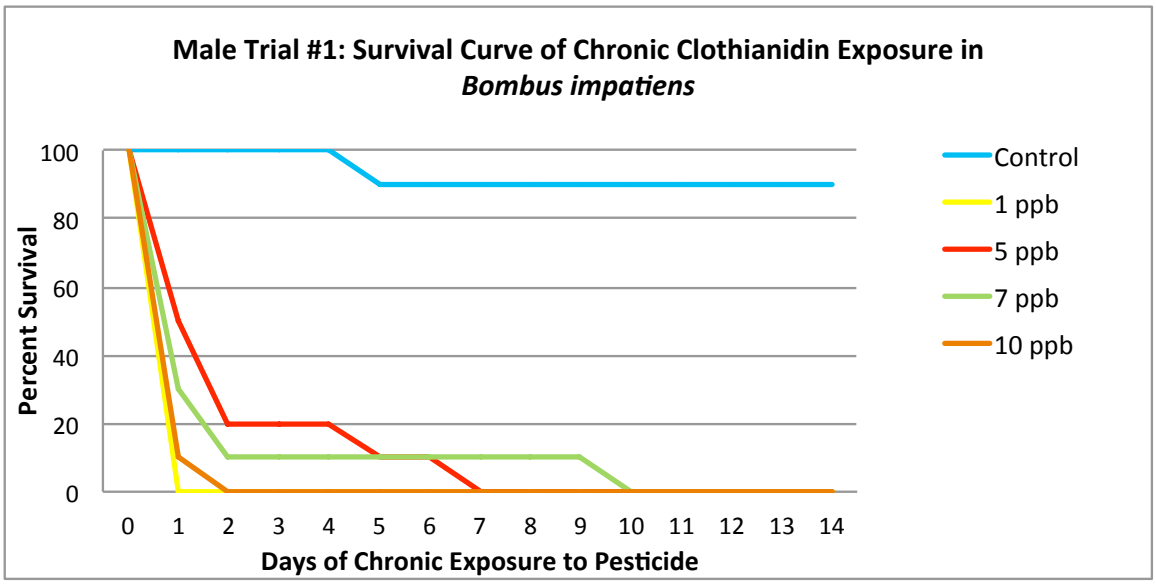

Figure 4: Male Trial 1 survival curve in *Bombus impatiens*

Male Trial 2

Acclimation period for Male Trial 2 began on 22 January 2013, for a period of 3 days with 57 bees. During this period, one bee had escaped into the Percival chamber, found the next day and placed back in his cup. In the future, the attachment of the mesh for the lids was monitored more closely. The trial began on 25 January 2013 and the drug was prepared the same day. With this trial, we began the practice of marking non-eating bees with pink tape after noticing sticky residue and a bubble of honey shortly after. This practice was started in the hopes of identifying which bees were dying from starvation, rather than pesticide exposure. During this trial, we observed sticky residue on the cups, potentially from temperature changes in the Percival chamber when alternating from light and dark cycles. In future trials, the temperature levels remained constant throughout both cycles.

Following the first dose, we observed 50% mortality in both the 7 and 10ppb groups. On Day 5 of this trial, 50% of the controls died. It was suspected to be a result of the fan within the Percival chamber, beginning the practice of placing a piece of foam between the bees and the fan, to avoid accidental chilling or stress. Due to these deaths, the control group was adjusted to represent the 5 remaining bees as a complete group, as shown in Figure 5. Once all control bees had died, the trial was prematurely ended and remaining bees were euthanized on 5 February 2013.

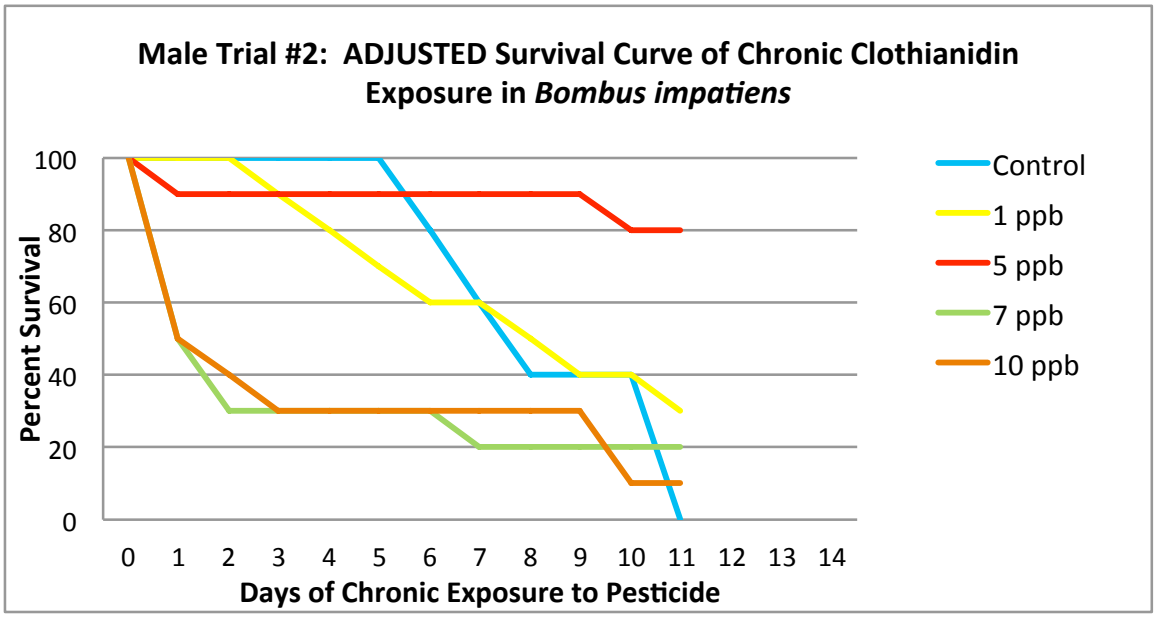

Figure 5: Male Trial 2 Adjusted Survival Curve in *Bombus impatiens*

Male Trial 3

Due to environmental concerns regarding Male Trial 2, the group placement on trays and throughout the Percival chamber was randomized from this point onwards. Acclimation for this trial began on 1 February 2013, where there were only enough supplies to support 50 bees. The following day, 4 newly collected bees were added into the acclimation period, totaling 53 bees after removing one for not eating. Drug solutions were prepared on 4 February 2013. Day 1 occurred on 6 February 2013. A pattern began to emerge in respect to anticipated deaths in male bees, with approximately 50% mortality in the 10ppb group after 1 exposure, as seen in Figure 6 below. On Day 2, it was noted that 1 bee was missing from the 1ppb group and 1 from the 5ppb group due to miscounting. These bees were added into the groups without an acclimation period, and remained 1 day behind the dosing schedule. This trial concluded on 19 February 2013.

During this trial, it was noted that the drug solutions began making a "popping" sound. This could be an indication of bacterial contamination. Control deaths were gradual and appear to be from natural causes. However, they could be a result of

this possible bacterial contamination. Also, with this trial we began monitoring the eating habits of groups, which can be seen in Figure 7. This starvation is self-induced and the bees are still provided with food daily regardless of their eating habits.

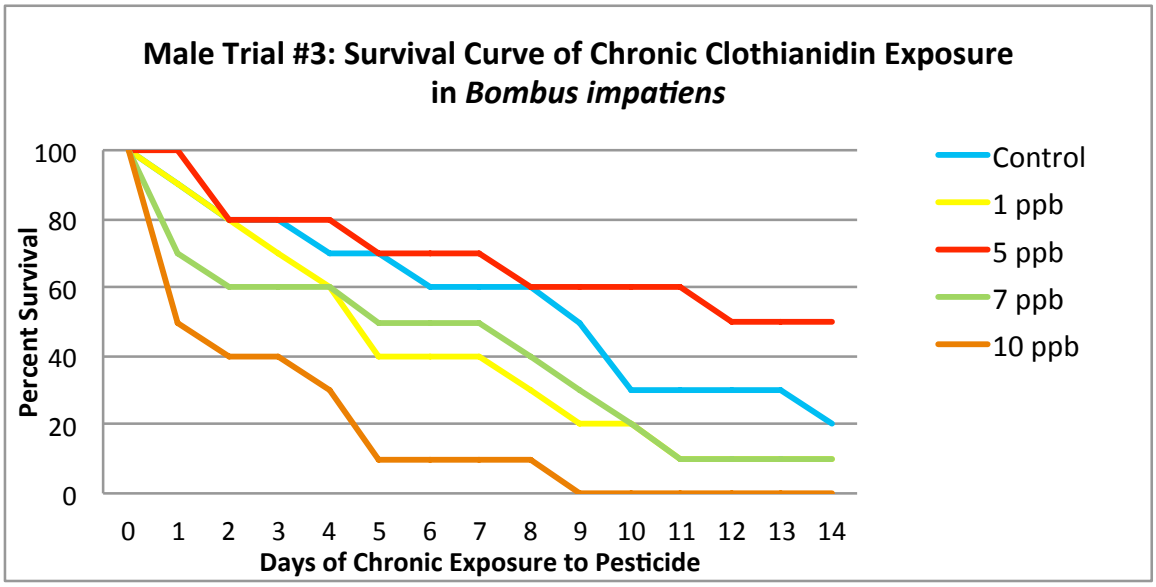

Figure 6: Male Trial 3 Survival Curve in *Bombus impatiens*

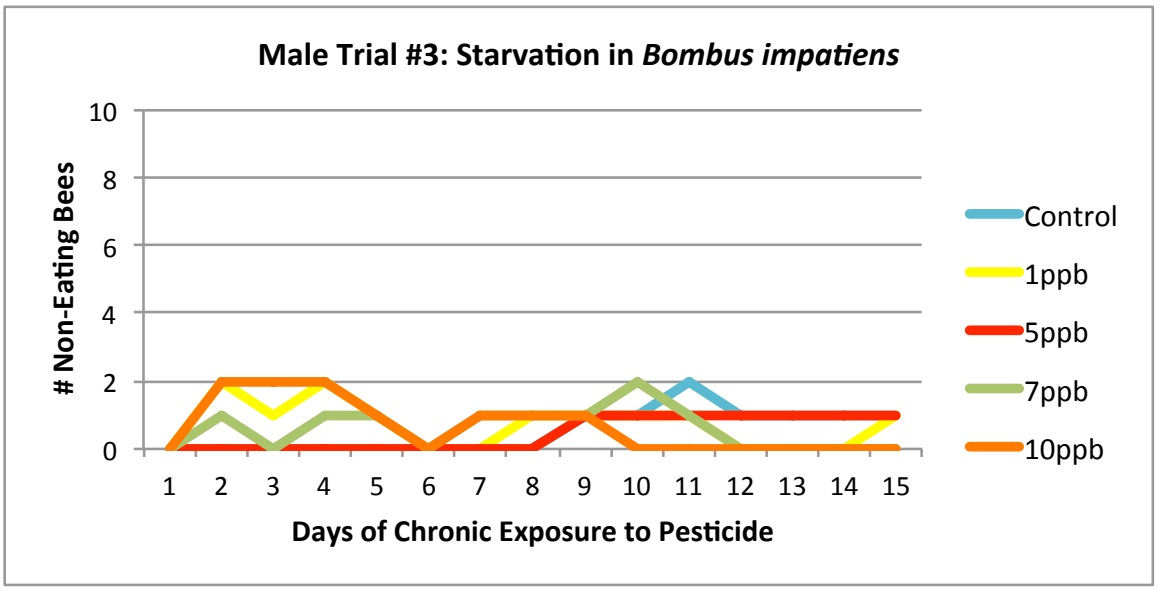

Figure 7: Male Trial 3 Starvation in *Bombus impatiens*

Female Trial 2

Acclimation for Female Trial 2 lasted a period of 5 days starting with 64 bees on 16 February 2013. Day 1 began on 21 February 2013, with pollen refilled if necessary. The drug solutions were created the same day. As seen in Figure 8 below, the female workers in the 10ppb group reached 50% mortality after 5 exposures. This trial concluded as scheduled with no complications, on 7 March 2013. In Figure 9,

note the drastic starvation in the 1ppb group, this could imply a sensory detection of pesticide in their food.

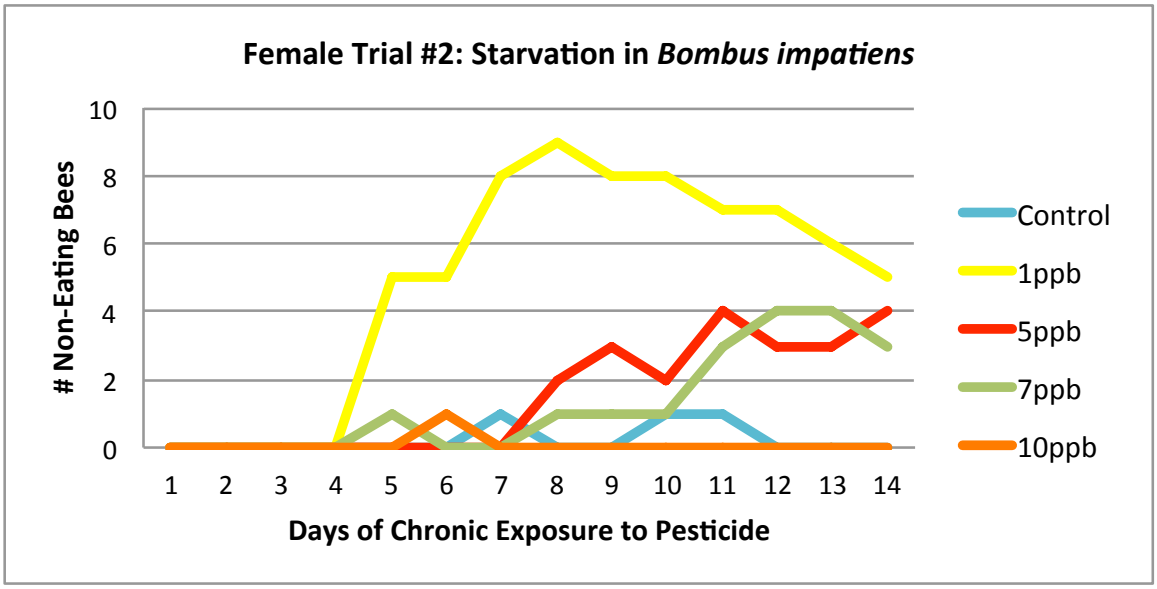

Figure 8: **Female Trial 2 Survival Curve in *Bombus impatiens***

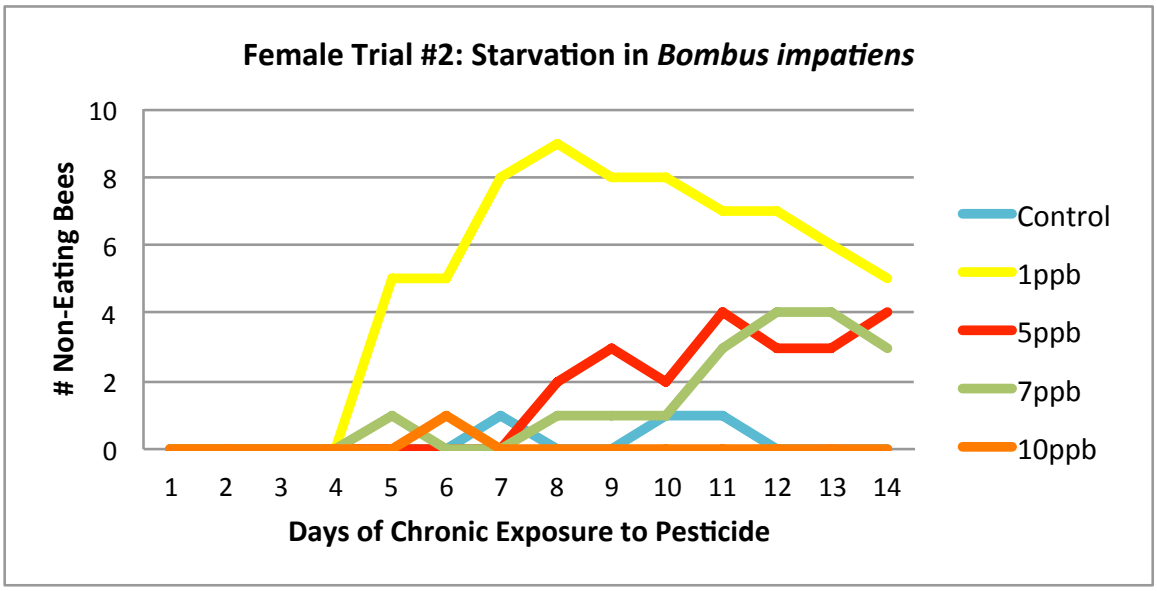

Figure 9: **Female Trial 2 Starvation in *Bombus impatiens***

Queen Trial 1

As two colonies in lab began producing queens, we were able to begin a virgin queen trial. These queens were separated from their colonies as soon as they emerged from the colony, to prevent mating. They were they group housed in plastic cups inside the Percival until we had acquired enough queens to begin the acclimation period. This period began on 20 February 2013, lasting for 6 days with 40 queen

bees. Due to these decreased numbers, we ran the trial with 8 bees/group with Controls, 5, 7 and 10ppb. We were more interested in the higher concentrations and in keeping the group totals as close to 10 bees/group as possible, and therefore decided not to include a 1ppb group. The drug was prepared on 21 February 2013. While the queens were being fed a greater volume than the female workers and males, the concentrations remained the same. Larger feeders were created for the queens, effectively holding the 150µl solution allowing for proper drug delivery.

As seen in Figure 10, the queens experienced 50% mortality at 5 exposures in the 10ppb group. The first 14 days are shown here. However, this trial concluded on 23 March 2013, after feeding was continued until the last 7ppb queen had died, totaling 23 days. Starvation was predominantly observed in the 5ppb and 7ppb groups, as seen in Figure 11. This data cannot be adequately compared to the female workers, as there was no 1ppb group.

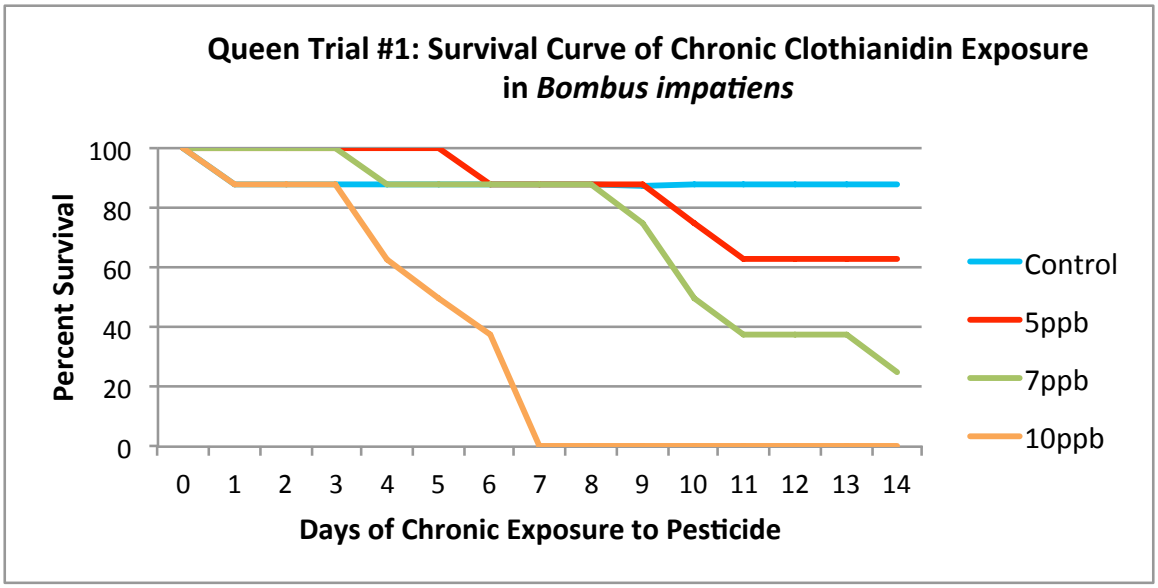

Figure 10: Queen Trial 1 Survival Curve in *Bombus impatiens*

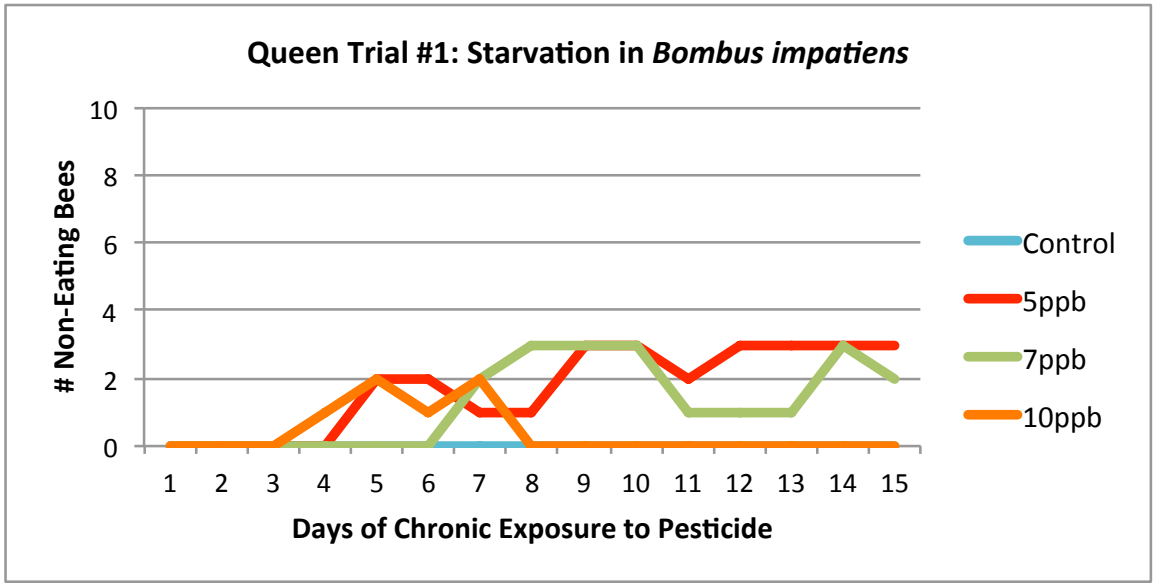

Figure 11: Queen Trial 1 Starvation in *Bombus impatiens*

Male Trial 4

Acclimation for Male Trial 4 began on 20 February 2013, with 56 males. With this trial, we began to track the approximate ages of the males, though it did not factor into our group forming decisions. On the fourth day of acclimation, there were no longer enough bees to hold the trial. Additionally, approximately 10 bees were no longer eating, providing a concerning future for this trial. On 26 February, 20 male bees were added to the acclimation period. With increasing deaths seen in the initial group of 56, two more additions were made with 13 new males on 1 March 2013 and 24 new males on 10 March 2013. As only 8 of the original 56 remained, we decided to exclude these bees from the trial groups under suspicious circumstances regarding death and starvation. All concentration groups contained bees from 26 February – 10 March 2013. The trial began on 12 March 2013.

Additional complications arose when the drug solutions did not appear to have an effect on the bees by Day 3, using the three previous trials as models for successful drug delivery. The trial was prematurely ended, and restarted with the same bees and newly made drug on the following day, 15 March 2013. The deceased bee from the 5ppb group was replaced with an un-acclimated bee. Following this restart, the anticipated deaths were experienced in the 10ppb group and drug delivery was deemed successful. The results of this trial can be found in Figure 12 below.

Self-induced starvation was observed in this trial, particularly in the 1ppb and 5ppb groups, as seen in Figure 13. This starvation could explain deaths found at these concentrations, particularly the higher frequency of mortalities in the 1ppb group when compared to 5ppb, a higher concentration.

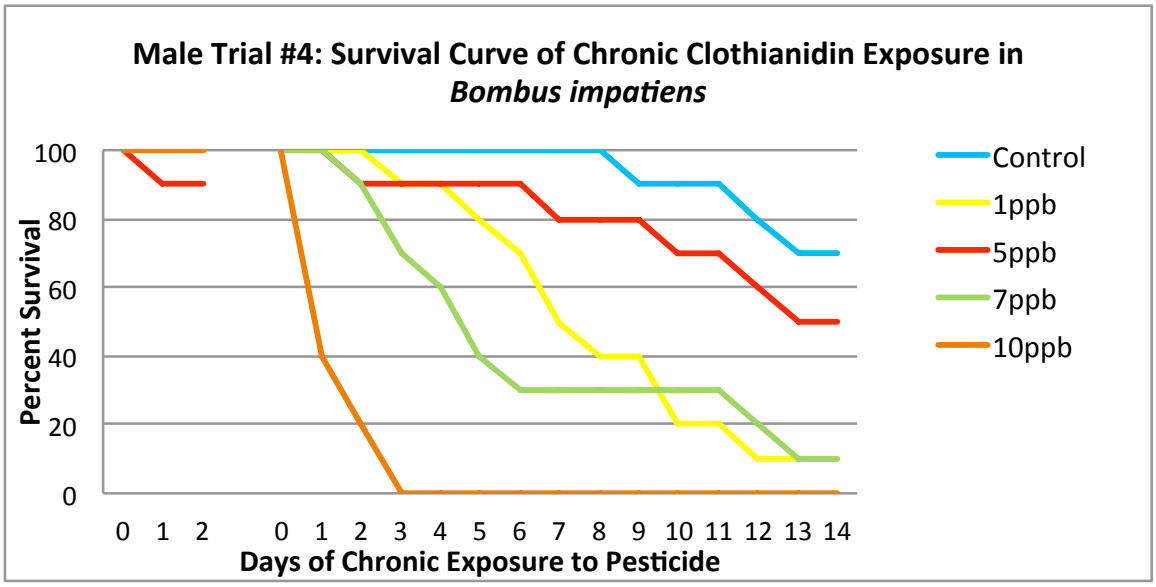

Figure 12: Male Trial 4 Survival Curve in *Bombus impatiens*

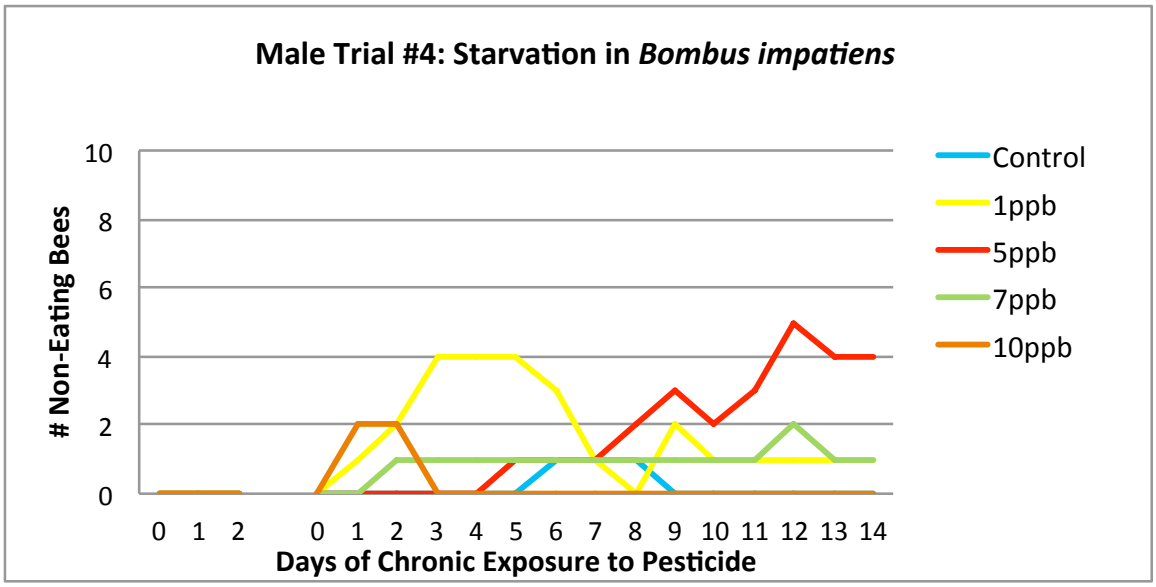

Figure 13: Male Trial 4 Starvation in *Bombus impatiens*

Summary of Results

Graphical representations of the trials can be found in Figures 10, 11 and 12 below. Standard error bars have been added. As previously mentioned, the number of trials per bee caste is not equal, and it is important to factor into interpreting the data found below. The Queen trials do not have standard error bars, as only one trial was included.

During certain trials, we observed and recorded self-induced starvation in all bee castes examined. This starvation could be a factor in fatalities during the trial. The causation for the starvation is unknown at this point in time.

Note the 50% mortality in the 10ppb group after experiencing 5 exposures to Clothianidin in Figure 14. It had been anticipated that the queens would be more resistant, with their ability to hibernate allowing for greater fat supplies, creating a heartier bee. However, they appear to be similarly affected by this pesticide as the female workers, found in Figure 15. With additional data from future trials, it is suspected to remain similar to the female workers. If the queen dies, the colony will no longer function properly.

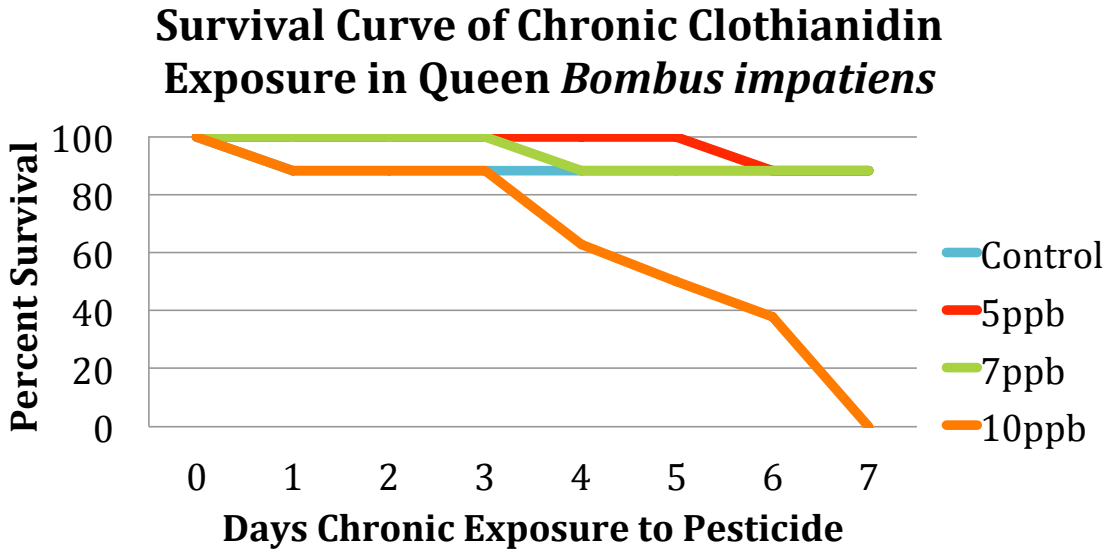

Figure 14: Queen Survival Curve (n=1) in *Bombus impatiens*

Female workers, as previously mentioned, experience 50% mortality in the 10ppb group at 4 exposures. This graph contains the data from two trials, and therefore needs further data to solidify the trends seen here in Figure 15. The implications of female workers dying after this few exposures means there will be a decline in worker bees to care for and feed the larvae and queen, undermining the infrastructure of the colony.

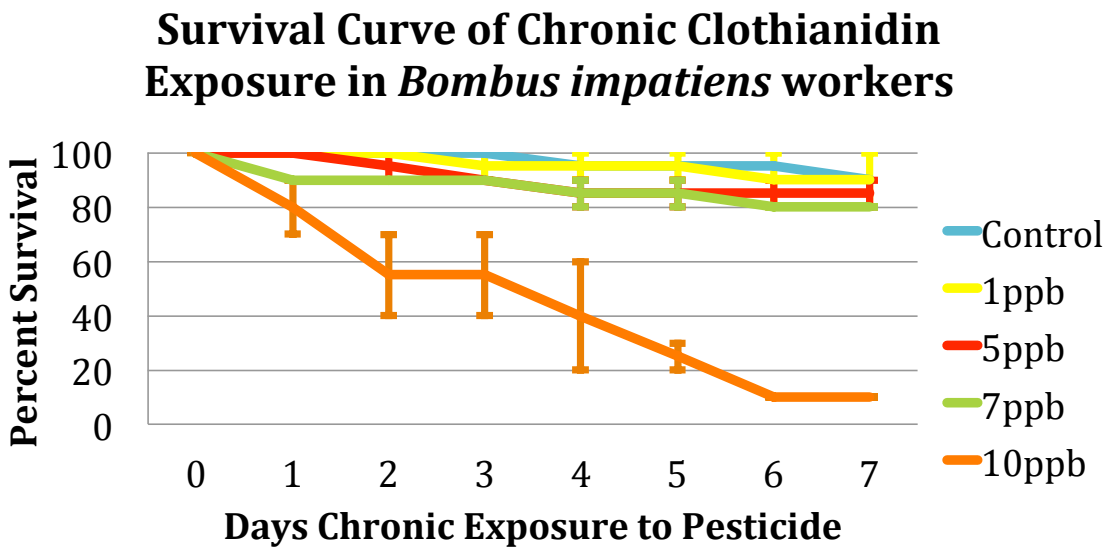

Figure 15: **Female Survival Curve (n=2) in *Bombus impatiens***

The male survival curves to Clothianidin differ greatly when compared to the queen and female workers. Note the 50% mortality in the 10ppb group after only 1 exposure, as seen in Figure 16. Also note the 50% mortality in the 7ppb group at 4 exposures, an unseen result in the queen and female trials. The implications of this are dangerous when looking at the lifecycle of a colony. If the males are leaving the colony to mate with newly emerged queens and die after one feeding, the number of fertilized queens who enter hibernation could drastically fall, meaning fewer and fewer colonies can be established in the spring.

A possible explanation for this difference is the ploidy of the males versus the females. Males are unfertilized eggs, meaning they are haloid (1n). Diploid females (2n) could have a genetic advantage in this situation. They are designed to survive for an upwards of several months, performing various tasks supporting the colony, while males are designed to survive for several weeks and are only for reproductive purposes.

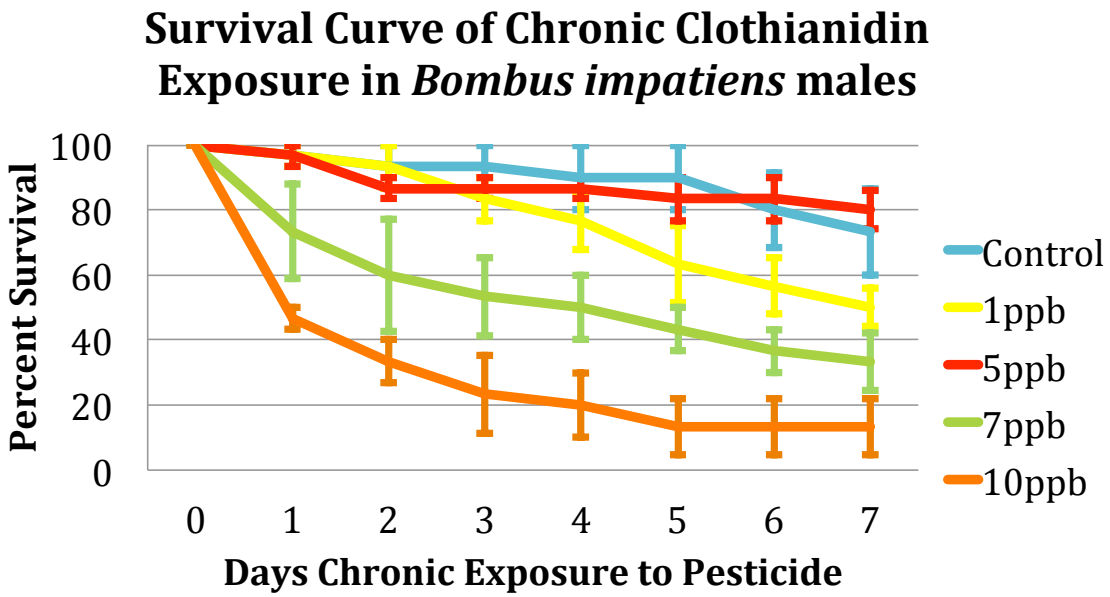

Figure 16: **Male Survival Curve (n=4) in *Bombus impatiens***

Conclusions

Chronic exposure to ecologically-relevant concentrations of Clothianidin results in high mortality rates in bumblebee queens, workers and males. Male bumblebees are at a higher risk for pesticide-related mortality than females.

To provide a well-rounded examination of the effects of Clothianidin on *Bombus impatiens* and the different castes within their colonies, the remaining trials will be conducted before publication, with the goal of 10 trials per caste. Furthermore, further analysis into the self-induced starvation in bees should be examined, as it could be responsible for certain fatalities during these trials. Currently this data is inconclusive, but intriguing and should be subject to supplementary analysis.

Future research plans aim for a greater understanding of the systemic neonicotinoid Clothianidin. The sub-lethal effects of this pesticide on bumblebee cognition and behavior will be examined. Similar trials will be conducted with monarch butterflies (*Danus plexippus*) to examine chronic oral exposure to Clothianidin as a possible treat to their populations. Testing into whether bees can detect contamination via sensory functions could be beneficial in creating a better understanding of how these pesticides affect our pollinators. Additionally, we will measure the levels of Clothianidin in flowers to assess the threat to wild bumblebee populations.

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Appendix I – Clothianidin Drug Preparation Calculations

Stock Solution: weigh 0.005g Clothianidin using lab scale

$$\frac{0.005g\ Clothianidin}{1mL\ DMSO} = 5x10^6 ng/mL$$

Serial Dilutions:

$5x10^4 = 10\mu l\ 5x10^6 stock\ solution\ into\ 990\mu l\ DMSO$
$5x10^2 = 10\mu l\ 5x10^4 solution\ into\ 990\mu l\ DMSO$
$5x10^1 = 30\mu l\ 5x10^2 solution\ into\ 270\mu l\ DMSO$

Feeding Solutions:

$Control = 1mL\ 30\%\ honey\ solution$
$1ppb = 20\mu l\ 5x10^1 solution\ into\ 980\mu l\ 30\%\ honey\ solution$
$5ppb = 10\mu l\ 5x10^2 solution\ into\ 990\mu l\ 30\%\ honey\ solution$
$7ppb = 14\mu l\ 5x10^2 solution\ into\ 986\mu l\ 30\%\ honey\ solution$
$10ppb = 20\mu l\ 5x10^2 solution\ into\ 980\mu l\ 30\%\ honey\ solution$

Adjusted Feeding Solutions:

$$\left(10\frac{bees}{group}\right) * \left(\frac{75\mu l}{feeding}\right) * 14\ day\ trial = 10.5mL\ required$$

Making 12mL per group for some flexibility

$Control = 12mL\ 30\%\ honey\ solution$
$1ppb = 240\mu l\ 5x10^1 solution\ into\ 11{,}760\mu l\ 30\%\ honey\ solution$
$5ppb = 120\mu l\ 5x10^2 solution\ into\ 11{,}880\mu l\ 30\%\ honey\ solution$
$7ppb = 168\mu l\ 5x10^2 solution\ into\ 11{,}832\mu l\ 30\%\ honey\ solution$
$10ppb = 240\mu l\ 5x10^2 solution\ into\ 11{,}760\mu l\ 30\%\ honey\ solution$

Feeding solutions were stored in the refrigerator, dilutions and stock solutions in the dark.

Appendix II – Feeding Logs

Female Trial #1: Survival Curves for Clothianidin LD50 (75ul in 30% honey daily)

Day	Day 1 (01/01/13)										Day 2 (01/02/13)										Day 3 (01/03/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Low - 1 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medium - 5 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
High - 7 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
Lethal - 10 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				X	X	X	X						

Day	Day 4 (01/04/13)										Day 5 (01/05/13)										Day 6 (10/06/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
Low - 1 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
Medium - 5 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X		
High - 7 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X		
Lethal - 10 ppb	X	X	X	X							X	X									X	X								

Day	Day 7 (01/07/13)										Day 8 (01/08/13)										Day 9 (01/09/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
Low - 1 ppb	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X		
Medium - 5 ppb	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X		
High - 7 ppb	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X		
Lethal - 10 ppb	X										X										X									

Day	Day 10 (1/10/13)										Day 11 (1/11/13)										Day 12 (1/12/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		
Low - 1 ppb	X	X	X	X	X	X	X				X	X	X	X	X	X	X				X	X	X	X	X					
Medium - 5 ppb	X	X	X	X	X	X					X	X	X	X	X	X					X	X	X	X	X	X				
High - 7 ppb	X	X	X	X	X	X	X	X			X	X	X	X	X	X					X	X	X	X	X					
Lethal - 10 ppb	X										X										X									

Day	Day 13 (1/13/13)										Day 14 (1/14/13)										Day 15 -no dose									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X			X	X	X	X	X	X														
Low - 1 ppb	X	X	X	X	X						X	X	X																	
Medium - 5 ppb	X	X	X	X	X	X					X	X	X	X	X	X														
High - 7 ppb	X	X	X	X	X						X	X	X	X	X															
Lethal - 10 ppb	X																													

Female workers acclimated to cups for several days until no deaths for 48hrs. Then given 75ul of 30% honey solution w/listed concentrations of pesticide 1x per day over 2 week period. Feedings fell between 9am and 11am each day under red light. While not being actively fed, bees kept in percival incubator on 12-hr light cycle (8am, day, 24C, 50% humidity - 8pm, night, 22C, 50% humidity).

Female Trial #2: Survival Curves for Clothianidin LD50 (75ul in 30% honey daily)

Day	Day 1 (2/21/13)										Day 2 (2/22/13)										Day 3 (2/23/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2		4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Low - 1 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medium - 5 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
High - 7 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
Lethal - 10 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X			

Day	Day 4 (2/24/13)										Day 5 (2/25/13)										Day 6 (2/26/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Low - 1 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	/	/	/	/	/	X	X	X	X	X	/	/	/	/	/
Medium - 5 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
High - 7 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	/		X	X	X	X	X	X	X	X	X	
Lethal - 10 ppb	X	X	X	X	X	X	X				X	X	X	X	X	X					X	X	/							

Day	Day 7 (2/27/13)										Day 8 (2/28/13)										Day 9 (3/1/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	/	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X				
Low - 1 ppb	X	X	/	/	/	/	/	/	/	/	X	/	/	/	/	/	/	/	/	/	X	/	/	/	/	/	/	/	/	
Medium - 5 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	/	/		X	X	X	X	X	X	/	/	/	
High - 7 ppb	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	/			X	X	X	X	X	X	X	/		
Lethal - 10 ppb	X										X																			

Day	Day 10 (3/2/13)										Day 11 (3/3/12)										Day 12 (3/4/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	/						X	X	X	X	/						X	X	X	X	X					
Low - 1 ppb	X	/	/	/	/	/	/	/	/		X	/	/	/	/	/	/	/			X	/	/	/	/	/	/	/	/	
Medium - 5 ppb	X	X	X	X	X	X	X	/	/		X	X	X	X	X	/	/	/	/		X	X	X	X	X	/	/	/		
High - 7 ppb	X	X	X	X	X	X	X	/			X	X	X	X	/	/	/				X	X	X	/	/	/	/			
Lethal - 10 ppb																														

Day	Day 13 (3/5/13)										Day 14 (3/6/13)										Day 15 - no dose									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X								X	X	X																	
Low - 1 ppb	X	/	/	/	/	/	/				X	/	/	/	/	/														
Medium - 5 ppb	X	X	X	X	X	/	/	/			X	X	X	X	/	/	/	/												
High - 7 ppb	X	X	X	/	/	/	/				X	X	/	/	/															
Lethal - 10 ppb																														

Females acclimated to cups for several days until no deaths for 48hrs. All cups recieved small ball pollen for feeding which was replaced as necessary. Females given 75ul of 30% honey solution w/listed concentrations of pesticide 1x per day over 2 week period. Feedings fell between 2pm and 4pm each day under red light. While not being actively fed, bees kept in percival incubator on 12-hr light cycle (12pm, day, 24C, 50% humidity - 12am, night, 24C, 50% humidity).

Male Trial #1: Survival Curves for Clothianidin LD50 (75ul in 30% honey daily)

Day	Day 1 - 1/19/13										Day 2 - 1/20/13										Day 3 - 1/21/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Low - 1 ppb	X	X	X	X	X	X	X	X	X	X																				
Medium - 5 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						X	X								
High - 7 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X								X									
Lethal - 10 ppb	X	X	X	X	X	X	X	X	X	X	X																			

Day	Day 4 - 1/22/13										Day 5 - 1/23/13										Day 6 - 1/24/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Low - 1 ppb																														
Medium - 5 ppb	X	X									X	X									X									
High - 7 ppb	X										X										X									
Lethal - 10 ppb																														

Day	Day 7 - 1/25/13										Day 8 - 1/26/13										Day 9 - 1/27/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
Low - 1 ppb																														
Medium - 5 ppb	X																													
High - 7 ppb	X										X										X									
Lethal - 10 ppb																														

Day	Day 10 - 1/28/13										Day 11 - 1/29/13										Day 12 - 1/30/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
Low - 1 ppb																														
Medium - 5 ppb																														
High - 7 ppb	X																													
Lethal - 10 ppb																														

Day	Day 13 - 1/31/13										Day 14 - 2/1/13										Day 15 -no dose - 2/2/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
Low - 1 ppb																														
Medium - 5 ppb																														
High - 7 ppb																														
Lethal - 10 ppb																														

Males acclimated to cups for several days until no deaths for 48hrs. Then given 75ul of 30% honey solution w/listed concentrations of pesticide 1x per day over 2 week period. Feedings fell between 2pm and 4pm each day under red light. While not being actively fed, bees kept in percival incubator on 12-hr light cycle (12pm, day, 24C, 50% humidity - 12am, night, 22C, 50% humidity).

Male Trial #2: Survival Curves for Clothianidin LD50 (75ul in 30% honey daily)

Day	Day 1 (1/25/13-CE)										Day 2 (1/26/13-MM)										Day 3 -1/27/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Low - 1 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medium - 5 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
High - 7 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						X	X	X							
Lethal - 10 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						X	X	X	X						

Day	Day 4 -1/28/13										Day 5 - 1/29/13										Day 6 -1/30/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						X	X	X	X	X					
Low - 1 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X			X	X	X	X	X	X	X			
Medium - 5 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
High - 7 ppb	X	X	X								X	X	X								X	X	X							
Lethal - 10 ppb	X	X	X								X	X	X								X	X	X							

Day	Day 7 -1/31/13										Day 8 -2/1/13										Day 9 -2/2/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X							X	X	X								X	X								
Low - 1 ppb	X	X	X	X	X	X					X	X	X	X	X	X					X	X	X	X	X					
Medium - 5 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
High - 7 ppb	X	X	X								X	X									X	X								
Lethal - 10 ppb	X	X	X								X	X	X								X	X	X							

Day	Day 10 - 2/3/13										Day 11 2/4/13										Day 12 -2/5/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X									X	X																		
Low - 1 ppb	X	X	X	X							X	X	X	X							X	X	X							
Medium - 5 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X		
High - 7 ppb	X	X									X	X									X	X	trial ended, bees euthanised							
Lethal - 10 ppb	X	X	X								X										X									

Day	Day 13 2/6/13										Day 14 -2/7/13										Day 15 -no dose -2/8/13									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb																														
Low - 1 ppb																														
Medium - 5 ppb																														
High - 7 ppb																														
Lethal - 10 ppb																														

Males acclimated to cups for several days until no deaths for 48hrs. Then given 75ul of 30% honey solution w/listed concentrations of pesticide 1x per day over 2 week period. Feedings fell between 2pm and 4pm each day under red light. While not being actively fed, bees kept in percival incubator on 12-hr light cycle (12pm, day, 24C, 50% humidity - 12am, night, 22C, 50% humidity).

Male Trial # 3: Survival Curves for Clothianidin LD50 (75ul in 30% honey daily)

Day	Day 1 (2/05/13) -CE										Day 2 (2/06/13) - MM										Day 3 (2/07/13) - CE									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		
Low - 1 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	/	/		X	X	X	X	X	X	X	/		
Medium - 5 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
High - 7 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	/				X	X	X	X	X	X				
Lethal - 10 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	/	/						X	X	/	/						

Day	Day 4 (2/08/13) - MM										Day 5 (2/09/13) - MM										Day 6 (2/10/13) - CE									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X				X	X	X	X	X	X	X			
Low - 1 ppb	X	X	X	X	X	/	/				X	X	X	X	X	/					X	X	X	X						
Medium - 5 ppb	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X			X	X	X	X	X	X	X			
High - 7 ppb	X	X	X	X	X	/					X	X	X	X	X	/					X	X	X	X	X					
Lethal - 10 ppb	X	X	/	/							X	X	/								X									

Day	Day 7 (2/11/13)										Day 8 (2/12/13)										Day 9 (2/13/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X					X	X	X	X	X	X					X	X	X	X	X	/				
Low - 1 ppb	X	X	X	X							X	X	X	/							X	X	/							
Medium - 5 ppb	X	X	X	X	X	X	X				X	X	X	X	X	X	X				X	X	X	X	X	/				
High - 7 ppb	X	X	X	X	/						X	X	X	X	/						X	X	X	/						
Lethal - 10 ppb	/										/										/									

Day	Day 10 (2/14/13)										Day 11 (2/15/13)										Day 12 (2/16/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	/						X	/	/								X	X	/							
Low - 1 ppb	X	/									X	/									X									
Medium - 5 ppb	X	X	X	X	X	/					X	X	X	X	X	/					X	X	X	X	X	/				
High - 7 ppb	X	/	/								X	/									X									
Lethal - 10 ppb																														

Day	Day 13 (2/17/13)										Day 14 (2/18/13)										Day 15 - no dose									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	/								X	X	/								X	/								
Low - 1 ppb	X										X										/									
Medium - 5 ppb	X	X	X	X	/						X	X	X	X	/						X	X	X	X	/					
High - 7 ppb	X										X										X									
Lethal - 10 ppb																														

Males acclimated to cups for several days until no deaths for 48hrs. Then given 75ul of 30% honey solution w/listed concentrations of pesticide 1x per day over 2 week period. Feedings fell between 2pm and 4pm each day under red light. While not being actively fed, bees kept in percival incubator on 12-hr light cycle (12pm, day, 24C, 50% humidity - 12am, night, 22C, 50% humidity).

Male Trial #4: Survival Curves for Clothianidin LD50 (75ul in 30% honey daily)

Day	Day 1										Day 2										Day 3									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Low - 1 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medium - 5 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
High - 7 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lethal - 10 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Day	Day 1 (3/15/13)										Day 2 (3/16/13)										Day 3 (3/17/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Low - 1 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	/	X	X	X	X	X	X	X	X	/	/
Medium - 5 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
High - 7 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	/	
Lethal - 10 ppb	X	X	X	X	X	X	X	X	X	X	X	X	/	/							/	/								

Day	Day 4 (3/18/13)										Day 5 (3/19/13)										Day 6 (3/20/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Low - 1 ppb	X	X	X	X	X	/	/	/	/		X	X	X	X	X	/	/	/	/		X	X	X	X	/	/	/	/		
Medium - 5 ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	/	
High - 7 ppb	X	X	X	X	X	X	/				X	X	X	X	X	/					X	X	X	/						
Lethal - 10 ppb																														

Day	Day 7 (3/21/13)										Day 8 (3/22/13)										Day 9 (3/23/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X	/	X	X	X	X	X	X	X	X	X	/	X	X	X	X	X	X	X	X	X	/
Low - 1 ppb	X	X	X	X	/	/	/				X	X	X	X	/						X	X	X	X						
Medium - 5 ppb	X	X	X	X	X	X	X	X	/		X	X	X	X	X	X	X	/			X	X	X	X	X	X	/	/		
High - 7 ppb	X	X	/								X	X	/								X	X	/							
Lethal - 10 ppb																														

Day	Day 10 (3/24/13)										Day 11 (3/25/13)										Day 12 (3/26/13)									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	
Low - 1 ppb	X	X	/	/							X	/									X	/								
Medium - 5 ppb	X	X	X	X	X	/	/	/			X	X	X	X	X	/	/				X	X	X	X	/	/	/			
High - 7 ppb	X	X	/								X	X	/								X	X	/							
Lethal - 10 ppb																														

Day	Day 13 (3/27/13)										Day 14 (3/28/13)										Day 15 - no dose									
Bee #	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Control - 0ppb	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X													
Low - 1 ppb	/										/																			
Medium - 5 ppb	X	/	/	/	/	/					X	/	/	/	/															
High - 7 ppb	/	/									/																			
Lethal - 10 ppb																														

Males acclimated to cups for several days until no deaths for 48hrs. All cups recieved small ball pollen for feeding which was replaced as

Queen Trial #1 Survival Curves for Clothianidin LD50 (150ul in 30% honey daily)

Day	Day 1 - 2/27/13								Day 2 - 2/28/13								Day 3 - 3/1/13							
Bee #	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Control - 0ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Medium - 5 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
High - 7 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lethal - 10 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	

Day	Day 4 - 3/2/13								Day 5 - 3/3/13								Day 6 - 3/4/13							
Bee #	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Control - 0ppb	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Medium - 5 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	/	/	X	X	X	X	X	X	/	/
High - 7 ppb	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Lethal - 10 ppb	X	X	X	X	X	X	/		X	X	X	/	/				X	X	X	/				

Day	Day 7 - 3/5/13								Day 8 - 3/6/14								Day 9 - 3/7/15							
Bee #	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Control - 0ppb	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Medium - 5 ppb	X	X	X	X	X	X	/		X	X	X	X	X	X	/		X	X	X	X	/	/	/	
High - 7 ppb	X	X	X	X	X	/	/		X	X	X	X	/	/	/		X	X	X	X	/	/	/	
Lethal - 10 ppb	X	/	/																					

Day	Day 10 - 3/8/13								Day 11 - 3/9/13								Day 12 - 3/10/13							
Bee #	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Control - 0ppb	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Medium - 5 ppb	X	X	X	X	/	/	/		X	X	X	X	/	/			X	X	/	/	/			
High - 7 ppb	X	X	X	/	/	/			X	X	/						X	X	/					
Lethal - 10 ppb																								

Day	Day 13 - 3/11/13								Day 14 - 3/12/13								Day 15 - 3/13/13							
Bee #	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Control - 0ppb	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Medium - 5 ppb	X	X	/	/	/				X	X	/	/	/				X	X	/	/	/			
High - 7 ppb	X	X	/						/	/	/						/	/						
Lethal - 10 ppb																								

Day	Day 16 - 3/14/13								Day 17 - 3/15/13								Day 18 -MM - 3/16/13							
Bee #	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Control - 0ppb	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Medium - 5 ppb	X	X	/	/	/				X	X	/	/					X	X	X	X				
High - 7 ppb	/	/							/	/							/							
Lethal - 10 ppb																								

Day	Day 19 - 3/17/13								Day 20 - 3/18/13								Day 21 - 3/19/13							
Bee #	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Control - 0ppb	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Medium - 5 ppb	X	X	X	X					X	X	X	X					X	X	X	X				
High - 7 ppb	/								/								/							
Lethal - 10 ppb																								

Day	Day 22 - 3/20/13								Day 23 -3/21/13								Day 24-3/22/13							
Bee #	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Control - 0ppb	X	X	X	X	X	X	X		X	X	X	X	X	X			X	X	X	X	X	X		
Medium - 5 ppb	X	X	X	X					X	X	X	X					X	X	X	X				
High - 7 ppb	/								/															
Lethal - 10 ppb																								

Queens acclimated to cups for several days until no deaths for 48hrs. Then given 150ul of 30% honey solution w/listed concentrations of pesticide 1x per day over 2 week period. Feedings fell between 2pm and 4pm each day under red light. While not being actively fed, bees kept in percival incubator on 12-hr light cycle (12pm, day, 24C, 50% humidity - 12am, night, 22C, 50% humidity).