LOW-DOSE PESTICIDE EFFECTS IN *APIS MELLIFERA:* TESTING HORMESIS AND REVIEWING CHANGING TRENDS IN HONEY BEE TOXICOLOGY RESEARCH

by

Alexa Stack Mills

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Dedication Page

I dedicate this to my late stepfather Amos Joseph King and my niece Valerie Cynthia Katherine Colpitts.

Joe, I miss you every day and I wish you could have seen me complete this degree. Thank you for choosing my sisters and I, for supporting and loving us until the end. We all wish you could have met Valerie; you would have loved her.

Valerie, I can't wait to watch you grow up. You can do anything you set your mind to, no matter the hurdles you face.

Table of Contents

List of Tables

List of Figures

Abstract
Honey bees (*Apis mellifera*) dominate crop pollination worldwide and are often considered the most important pollinator of agricultural crops. As pollinators, honey bees are likely to encounter insecticides in the field. Neonicotinoids are widely used insecticides that have been at the center of the pollinator-pesticide debate. Hormesis is a biphasic dose response whereby exposure to low doses of a stressor can stimulate biological processes. Insecticide-induced hormesis has been recorded in insects and bees. My thesis examined the idea of hormesis within honey bees. Specifically, I examined whether low doses of a neonicotinoid would stimulate honey bee longevity. Groups of honey bees were individually treated with doses of imidacloprid and kept in bee cages. Their survival was recorded every day until the end of the experiments to determine longevity. In this thesis, I also examined the shifting focus of honey bee toxicology over the decades in an attempt to observe how research has changed, and determine how prevalent insecticide-induced hormesis is within the honey bee literature. I conducted a search using Web of Science to broadly examine pesticide toxicology research on pollinators and then specifically *Apis* species from 1950-2019. Another, more comprehensive search was done where I examined a total of 73 papers from three specific journals, published between 2000 and 2019, all of which examined honey bees and neonicotinoids. Exposure to low doses of imidacloprid did not result in stimulation of honey bee longevity. I found that most toxicology research surrounding pollinators and *Apis* species, alike, was published after 2010. I found there to be a focus on harmful sublethal effects and less of a focus on stimulatory effects or hormesis. In studies examining honey bees and neonicotinoids, I found the number of studies that used probable exposure scenarios was consistent throughout the years. Studies that found neonicotinoids to have no and negative effects on honey bees was another consistent pattern over the time span I examined. The possibility of hormetic responses in honey bees has been overlooked in the literature. By studying hormesis new information into how bees adapt to exposure to neonicotinoids could be revealed. This could provide impactful insights for those involved in the pesticide-pollinator debate.

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Chapter 1: General Introduction

1.1 Pollination, Bees, and Insecticides

Pollination is an ecosystem service and production practice that is widely used and depended upon by many farmers around the world for crop production (Gallai et al., 2009; Jaffé et al., 2009). Pollination services are provided by wild, free-living animals as well as managed bees. Insects have been estimated to supply pollination services worth up to US $215 billion per year (Gallai et al., 2009). Social and solitary bees, wasps, flies, beetles, butterflies, and moths comprise the vast majority of the world's pollinators (Vanbergen et al., 2013). However, bees are the main driving force behind the successful pollination of the vast majority of agricultural crops and wild plants (Potts et al., 2010). They are estimated to be responsible for 35% of global food production (Klein et al., 2007). Given mounting concerns of alleged declines in managed and wild pollinator populations, and considering their economic importance, studies surrounding the potential effects of insecticides on pollinators have increased rapidly in the past ten years.

The European honey bee (*Apis mellifera* Linneaus (Hymenoptera: Apidae)) is the most common managed pollinator in North America and is capable of increasing yield in 96% of animal-pollinated crops (Aguilar et al., 2006). Honey bees dominate crop pollination worldwide and are often considered the most important pollinator of agricultural and horticultural crops (Abrol, 2012; Potts et al., 2010). When wild bees do not visit agricultural fields, or their populations are inadequate to visit all flowers, managed honey bees are often the best solution for farmers to ensure crop pollination. Therefore, over time farmers have incorporated honey bees into their pollination

programs (Klein et al., 2007). The body size and proboscis length of honey bees enables them to forage on and pollinate many types of flowers and crops (Abrol, 2012).

European honey bees are eusocial, holometabolous insects of the family Apidae. They live in large colonies that typically include one queen and all her offspring, which typically includes ~20,000-40,000 female worker bees and ~200-300 male drones (Elekonich and Roberts, 2005). Being haplodiploid, females (queens and workers) arise from diploid (fertilized) eggs, and males (drones) arise from haploid (unfertilized) eggs. The dynamics within a colony are very complex, but the division of labour between worker bees assures things run as smooth as possible. A worker bee can have between five to eleven different duties to perform throughout their lifespan; this is called temporal polytheism (Elekonich and Roberts 2005; Winston, 1987). In most cases, foraging is the duty of the older worker bees (~15+ days old; Elekonich and Roberts, 2005). The transition from an "in hive" bee to a foraging bee is quite drastic, as the conditions within the hive differ greatly from those outside of it. Transitioning takes place from a dark, stable, safe, and warm environment where they are surrounded by their sisters to the bright and busy outdoors where they are susceptible to predators, weather changes, parasites, and pesticides (Vanbergen et al., 2013; Winston 1987).

Due to the eusocial nature of honey bee hives, stressors that foragers are exposed to could be brought back into the hive, including insecticides (Schneider et al., 2012). This may be problematic because when a forager returns to the hive, it "unloads" what it has collected to an in-hive worker bee and the substance (pollen or nectar), along with the insecticide, will then be integrated into the hive in one way or another (Colin et al., 2004). Pollen and nectar are the two primary food sources that honey bees rely on, with

pollen providing protein and essential amino acids, and nectar providing the carbohydrates required to meet energy needs (Rodney and Purdy, 2020). Regardless of the substance that is returned to the hive, it is highly likely there will be some pesticidal residue or contamination as pesticides are so widely used. Depending on the concentration or dose of the chemical that is brought in, there may be observable effects at the individual or colony level. The majority of research on insecticides and honey bees examines adverse effects, which is appropriate as there is ample evidence that at certain doses insecticides can be harmful to honey bees.

1.2 Development of Insect Toxicology

The study of insect toxicology and insecticide dose-response has a rich scientific history. Papers dating as far back as 1956 examine a wide variety of research topics on these subjects (Brann, 1956; Cutler, 2013; Gunther and Blinn, 1956; Hoskins and Gordon, 1956; Kearns, 1956; Martin, 1956). This trend continues today with hundreds of papers published every year on these same subjects and more in emerging related fields. However, studies in insect toxicology with pest and beneficial insects have traditionally focused mostly on high doses and lethal effects (Calabrese, 2005b; Guedes et al., 2016). The traditional laboratory method for estimating the side effects of pesticides on different insects was to determine a median lethal dose (LD_{50}) or lethal concentration (LC_{50}). This measure is the median dose or concentration that kills 50% of individuals over a set period of time, typically 24 or 48 hours. Arguably, the reason why high doses have dominated toxicology testing has been rooted in the strong underlying assumption of a threshold dose–response model (discussed in section 1.4; Calabrese, 2005b). However,

focusing solely on high doses and acute mortality is a large oversimplification of the potential consequences associated with pesticide exposure.

The importance of examining lower doses has long been realized and different methods have been employed to account for this (Calabrese, 2005b; Croft and Brown, 1975; Haynes, 1988; Ripper, 1956; Stark and Banks, 2003). In particular, the highest concentration where no effects are observed is referred to as the no observable effects concentration (NOEC) or level (NOEL; Calabrese, 2005b; Calabrese and Baldwin, 2003; Figure 1.1)). This measure is broadly used when examining sublethal effects as it determines the concentration or level below which no effects will be observed (Calabrese and Baldwin, 2003). It is important to employ this method and examine sublethal effects because although pesticides are typically applied to cause rapid death of pest species, residues degrade over time on plants and animals, as well as in water and soils (Badji et al., 2007; Desneux et al., 2005; Guedes et al., 2016). This results in sublethal exposures. Further, nontarget species, including secondary insect pest species, and beneficial insects can be exposed to sublethal concentrations of pesticides, leading to unforeseeable consequences (Cordeiro et al., 2013; Cutler et al., 2022; Desneux et al., 2007; Guedes et al., 2016; Haddi et al., 2015).

Natural enemies are a group of beneficial insects that have arguably received the most interest when examining sublethal effects. These include insects that kill, and/or consume pest species. For this reason, they are important in pest management, providing biological control. This group of insects spend a significant amount of their life searching for hosts or prey, making navigation and orientation very important (Desneux et al., 2007). Therefore, when exposed to an insecticide that targets the nervous system they

may be particularly impeded as navigation depends on nervous transmissions. In examining sublethal effects, Longley and Jepson (1996) found that when exposed to field realistic doses of deltamethrin on filter paper, an aphid parasitoid wasp (*Aphidius rhopalosiphi* deStefani-Perez (Hymenoptera: Braconidae)) reduced the time spent searching for a host, spent significantly more time grooming, and were found to actively avoid insecticide treated vegetation. Many beetles also act as natural enemies; for example, coccinellid species (ladybird beetles) are used as a biological control of aphids and have been shown to alter their feeding behaviours after exposure to insecticides (Signh et al., 2004). Further, natural enemies are exposed to pesticides by direct or indirect contact with sprayed areas or through ingestion of contaminated prey (Santos-Junior et al., 2019). Exposure to pesticides could cause mass death in natural enemy populations, which can directly affect the agroecosystem, potentially causing an imbalance in favor of pest infestation and resurgence, thereby decreasing crop quality (Cutler et al., 2022; de Castro et al., 2015; Guedes et al., 2022a; Serrão et al., 2022). Therefore, fully investigating the sublethal effects associated with different pesticides is imperative to maintaining agroecosystems.

Pollinators have also become one of the most studied groups of insects regarding sublethal effects. Pollinators are another large group of beneficial insects as they provide us with pollination services. They are susceptible to sublethal exposure as they are very likely to interact directly with crops that have been treated with pesticide. Pesticides that affect pollinator physiology, such as neurotoxic insecticides, can cause acute or chronic toxicity. These include the insecticides organophosphates (Dorneles et al., 2017),

neonicotinoids (Tosi et al., 2016), and pyrethroids (Mokkapati et al., 2021; Peterson et al., 2021), among others (Barbosa et al., 2015a; Barbosa et al., 2015b; Tomé et al., 2015).

Many studies have demonstrated that pesticides can cause negative chronic or sublethal effects in bees. For example, Henry et al. (2012, 2015) demonstrated that field exposure to sublethal amounts of insecticides can have negative effects on honey bees including decreased survival and foraging success at both the colony and individual level. Tsvetkov et al. (2017) showed that bees chronically exposed to a neonicotinoid had shorter foraging times, a decrease in hygienic behaviour, and a higher proportion of queenless hives later in the summer. Similarly, Goñalons and Farina (2015) found that young honey bees exposed to field-realistic doses of imidacloprid had impaired associative learning. Effects like these are widespread throughout honey bee toxicology literature.

Effects on foraging activity and locomotion have been observed frequently in bees exposed to pesticides (Charreton et al., 2015; Henry et al., 2012; Stanley et al., 2016; Yang et al., 2008). There are many instances of negative effects including hypo- or hyper- activity and involuntary movements that impede mobility (Lunardi et al., 2017). Changes in the flight ability of pollinators after exposure could be related to flight muscles being uncoordinated (Kenna et al., 2019). Other effects such as repellency and irritation can be caused by an unpleasant odor that is emitted when a pesticide is applied (Stejskalová et al., 2021). Negative effects within the hive have also been examined such as reduced brood development (Laycock et al., 2012). Gill et al. (2012) found that bumble bees exposed to two pesticides showed a higher worker mortality which ultimately led to reductions in brood development and colony success. However, in some

cases improbable exposure scenarios are used whether it be using doses that are too high (not field realistic), or only providing food that is laced with pesticides for a prolonged period of time (Aufauvre et al., 2014; Catae et al., 2018).

There has also been plenty of research that suggests exposure to pesticides treated crops showed no observable effects at the colony or individual level (Cutler et al., 2014; Schneider et al., 2012). For example, after a three week exposure period to flowering clothianidin seed-treated canola, honey bees showed no long term effects with regard to mortality, longevity, or brood development and there were no residues of clothianidin found in any beeswax samples (Cutler and Scott-Dupree, 2007). Small residues of imidacloprid were found in hives that had been exposed to seed treated maize over the course of a summer. Although imidacloprid was present in wax samples in the hive, there was no observable effect on honey bee health and there was no significant difference in mortality between the control and treated groups (Nguyen et al., 2009). Further, Traver et al., (2018) found that honey bees that were already infected with varroa mite (*Nosema ceranae* (Fries) (Dissociodihaplophasida: Nosematidae) showed no impairment in individual or social immunity after exposure to field relevant concentrations of different pesticides and fungicides (*tau*-fluvalinate, cholorthalonil, and Fumagilin-B). Examining other pollinators have also shown minimal effects of pesticides at field realistic doses. *Osmia bicornis* (Linnaeus) (Hymenoptera: Megachilidae) food consumption or survival was not affected in adult females after chronic exposure to different doses of Roundup® and clothianidin either alone or combined (Strobl et al., 2020).

Exposure to sublethal doses of insecticides have also been shown to cause stimulatory effects in bees. Studies have shown that at sublethal doses chemical stressors

can have a stimulatory effect on bee memory, survival, and queen production (Köhler et al., 2012a; Moffat et al., 2016; Thany and Gauthier, 2005). Decourtye et al. (2003) found that winter honey bees exposed to low concentrations of an imidacloprid metabolite (5-OH-imidacloprid), and imidacloprid had reduced mortality, and a higher reflex response, respectively. Acute exposure to a low dose of the neonicotinoid thiamethoxam has also been found to increase flight speed, distance and duration in the eastern honey bee (*Apis cerana*; Ma et al., 2019). Bumble bee (*Bombus terrestris* (Linnaeus) (Hymenoptera: Apidae)) colonies reared on a sugar solution laced with small doses of thiamethoxam produced more foragers that collected more pollen and learned to manipulate flowers faster than the control bees (Stanley and Raine, 2016). Interestingly, Stanley et al. (2015) found that individual bumblebees exposed to thiamethoxam visited more flowers, but on the colony level showed an overall reduction in pollination services. Likewise, bumblebees chronically treated over nine days with thiamethoxam showed stronger buzz pollination efforts, collecting more pollen at the beginning of an experiment in comparison to later in the experiment, ultimately leading to a decrease in pollination services (Whitehorn et al., 2017)

Arthropods can be affected both directly and indirectly by the application of pesticides. Direct effects include effects that are without intermediation (i.e. a direct exposure leading to a direct response). Indirect effects include effects that are mediated by another organism (Cutler et al., 2022; Guedes et al., 2016). Pesticides supress arthropod populations by interacting with a primary site of action within an individual organism, impairing at least one of its basic physiological functions, which ultimately leads to death (Casida and Durkin, 2013). However, most pesticides are likely to interact

with at least one secondary site of action, which may not lead to death but could cause sublethal effects that alter homeostasis and interfere with other physiological or biological processes (Cutler et al., 2022; Desneux et al., 2007; Guedes et al., 2016). Therefore, investigating all aspects of sublethal effects with regard to honey bees is of interest for economic, environmental, and sustainability reasons.

1.3 Neonicotinoids

Neonicotinoids are a class of insecticides that target the central nervous system of insects and induce continuous excitation of the neuronal membranes, which leads to exhaustion, paralysis, and death (Matsuda et al., 2001; Simon-Delso et al., 2015). The first neonicotinoid, imidacloprid, came onto the market in 1991 and since 1999 has been the most widely used insecticide each year until at least 2018 (Casida, 2018; Cressey, 2017). The group is currently responsible for a quarter of the global insecticide market share, despite its current use restrictions in Europe (Sparks et al., 2020). These insecticides were originally used to control sucking insect pests but have expanded to target a variety of agricultural and horticultural pests (Millar and Denholm, 2007; Tomizawa and Casida, 2005). Neonicotinoids target the nicotinic acetylcholine receptors (nAChRs) in nerve cells of both vertebrates and invertebrates (Casida, 2018; Millar and Denholm, 2007). The nAChRs are receptor polypeptides that typically respond to the neurotransmitter acetylcholine (Tomizawa and Casida 2005). However, nicotine and neonicotinoids act as agonists on these receptors by binding and causing overstimulation of nerve cells which can lead to death (Jeschke and Nauen, 2008). Imidacloprid in particular behaves as a partial agonist of the nAChRs in the Kenyon cells of the honey bee mushroom body, as well as the antennal lobe neurons (Déglise et al., 2002). In antennal lobe neurons, there

are two types of currents reported: type I nAChR currents, which exhibit slow desensitization, and type II currents, which exhibit fast desensitization, strongly suggest the presence of at least two different types of nAChRs (Simon-Delso et al., 2015). `The nAChRs are the most abundant excitatory neurotransmitter receptors in insects, whereas glutamate plays this role in vertebrates making the toxicity risk of neonicotinoids to vertebrates minimal and making insects the perfect target (Bass and Field, 2018; Jeschke and Nauen, 2008).

Neonicotinoids can be applied as seed dressings, as a foliar spray, and in furrow. The systemic nature and water solubility of neonicotinoids allows the insecticide to be absorbed by plants through roots and be transported throughout plant tissue. This gives this class of insecticides an advantage in pest control as they protect all parts of the plant. This is advantageous because foliar sprays of non-systemic compounds are not efficient at controlling pests that feed on or bore into the tissues or roots of plants (Goulson, 2013). Considering neonicotinoids are integrated into all tissues of plant, it is not surprising that they are also found in the nectar and pollen of those plants (Blacquière et al., 2012). This is of concern to pollinators as they could be exposed to different doses of neonicotinoids in this manner and, as outlined in previous sections, this can cause a variety of sublethal and lethal effects.

Questions concerning the effect of neonicotinoids on pollinators surfaced in the mid 2000s and by 2011, neonicotinoids were thought to be a contributing factor to the alleged declines in honey bee colonies, particularly in the European Union (EU). It was not until 2013 that the use of three neonicotinoids (imidacloprid, thiamethoxam, and clothianidin) as seed dressings in crops that attract pollinators, were banned by the EU in

an attempt to mitigate concerns surrounding pollinator decline (European Union, 2013). This was based on evidence that reported sublethal effects in pollinators in response to neonicotinoids (European Union, 2013). This ban was revaluated and extended in 2018 after the European Food and Safety Authority published a comprehensive report (EFSA, 2018). This included and updated risk assessment for the three neonicotinoids mentioned above. The report concluded that there was scientific evidence that neonicotinoids do contribute to the decline in honey bee colonies, and thus the ban was extended (Cressey, 2017; Wood and Goulson, 2017). Two years before this extension, the French parliament was the first in the world to ban the use of neonicotinoids for crop protection (Biodiversity Act, 2016); the ban taking effect in September 2018. These bans are controversial as there has been conflicting evidence suggesting neonicotinoids have no effects, or even stimulatory effects, on bees (Barascou et al., 2021; Cutler et al., 2014; Colin et al., 2019; Meikle et al., 2022; Traver et al., 2018). This is of concern because farmers in the EU and France could be suffering crop, and therefore economic, losses that could be avoided using neonicotinoids. Alternatives have been considered to replace neonicotinoids (sulfoximines; Watson et al., 2021), but right now the most effective replacement are simply other chemical insecticides, typically of previous generations (e.g. pyrethroids; Jactel et al., 2019), these insecticides also come along with possible sublethal effects on pollinators (Charreton et al., 2015; Mokkapati et al., 2021).

1.4 Hormesis

Hormesis is a biphasic dose response phenomenon, whereby exposure to low doses of a stressor can stimulate biological processes (Calabrese, 2010, 2013). The concept of hormesis and stimulatory responses to stress was established in the 1880s with Hugo

Schultz's research on yeast (Calabrese 2018). He found that after exposure to different types of acids, fermentation was stimulated and resulted in an increase in carbon dioxide emissions (Henschler, 2006). The term "hormesis" was first coined in 1943 by Southam and Erhlich who observed that red cedar extracts inhibited fungal metabolism in high doses, but enhanced it in low doses (Southam and Ehrlich, 1943). However, hormesis has been relatively unknown and understudied in the science community until recently.

In the past, the idea of a dose response relationship has taken the form of two main models in toxicology (Calabrese and Baldwin, 2003). The threshold model, which states that there is a threshold where exposure to a stressor causes an effect (Cox, 1987; Figure 1.1). The second model is the linear non-threshold model, which assumes that at low doses there is a linear relationship between dose and risk that terminates at zero (Calabrese 2013; Figure 1.1). Neither of these models explain stimulatory responses that are observed after exposure to a low dose of a stressor. Hormesis is a model that encompasses this phenomenon. There are two forms of the hormetic dose-response model that can be expressed. Depending on the stressor and the endpoint being measured, the dose-response curve will take the form of a J or an inverted U shape (Calabrese and Baldwin, 2002). The inverted U shaped curve presents itself when stimulation in endpoints like survival is seen at low doses, but increased inhibition (e.g., decreased survival) is seen at high doses (Calabrese and Baldwin, 2002). The J shaped curve would be seen with a dysfunctional endpoint like disease incidence. Here you could see responses where low doses of stressor will reduce dysfunction, but high doses increase dysfunction (Ayyanath, 2013; Calabrese and Baldwin, 2002; Figure 1.1). In both cases of the hormetic dose response model, the effect will be seen around the No Observable

Effects Concentration (NOEC), which is the concentration or dose at which no effects will be observed in response to a stressor (Figure 1.1).

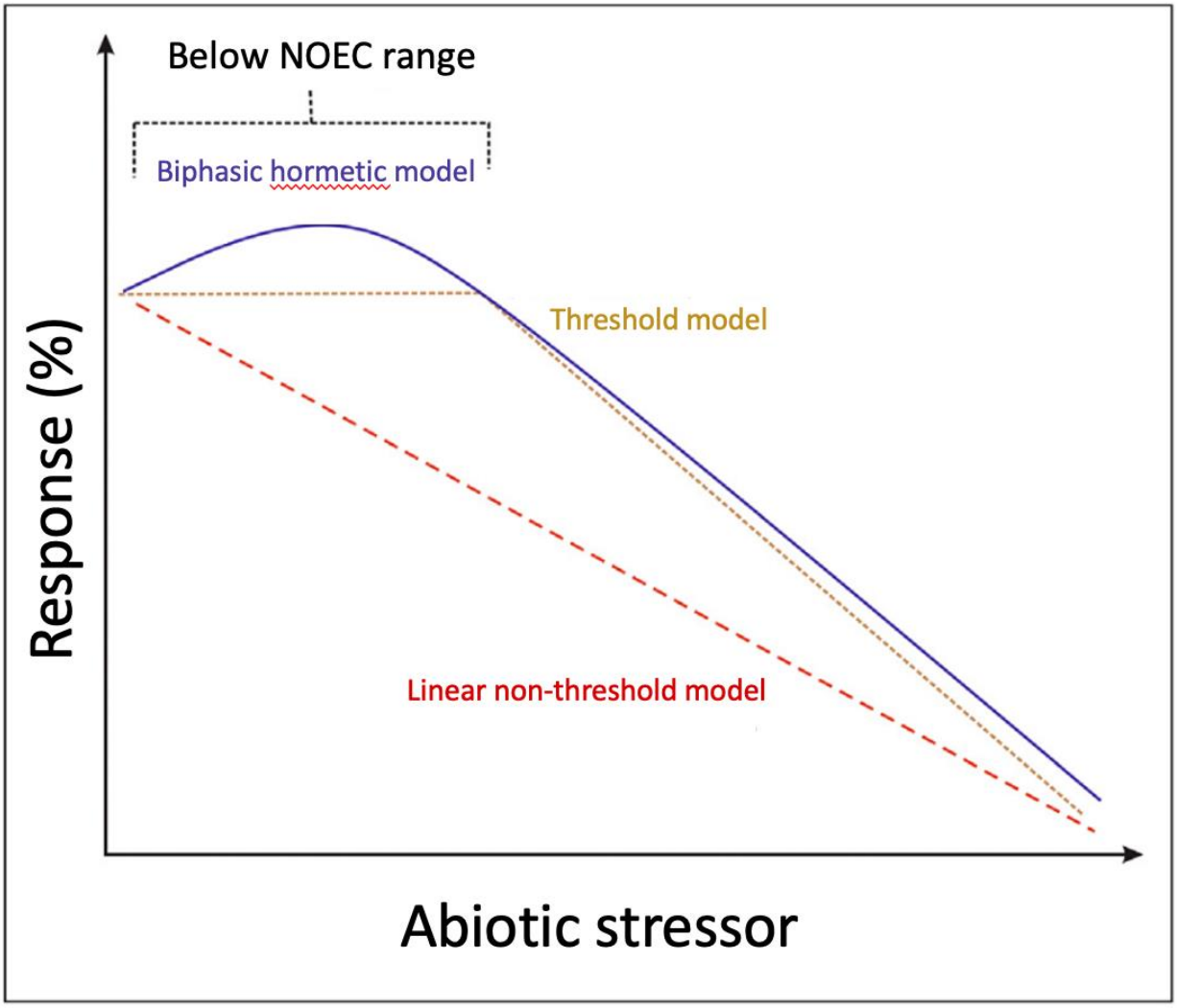

Figure 1.1. Schematic of monophasic and biphasic dose–response models for a given abiotic stressor. Red dashed line indicates the linear non-threshold model, brown dotted line represents the threshold model, and the blue solid line represents the biphasic hormetic model. The range below the no observable effects concentration (NOEC) is indicated. Figure modified from Guedes et al. (2022a).

Hormesis can be considered an evolutionary adaptation as it arises from various, diverse stressors that an organism deals with (Parsons, 2001). It has been observed in

many different organisms and taxa across multiple biological endpoints following exposure to a variety of different stressors (Agathokleous and Calabrese, 2019; Cutler et al., 2022; Saitanis and Agathokleous, 2019; Vargas-Hernandez et al., 2017; Zhou et al., 2019). Since 2010, interest in insecticide-induced hormesis has increased substantially (Cutler, 2013). There is now a large amount of evidence showing that hormesis occurs in insects when exposed to a low dose of insecticide, resulting in, for example, stimulated reproduction, increased size, or increased longevity (Cutler and Guedes, 2017; Cutler, 2013; Cutler et al., 2022). The majority of hormesis research involving insects has focused on pest species, with ramifications for agricultural challenges like pest resurgence or insecticide resistance (Cutler et al., 2009; Guedes et al., 2017; Guedes et al., 2022a; Morse, 1998). For example, Cutler et al. (2009) showed that exposure to imidacloprid stimulates reproduction in the green peach aphid (*Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Similarly, Guedes et al. (2010) found a low dose of deltamethrin caused a peak in population growth rate of the highly-pyrethroid resistant strain of the maize weevil (*Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). Exposure to low doses of imidacloprid via dipped leaf disks sped up the development and increased the fecundity of the western flower thrips (*Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae; Cao et al., 2019). Similarly, there is research showing that low amounts of chemical stress can stimulate longevity or reproduction in insects other than pest species. For example, *Galleria melonella* (Linnaeus) (Lepidoptera: Pyralidae)is an important insect model in many areas of research. Wojda et al. (2009) found that when *G. melonella* larvae were exposed to mild heat shock (38 °C for 30 min) before being infected with a fungus, their lifespan was extended; which the authors attributed to higher

expression of antimicrobial peptides and higher antifungal and lysozyme activities in the heat-shocked animals. Similarly *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), had an increased lifespan after exposure to repeated heat stress (Hercus et al., 2003).

Given that insecticide-induced hormesis occurs in many other insect taxa, it seems plausible that it should also occur in bees (Cutler and Rix, 2015). Honey bees (*Apis cerana*) that were orally exposed to thiamethoxam in sugar solution at a dose of 10 ppb showed significantly higher average homing time, mean flight velocity, flying distance, and flying duration than the control (Ma et al., 2019a). Similarly, honey bees orally exposed to sublethal doses of thiacloprid and two strains of the bacterium *Enterococcus faecalis* (Andrews and Horder) ((Lactobacillales: Enterococcus); found in manure)) simultaneously had a significantly higher survival rates eleven days post exposure compared to controls (Dickel et al., 2018). This study also reported higher immune response in the form of increased antibacterial activity. Sugar syrup with a concentration of 5 ppb of imidacloprid was fed to colonies over a six week span and those that were exposed showed more capped brood than the control (Colin et al., 2019).

1.5 Objectives and Hypotheses

It is essential for organisms to be able to adapt to changing environmental conditions to survive. Hormesis is considered an adaptive, evolutionary response to stress and has been observed in multiple different taxa. Insecticide-induced hormesis is an important field of research and primarily focuses on pest species, as hormetic exposure to pesticides can have ramifications in terms of pest outbreaks (Guedes et al., 2022a). However, research surrounding hormesis in beneficial or non-pest species is also of importance as it will

provide insight into how low doses of insecticide may stimulate certain biological processes in insects.

Bees are important pollinators and can be exposed to sublethal doses of insecticides while foraging. Neonicotinoids are considered one of the most important insecticides with regard to overall sales and use; they accounted for 24% global insecticide market in 2018 (Sparks et al., 2020). Neonicotinoids are also analogues of the natural compound nicotine which is considered a stimulant (Tomizawa and Casida, 2005). Nicotine has been shown to cause stimulatory effects in honey bees such as an increase in learning, memory retention, and longevity (Köhler et al., 2012a; Thany and Gauthier 2005). Nicotine is toxic to honey bees at high doses, but has been shown to cause stimulatory effects at low doses. Considering this, the objective of my second chapter was to examine if low doses of the neonicotinoid imidacloprid would stimulate honey bee longevity. I hypothesized that low doses of a nicotine analogue, the neonicotinoid imidacloprid, would cause hormetic (stimulatory) effects in honey bee longevity.

My research was supposed to involve further experiments considering hormesis and learning in honey bees. These experiments (longevity and learning) were to be coupled with examining gene expression in honey bee tissues associated with longevity (fat body) and learning (brain). However, due to the COVID-19 pandemic, I was unable to complete the two other experimental aspects of my research. Therefore, as an alternative I completed a systematic review of literature that focuses on *Apis* spp., their interactions with pesticides, and how honey bee toxicology research has changed over decades. This was broken down into two sections. The first examined how many papers were published

each decade between the years 1950 – 2019 that included keywords related to pesticides, honey bees and different responses (e.g. sublethal, acute, chronic toxicity etc.). The second section specifically focused on neonicotinoids and honey bees between the years 2000 and 2019. I examined trends in toxicology research to determine if patterns emerge (e.g., possible biases or deficiencies) and drew broader conclusions about the honey bee's relationship with neonicotinoids.

The overall objective of my research aimed to further understand and explain the relationship between honey bees and pesticides. Hormesis is a phenomenon that could provide insights into biological, physiological, and economic benefits with regard to honey bees and other beneficial insects, as well as improve fundamental understanding of how bees respond to low doses of chemical stressors. Further, examining how research and trends have changed over the decades could be integral in understanding how knowledge and possible biases have developed and continue to develop over time. It may also provide further insight into pollinator risk assessment.

Chapter 2: Can Low Doses of the Neonicotinoid Imidacloprid Cause a Hormetic Response in Honey Bee Longevity?

2.1 Introduction

Hormesis is an evolutionarily conserved, adaptive response to stressful conditions (Calabrese et al., 2007). In general, the hormetic response has been shown to stimulate many biological processes, such as increasing reproduction, memory and learning, and longevity (Cutler, 2013). Insecticide-induced hormesis in insects has become more widely known in toxicology research in the last decade (Cutler and Guedes, 2017; Cutler, 2013; Cutler et al., 2022) and hormesis in honey bees has become an area of interest (Cutler and Rix 2015). Honey bees are economically important insects that are widely managed worldwide. In recent years, there have been increasing concerns about pollinator decline around the world, in North America and Europe in particular (Kevan and Phillips, 2001; Williams and Osborne, 2009).

Bees can be exposed to insecticides, and among these the neonicotinoid insecticides have received much attention. Neonicotinoids are a common group of insecticides that are used to control a variety of insect pests (Jeschke and Nauen, 2008). High doses of insecticides can certainly be detrimental to bees, and inhibition of biological functions following sublethal exposure is demonstrated throughout the literature using a wide range of endpoints (e.g. survival, foraging, learning, etc.). However, given that insecticide-induced hormesis occurs in many other insect taxa, it seems plausible that it should also occur in bees. This may be particularly true with analogs of nicotine (i.e. neonicotinoid insecticides), given nicotine can be a stimulant in low doses (Köhler et al., 2012a; Palmer-Young et al., 2017; Thany and Gauthier, 2005). Stimulatory effects with low doses of nicotine and its analogues occur despite that fact

that high doses of nicotine are clearly lethal to bees (Bounias et al., 1995; Colin et al., 2019; Dickel et al., 2018; Ramanaidu and Cutler, 2013; Wong et al., 2018; Wright et al., 2013). Here, I examined if exposure to low doses of a nicotine analogue, imidacloprid, would produce hormetic effects in the longevity of honey bees. I hypothesize that bee exposed to certain low doses of imidacloprid would live longer than control bees.

2.2 Materials and Methods

2.2.1 Insects

Worker honey bees (*Apis mellifera,* specifically Kona strain) were collected from hives maintained at the Dalhousie University Agricultural Campus in Truro, Nova Scotia. Hives were treated with Apivar® (Amitraz, 3.3% active ingredient (AI); Véto-pharma S.A, Courtaboeuf, France) in the spring and FormicPro™ (Formic acid, 42.25% AI; NOD Apiary Products Ltd., Frankford, ON, Canada) in the fall for varroa mite (*Varroa destructor* Anderson and Trueman (Mesostigmata: Varroidae) suppression. The hive used was considered very strong and young worker bees were collected by removing frames and gently brushing them into a container. This method was chosen in an attempt to ensure young worker bees, as ~60% of workers on frames will be one to two weeks old, and there is no significant difference in worker age between frames (van der Steen et al., 2012). This method of collection could not ensure all bees were of a homogeneous age, and their age was ultimately undefined with a range of 1-2 weeks. I ensured all individuals were evenly distributed among all experimental cages (see below), as similarly done in Williams et al. (2013). Bees were taken from the same hive throughout experiments and after collection they were immediately transported to the lab with ~100 bees per container. The containers used for transportation were round, ~7.6 cm tall, and

had a diameter of ~10.2 cm. They were vented on either side by holes covered in mesh and were otherwise empty.

2.2.2 Preliminary Bioassays and Cage Design

Prior to hormesis experiments, preliminary bioassays were conducted to determine the No Observable Effects Level (NOEL) and LD_{50} for imidacloprid on honey bees. The NOEL is considered the highest concentration where no effects are observed and LD_{50} is the median dose that kills 50% of individuals over a set period of time, typically 24 or 48 h. Determining the NOEL is important because chemical hormesis becomes more likely to occur below the NOEL (Calabrese 2005a). A 100 ml stock solution of 1000 ppm of imidacloprid (Admire® 240 SC, 240 g AI L-1; Bayer Crop Science Canada, AB, Canada) was prepared using distilled water and the surfactant (0.1% v/v) Polysorbate 80 (Tween 80; Sigma-Aldrich, Saint Louis, Missouri, United States of America). To determine the NOEL, bees were exposed to concentrations of 0 (control), 1, 10, 50, 75, 100, 500, 1000, and 2000 ppm of imidacloprid.

Over a one-month period, the preliminary bioassays were completed. The NOEL was determined by using a probit analysis to assess honey bee mortality 48 h post exposure. A probit analysis determines the NOEL value by taking the log of the dose response curve and transforming it into a linear function. This makes it easier to estimate the LD_{10} and LD_{50} (PROC PROBIT; SAS Institute 2013). In the first three preliminary trials, five treatment concentrations (0, 1, 10, 100, and 1000 ppm) were used. Each treatment contained four replicate cages for a given bioassay, giving a total of 12 replicates per concentration and 60 cages. Four additional treatment concentrations were added (50, 75, 500, and 2000 ppm). These additional treatment levels contained six

replicates each and two bioassay trials were completed: giving a total 12 replicates per concentration and 48 cages. Thus 108 cages were used for the preliminary bioassay probit analysis. However, some replicate cups were unusable with the majority of the bees dying immediately after treatment (i.e. >50% mortality within an hour after treatment). These cups were removed from the analysis, leaving 106 cages. Honey bee survival was recorded after 48 h and produced a probit line of mortality, LD values, χ^2 test statistics, 95% fiducial limits, and the NOEL using the PROC Probit procedure in SAS 9.4 (SAS Institute 2014).

Prior to exposure, honey bees needed to be immobilized. Individuals were separated into groups of ten, placed in a 9.6 mm petri dish lined with filter paper and assigned a treatment group. Bees were separated into the petri dishes by gently removing them from a small hole in the container that housed them using feather tweezers. The petri dishes that contained honey bees were placed in a freezer at ~ -21 °C for three min (Human et al., 2013). This immobilisation technique was chosen because the length of chilling exposure can influence phenotypic responses, and chilling for three min at -20 °C does not affect worker longevity, orientation, or foraging (Ebdai et al., 1980; Frost et al., 2011). Once immobilized, individual bees were exposed to 1 µl of the appropriate treatment using a PAX 100-3 micro-applicator (Burkard Scientific, Uxbridge, UK). The dose was applied directly to the dorsal side of the thorax, ensuring contact with the cuticle and minimal contact with hair/setae (Medrzycki et al., 2013).

Cages were constructed for bees to be kept in after exposure. These cages were clear 354.8 mL plastic cups with square ventilated “windows” (~ 5 cm x 5 cm) covered in very fine mesh. The cups were placed on top of the petri dishes described above. A 10 ml

plastic syringe with the tip removed was filled with 50% sucrose solution and inserted into the top of the cup, via a small hole for feeding (Huang et al., 2014). All "bee cup cages" (hereafter termed "cages") were kept in a growth chamber (ThermoFisher Scientific, Waltham, Massachusetts, USA) at 60% relative humidity in the dark at ~25 °C until the bioassay concluded.

2.2.3 Design and Details of Hormesis Toxicity Experiment

Eight treatment concentrations were used for the longevity bioassays: 0 (control), 0.0025, 0.025, 0.250, 1, 2, 2.5, and 25 ppm of imidacloprid applied using a micro-applicator. This translates to doses of 0 (control), 0.0025, 0.025, 0.250, 1, 2, 2.5, and 25 ng/bee. Doses were chosen by bracketing the NOEL value (2 ng/bee), incorporating two doses above the NOEL and several other doses down to 1000-fold below the NOEL. Each treatment level had four replicate cages, and ten bees per replicate; the experiment was repeated four times (four trials). Honey bees in each cage were observed daily, and mortality was assessed until fewer than 25% of bees remained in the control group (i.e. less than 10 bees among all four cages), as the control is the baseline for comparisons. Trials lasted between 28 and 35 days, depending on mortality. Bees were considered dead if they were motionless and did not respond at all to tapping near them on the side of their cage.

As described above, sister bees from one hive were kept in bee cages (Figure 2.1). The syringes containing the sugar syrup were changed every seven days. Additionally, a 1 g pollen ball was also added to each cage to meet the bees' protein needs. The pollen balls were made by pulsing 90 g of frozen pollen (Wild Country™, Horizon Group, Canada) in a coffee grinder and mixing the grinds with 10 mL of distilled water. The mixture was then rolled into 1 g balls and dipped in melted beeswax (Kittilsen's Honey

Ltd., Debert, Nova Scotia). They were then left to dry for ~2 h and kept in the freezer until needed (up to two months).

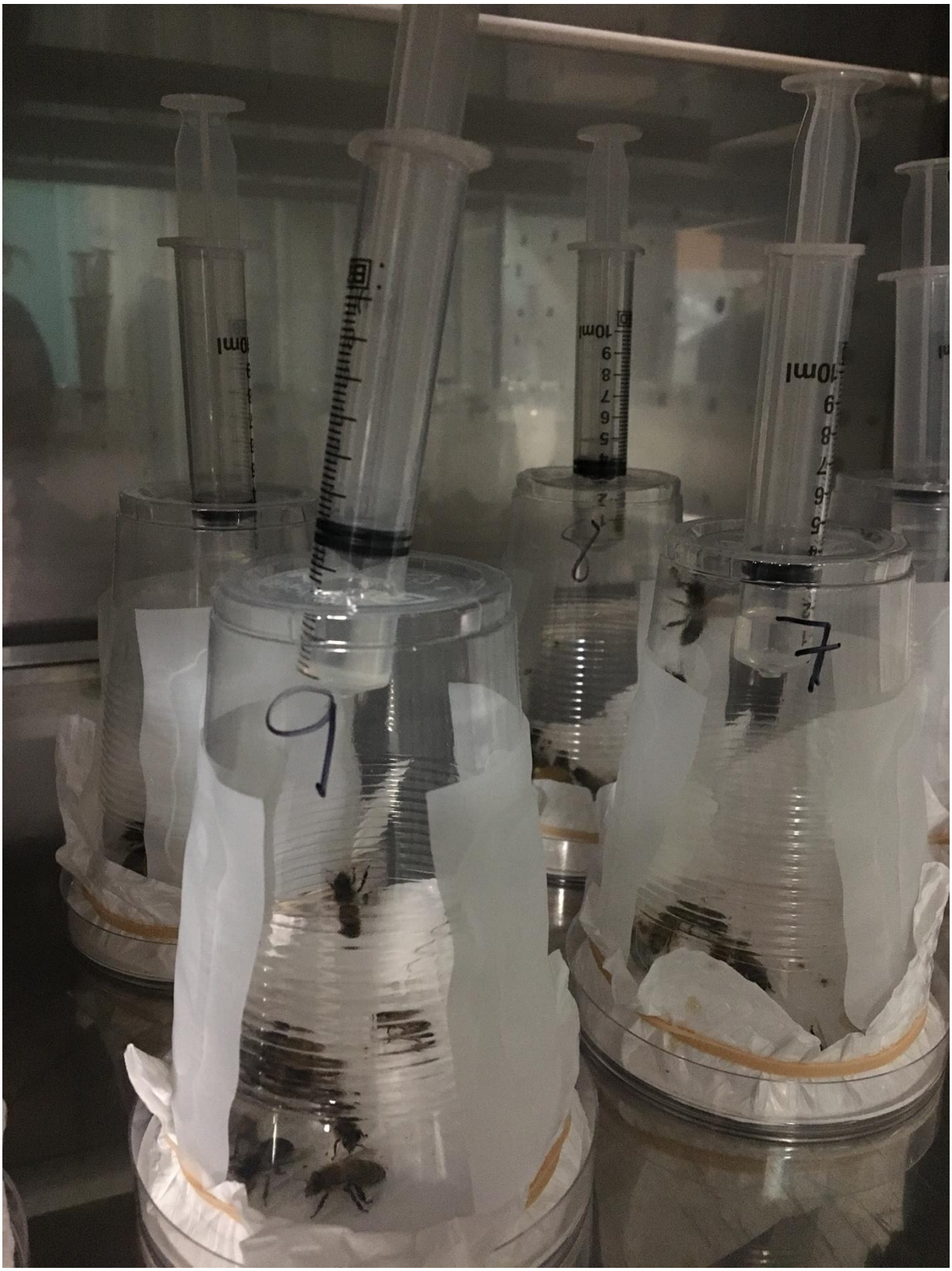

Figure 2.1. Bee cup cages used to hold and expose honey bees (*Apis mellifera*) throughout longevity experiments following imidacloprid treatments.

2.2.4 Statistical Analyses

The longevity data were analyzed by a survival analysis using the LIFETEST procedure with the Kaplan-Meier method in SAS (SAS Institute 2014). The Kaplan-Meier method estimates the fraction of individuals living for a certain amount of time in a treatment level, and so calculates the probability of surviving a given length of time (Goel et al., 2010). Using the χ^2 log-rank test the expected and observed numbers of events in each group are used to determine the χ^2 test statistic (Goel et al., 2010), which is then compared to the critical value, $\alpha = 0.05$. Furthermore, time to mortality can be right-censored when individuals are not followed until they die, meaning that the information that they are alive is used in the analysis, but not their death, since that time is unknown. Bees still alive at the end of a trial were censored. The Kaplan-Meier method also provides estimates of the median survival time for each treatment level (TL_{50} values; the median time required for 50% of the population to die (Appendix A)). Pairwise comparisons among the survival curves were tested using Holm-Sidak's tests to isolate the group(s) that were significantly different from one another (SAS Institute 2014). To further investigate survival patterns, TL_{50} estimates obtained from the LIFETEST were regressed against dosage levels. In this analysis there were some replicates that showed less than 60% survival after day 10. If this was the case, these replicates were removed from the analysis.

2.3 Results

2.3.1 Preliminary Bioassay

The probit results indicated an LD_{10} of 2.03 ng/bee and LD_{50} of 70.7 ng/bee. The χ^2 is significant ($p < 0.001$) indicating the probit model does not fit the data appropriately. I

compared my LD_{10} and LD_{50} to those found in other studies that examined honey bee toxicology with respect to imidacloprid. A range of 1.5-6 ng/bee has been reported for the LD_{10} and a range of 42-104 ng/bee for the LD_{50} (Decourtye et al., 2004; Nauen et al., 2001; Ramirez-Romero et al., 2008; Schmuck et al., 2003; Suchail et al., 2001). My LD_{10} and LD_{50} values for imidacloprid fall within this range. I then used my results to choose a range of doses for my hormesis tests (0-25 ppm).The slope calculated by the probit analysis is indicative of how homo- or heterogeneous a population is in their response to a stressor. The slope calculated here is less than one, indicating a fair amount of heterogeneity in response to imidacloprid within the population of bees used.

Table 2.1. Contact toxicity of honey bees (*Apis mellifera*) 48 h after topical exposure of 1 µl of imidacloprid (using concentrations of 0 to 2000 ppm) applied via a micro-applicator. LD is lethal dose until 10% and 50% of bees died; FL are fiducial limits.

Number of experimental units (*n*)	**LD_{10} (ng/µl) (95% FL)**	**LD_{50} (ng/µl) (95% FL)**	**Slope (± SE)**	**χ^2 (p)**
94	2.03 (0.097 - 7.85)	70.7 (25.8 – 169.4)	0.83 (± 0.07)	43.7 (<.0001)

2.3.2 Longevity Experiment

Results from the LIFETEST revealed a significant difference between survival curves (Figure 2.2; log-rank $\chi^2 = 76.52$, df = 7, $p < 0.001$). Multiple pairwise comparisons (Holm-Sidak method) detected a significant difference between the highest dose (25 ng/bee) and the control (0 ng/bee; $p < 0.001$). The highest dose also showed significant differences between three other doses (2.5, 0.025, and 2 ng/bee; all showed $p < 0.001$). All survival curves showed a similar pattern, relatively high survivorship (>80%) up until approximately day 19, followed by a gradual decline (Figure 2.2). In all curves, the TL_{50} only ranged from 23 to 26 days, with the highest dose of 25 ng/bee showing slightly

lower survivorship overall throughout the trials (Figure 2.2). The TL_{50} estimates regressed against imidacloprid dose did not provide significant relationship (linear nor non-linear) at $\alpha = 0.05$.

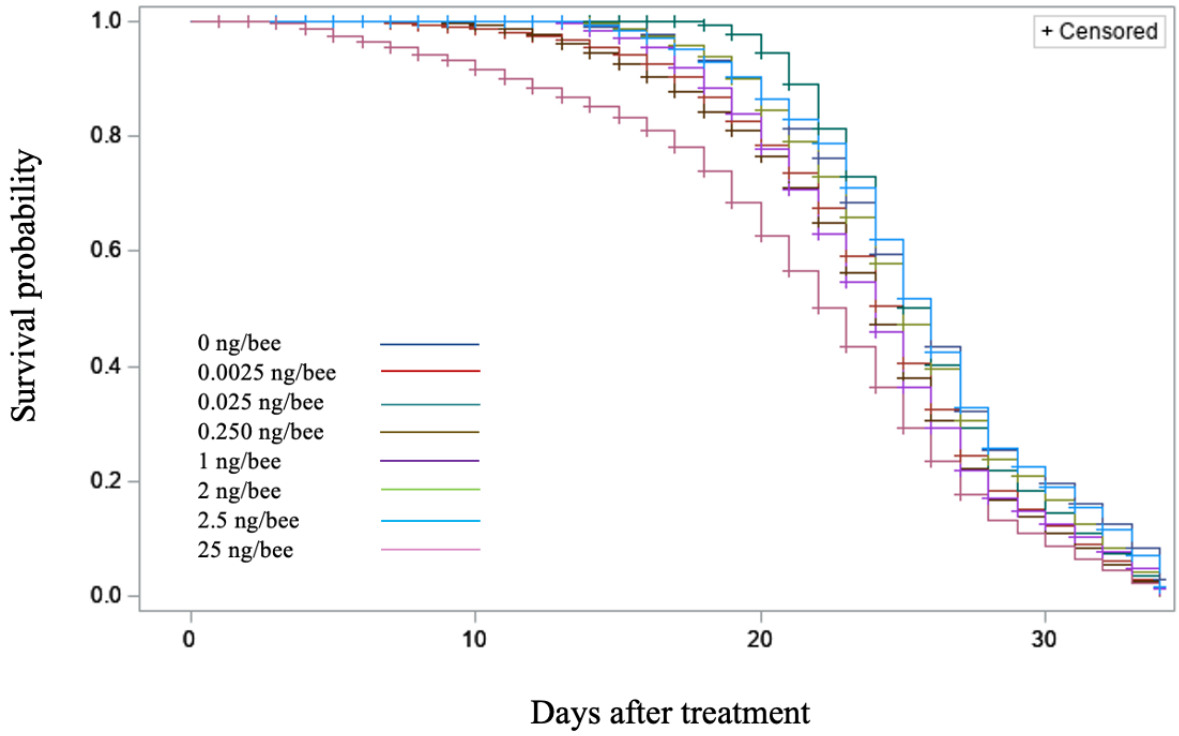

Figure 2.2. Survival probability curves of worker honey bees (*Apis mellifera*) after topical exposure to 1 µl of imidacloprid with varying doses (0–25 ng/bee). Each survival curve is representative of 16 replicate cages for experimental doses and 12 replicate cages for the control (each containing 10 bees).

2.4 Discussion

Pest management practices are widely used in Canada to protect crops from disease and damage. Use of insecticides can pose a threat to pollinators as residues can accumulate in the pollen and nectar of flowers and crops (Blacquière et al., 2012a). This is of concern for bees as insecticide residues can accumulate and persist in hives (Chmiel et al., 2020; Mullin et al., 2010). A considerable amount of research points to the idea that pesticides

cause harm to honey bees, and in certain doses this is the case (Decourtye et al., 2004; Tosi et al., 2017; Williamson et al., 2013). However, bees have been shown to have better memory, higher survival, better immune response, and an increased queen production after exposure to low amounts of different forms of insecticides (Cervantes and López-Martínez, 2022; Köhler et al., 2012a; Moffat et al., 2016; Mulvey and Cresswell, 2020; Palmer-Young et al., 2017; Strobl et al., 2020; Thany and Gauthier, 2005). Many of these studies use a nicotine-based compound, which leads to the possibility that nicotine and its analogues may act as a stimulant to honey bees. I therefore examined whether exposure to low doses of a nicotine-based pesticide could have stimulatory effects on honey bee longevity.

My preliminary bioassays used to determine lethal doses and the NOEL produced an LD_{50} of 70 ng/bee and a NOEL of 2 ng/bee. These values are comparable to those found in the literature. Suchail et al. (2001) found the LD_{50} of imidacloprid on honey bees to be 60 ng/bee. Similarly, Nauen et al. (2001) and Schmuck et al. (2003) found the LD_{50} for this insecticide to be between 49 – 102 ng/bee, and between 42 – 104 ng/bee, respectively. For the NOEL, studies found values ranging from 1.5 – 3.2 ng/bee (Decourtye et al., 2004; Nauen et al., 2001; Ramirez-Romero et al., 2008; Schmuck et al., 2003). Thus, my values for LD_{50} and NOEL are within the range of published values for honey bees, and enabled me to design my experiment. It is important to note that in determining the NOEL, a chi-square test is completed and to confidently assess the validity of your data, the χ^2 value should be insignificant. However, my χ^2 is significant, indicating the probit model does not fit the data appropriately. This could indicate a questionable NOEL and, in turn, questionable experimental doses. However, after

reviewing the literature stated above I felt confident in the doses I chose to complete the bioassays.

Honey bees in my experiment overall showed similar survivorship in relation to other studies. Bees in all but one group showed >80% survivorship until day 19, the highest dose (25 ng/bee) showed a dip below 80% three days earlier, at day 16 (Figure 2.2). This reflects a study done by Paris et al. (2020) where survivorship dipped below 80% at day 18 in the controls. Further, my results showed a decrease to ~50% survivorship in the controls at day 25 (Figure 2.2). This is consistent with other studies, where ~50% survivorship was observed at approximately the same time (Liao et al., 2020; Paris et al., 2020; Shi et al., 2019; Wong et al., 2018a). In studies examining honey bee longevity there appears to be two outcomes. Either honey bee survival begins to notably decrease after 15 days (Liao et al., 2020; Paris et al., 2020; Shi et al., 2019; Wong et al., 2018a) or the notable decrease begins around day 8 (Barascou et al., 2021; Crone and Grozinger, 2021; Köhler et al., 2012a, 2012b; Retschnig et al., 2015). The high survivorship I observed could be representative of the cages used. The cages I used met the requirements for a successful cage, as described by Huang et al. (2014). This included having ventilation, having a transparent window for observations, not being reused, and having an entry way for introduction or removal of materials.

I found significant differences between the 25 ng/bee dose and the control. This dose was the highest dose used in the experiment and therefore a lower survivorship was to be expected. There were also significant differences between this dose (25 ng/bee) and three other doses (2.5, 0.025, and 2 ng/bee). In Figure 2.2 it is evident that all survival curves other than the 25 ng/bee dose showed a similar trend with relatively high

survivorship that started to drop off after day 20. Considering the trend is consistent across most of the other groups, it makes sense there are differences between the highest dose group and the other three groups. Further, I found no significant differences between any of the mid to low doses of imidacloprid and the control, suggesting no evidence of hormesis with respect to longevity. Other research has found similar results to mine, where exposure to a pesticide showed no effect on bee longevity at the individual level (Gregorc et al., 2018; Retschnig et al., 2015). However, I did expect to see some evidence suggesting that hormesis is present in honey bees, as this has been recorded in other bee research (Johnson et al., 2012; Köhler et al., 2012a; Palmer-Young et al., 2017; Strobl et al., 2020; Wong et al., 2018a; Wright et al., 2013).

Stimulation at low doses regarding longevity has been shown in honey bees, however in these cases it appears to be associated with another stressor. Research shows that doses of imidacloprid (0 – 135 ppb) showed no stimulatory effects on honey bee longevity until co-exposure with common phytochemicals *p*-coumaric acid and quercetin (Wong et al., 2018). Liao et al. (2017) found similar results, where honey bee longevity was enhanced after co-exposure to the same phytochemicals and two pyrethroids (β-cyfluthrin and bifenthrin). This could be indicative of a postconditioning hormetic response. Postconditioning is a phenomenon that occurs when a low dose of a stressor is administered after a trauma, disease, or massive exposure and the dose response follows the hormesis model (Calabrese et al., 2013). There are many studies that show hormesis in insects is observable with one stressor (Cutler and Guedes, 2017; Rix and Cutler, 2020; Santos et al., 2018; Strobl et al., 2020). However, with respect to honey be longevity it could be that honey bees only show a hormetic response to pesticides if they have

previously been exposed to phytochemicals, or other stressors, *in vivo* and thus once exposed to both these and pesticides simultaneously. It is plausible that hormesis with respect to longevity may be better observed after a previous stressor is already present as there is minimal literature showing a direct hormetic effect between neonicotinoids and honey bee longevity. It may also be that hormesis is simply not expressed as an increase in longevity in bees, and perhaps other endpoints such as learning or foraging efficiency should be explored.

It is notable that hormesis can be particularly difficult to observe, as the effect is subtle and it requires the use of many doses (particularly surrounding the NOEL), frequent measurements over the span of an experiment, many subjects, and high replication to enhance statistical power (Calabrese and Baldwin, 2003). I believe I met most of these requirements, however, the use of more doses surrounding the NOEL may have yielded different conclusions to my present study. It is important to note that there were no significant negative effects observed in my study; honey bees in most groups survived a similar amount of time as the control group.

In conclusion, although it has been suggested that hormesis is present in bees (Cutler and Rix, 2015), I was unable to find definite evidence of hormesis in adult longevity to low doses of imidacloprid on honey bee longevity in my study. I did observe differences between the highest dose (25 ng/bee) and other groups (0, 0,025, 2, & 2.5 ng/bee). However, the difference between the highest dose and the control is not unexpected and due to the similarity between survival curves in all other groups, the differences are negligible. Overall, I think it is important that the hypothesis of hormesis in honey bees be investigated further to tease out possible hormetic trends. These may

include effects of co-exposure with physical stress, dietary stress, or parasitic stress in honey bee longevity. This would further the understanding of the dose-response relationship neonicotinoids have with honey bees, provide more information on insecticide-induced hormesis in general, and provide a broader perspective to the neonicotinoid and honey bee debate.

Chapter 3: A Systematic Review of the Shifting Focus of Honey bee Toxicology Research

3.1 Introduction

The focus of research on insects and pesticides has shifted more than once. Prior to the 1960s pesticides were generally viewed as a godsent for crop protection, with little attention paid to the possible adverse effects on nontarget organisms. *Silent Spring* was first published in 1962; Rachel Carson was among the first to publicly recognize the reality of the dangers surrounding the misuse of dichlorodiphenyltrichloroethane (DDT; Carson 1962). Carson's book sparked a change in how pesticides were viewed and managed, although pesticides remain the most influential and important pest management tool for most cropping systems around the world.

Exposure to toxicants can result in short-term acute affects, or longer term chronic or sublethal effects. Acute toxicity is the response of an organism 24-72 h after exposure to a toxicant. Traditionally, determining the acute toxicity of pesticides to insects relied largely on the determination of an acute median lethal dose or concentration (Desneux et al., 2007). Regulatory processes of pesticide risk assessment and pesticide registration in both the United States and the European Union encourage the use of acute mortality as the toxicity endpoint of interest for insects (Guedes et al., 2016). Though important, robust, and critical for risk assessment, solely relying on acute mortality as a toxicological endpoint is an oversimplification of the potential consequences associated with pesticide exposure. Sublethal effects in response to pesticide exposure have long been examined but were not studied as much as other endpoints such as measuring the lethality of pesticides. This is because lethality is a clearly important endpoint, whereas sublethal endpoints such as learning are more difficult to translate into risk assessments

(e.g the relevance and transferability of sublethal endpoints are not as clear). Sublethal effects are defined as effects (physiological or behavioural) that occur in organisms surviving exposure to a pesticide. A sublethal dose is a dose that causes no apparent mortality in an experimental population (Desneux et al., 2007).

Study of sublethal effects of pesticides has traditionally been based on a threshold dose-response model, which assumes there is a dose above which an adverse effect occurs, and below which no measurable response occurs. However, is it now understood that hormetic dose responses occur and probably represent the default dose-response model toxicology. Hormesis is a biphasic dose response phenomenon, whereby exposure to low doses of a stressor can stimulate biological processes (Calabrese, 2010, 2013). Hormesis has only recently became more abundant throughout toxicology literature and since 2010 interest in insecticide-induced hormesis has increased substantially (Calabrese 2005b; Cutler, 2013; Cutler et al., 2022; Guedes et al., 2022a). Hormesis is a biological process that can be represented by the hormetic dose response model, which explains or predicts stimulatory responses that are observed after exposure to low doses of a stressor. Hormesis has been observed in many different organisms and taxa across multiple biological endpoints following exposure to a variety of different stressors. Pesticide-induced hormesis occurs widely in insects (Cutler and Guedes, 2017; Cutler et al., 2022), and although it occurs in bees and other insect pollinators, the majority of hormesis research with insects has focused on pest species (Guedes et al., 2022a, 2022b).

Bees (Apoidea) are a group of beneficial insects that, as pollinators, are extremely important to the health of natural and manufactured (e.g. agricultural) ecosystems. Because pollinators are so important and often subject to pesticide exposure, they are

regularly incorporated into toxicological studies and pesticide risk assessments. Of these pollinators, the European honey bee (*Apis mellifera*) dominates crop pollination worldwide and is often considered the most important pollinator of agricultural and horticultural crops (Abrol, 2012; Potts et al., 2010). Exposure of bees to foliar applied (sprayed) pesticides is reduced by label restrictions that prohibit application of active ingredients toxic to bees when they are flying or when the crop is in bloom (Government of Canada 2022). However, when using systemic insecticides that are applied as seed treatments or in-furrow during planting, the insecticide may move into pollen and nectar during crop bloom, thereby potentially exposing bees or other insects that forage on those crops.

Neonicotinoids are insecticides that have been widely used since the 1990s. They are systemic in nature and water soluble, allowing the insecticide to be absorbed by plants through roots and be transported throughout plant tissue. This gives this class of insecticides an advantage in pest control as they can protect all parts of the plant. Considering neonicotinoids are integrated into all plant tissues, it is not surprising that they are also found in the nectar and pollen of those plants (Blacquière et al., 2012). The use of neonicotinoids has become controversial as there has been evidence to suggest they can negatively affect honey bee colonies (Charreton et al., 2015; Faucon et al., 2005; Laycock et al., 2012; Stanley and Raine, 2016; Stejskalová et al., 2021). In 2013 the use of three neonicotinoids (imidacloprid, thiamethoxam, and clothianidin) in crops that attract pollinators were banned in the EU (European Union, 2013). France followed in 2016, by banning the use of neonicotinoids for crop protection (Biodiversity Act, 2016), the ban taking effect in September 2018. These bans are an attempt to mitigate concerns

surrounding pollinator decline but can be considered controversial as there has been conflicting evidence emerge that suggest neonicotinoids show no effects, or even stimulatory effects on bees (Barascou et al., 2021; Cutler et al., 2014; Colin et al., 2019; Meikle et al., 2022; Traver et al., 2018).

In the present chapter, I examined how the study of honey bee pesticide toxicology has changed over decades. My first objective was to examine if there were shifts that occurred in pollinator and honey bee literature and if shifts did occur when were they, and what may have driven them. I also wanted to determine if hormesis has become prominent in the pollinator literature. Here, I used Web of Science and broadly examined pesticide toxicology research on pollinators and then specifically *Apis* species from 1950-2019. I hypothesized that there would be a noticeable shift from examining acute effects to examining sublethal effects, as this had been reported in the literature (Guedes et al., 2016). I also expected stimulation in response to neonicotinoid exposure to be common in later years of my review as increased attention has been given to insecticide-induced hormesis (Cutler and Guedes, 2017; Cutler et al., 2022).

My second objective aimed to characterize any patterns that may be present throughout peer-reviewed studies that examined honey bees and neonicotinoids. To examine this, also using Web of Science, I reviewed papers from three journals: Journal of Economic Entomology and Pest Management Science, which are highly read and cited entomology and pest management journals that publish many papers on bees and pesticides; and PLoS One, a multidisciplinary journal that has published many papers on the pollinator-neonicotinoid issue. Relevant papers from the years 2000-2019 were examined in these journals. These papers specifically focused on *Apis* spp. and

neonicotinoids. For each of these studies I recorded information that included but were not limited to: (i) the methodological approach, (ii) what kind of effects were reported (inhibitory, stimulatory, or none), (iii) what kind of experimental exposure scenarios were used (probable, improbable or field sampling studies (explained in Section 3.3.2)), and (iv) what endpoints were being examined. Here, I predicted there would be a similar number of studies reporting no effects and inhibitory effects on honey bees, and that there would be fewer studies reporting stimulation. I expected similar number of studies to report inhibitory or no effects on bees because there is ample evidence to suggest both sides within the literature. I expected a smaller number of studies reporting stimulation on bees because stimulatory effects and hormesis are topics that have only become more popular in recent years (Cutler, 2013; Cutler et al., 2022; Guedes et al., 2022a, 2022b; Rix and Cutler, 2022). Further, I predicted laboratory studies to be the most prominent because insect toxicology work is much easier to complete under laboratory conditions. Pesticidal treatments and bees can be better controlled, and field studies are more labour intensive and costly. I predicted more studies to have used probable exposure scenarios, as blatantly using improbable exposure scenarios would not be accepted scientifically. Regarding specific endpoints, I expected there would be many studies examining acute mortality, as that has been the most popular method used in risk assessments (Guedes et al., 2016).

3.2 Methods

3.2.1 Overarching Patterns in Peer-reviewed Studies

To examine how research in honey bee toxicology has shifted over the years, I searched the Web of Science Core Collection database, always using the term “pollinator”

combined with other varying toxicology related terms (Table 3.1). I separated my searches by decade, searching a term combination (e.g. “pollinator” AND “insecticide”) in each decade from 1950 until 2019. The same procedure was followed using the word “*Apis*” combined with the same toxicology terms (Table 3.2). For each search, I recorded the number of results obtained with the key search terms, as a means of estimating how much research attention each topic had received over the decades. The titles of these articles were briefly scanned and if a title did not seem appropriate the abstracts were scanned to ensure focus on the key words entered. If the article did not focus on the key words entered it was excluded from the total.

3.2.2 In-depth Examination of Literature on Honey Bees and Neonicotinoids

Due to neonicotinoids importance in insect pest management and perceived role in suspected pollinator declines, a more intensive and specific literature search was done in the Web of Science using the key words “*Apis*” and “neonicotinoid”, or specific active ingredients in this class, i.e., imidacloprid, clothianidin, thiamethoxam, acetamiprid, thiacloprid, nitenpyram, and nithiazine. These searches were restricted to two prominent entomological journals, Pest Management Science and Journal of Economic Entomology, and the journal PLoS One. Searches on neonicotinoids were limited to years 2000-2019, resulting in 103 articles. I then examined each original article in detail to ensure they were appropriate for this study, meaning the article had to contain either a direct measure of an effect of a neonicotinoid on honey bees (e.g., exposing honey bees to a neonicotinoid and recording a toxicological endpoint measure such as survival, longevity, homing ability, etc.), or included an environmental measure of relevance for exposure

assessments in pollinator risk assessment (e.g., measures of neonicotinoid residues in pollen, nectar, honey, etc.).

Original research articles were categorized based on whether they utilized laboratory, field, semi-field, or combined approaches. Laboratory studies were defined as experiments done in a greenhouse or a laboratory. Field studies included those that were done in the field, including those that removed honey bees or samples such as pollen, nectar, honey, or bee bread, etc. from the field for testing in the lab. Semi-field studies were defined as experiments that used cages or tunnels in the field. Combined studies were, for example, situations where the treatment applied to the study subjects was performed in the field and the effects were observed in the laboratory on the same study subjects, or vice versa (Lundin et al., 2015). Most studies I examined could be assigned to a single methodological approach, however, some studies used different approaches for different endpoints and were therefore classified into multiple categories. Review articles were considered a separate methodological category. I also recorded the type of effects that each paper reported whether they be inhibitory, stimulatory, or no effects. Inhibitory effects were classified as any effects stemming from neonicotinoid exposure that inhibited a biological function in honey bees at the individual or colony level (e.g. decreased longevity, downregulation of an important gene, or reduction in pollen or honey stores). Conversely, stimulatory effects were classified as any effects that stimulated honey bees at the individual or colony level (e.g. faster foraging times, higher affinity for learning, or increased food storage). No effects were classified as studies that showed no difference between treated bees and control bees (e.g. bees exposed to neonicotinoid showed the same learning behavior as the control bees).

I evaluated whether the studies reported using probable or improbable exposure scenarios. When evaluating this attribute, there were also studies that took random samples from different hives or natural environments to determine the levels of pesticides. These studies were labelled as "field sampling studies". Studies were considered probable if exposures were within the range that honey bees could most likely encounter in the field. Laboratory studies were considered improbable if the exposure would be unlikely to occur under field conditions. Due to different types of studies, there were different factors involved in deciding which papers used probable exposure scenarios and which did not. I used data from Europe (EFSA, 2012) and the United States (USEPA, 2012) on neonicotinoid levels found in pollen and nectar of treated plants. Concentrations in nectar are generally lower than those in pollen. Concentrations differ based on the way the neonicotinoid treatment was applied. When applied as seed dressings, general neonicotinoid concentrations in nectar range from <1 to 8.6 ppb (mean maximum level ±SE from 20 studies = 1.9 ± 0.5 ppb, (EFSA, 2012)) and concentrations in pollen range from <1 to 51 ppb (mean maximum level ±SE from 20 studies = 6.1 ± 2.0 ppb). Higher concentrations were found when neonicotinoids were applied directly to the soil (in furrow), ranging from 1 to 23 ppb in nectar and 9 to 66 ppb in pollen (USEPA, 2012). I used Mullin et al., (2010) to help determine improbable exposure scenarios. This study completed a comprehensive survey of pesticide residues from hives across 23 states and one Canadian province in 2007-2008 and provided 95th percentiles in pollen for specific compounds. Ninety fifth percentiles are concentrations of compounds that are only likely to be found in the field ~5% of the time. I took the 95th percentile of each of the compounds included in their report and used this as a cut-off dose (i.e. anything

above this was considered improbable). The 95th percentiles reported by Mullin et al., (2010) were as follows: imidacloprid = 41.3 ppb (SEM ± 19 ppb), thiacloprid = 108.7 ppb (SEM ± 7.2 ppb), thiamethoxam = 53.3 ppb (only 1 sample so no SEM), acetamiprid = 117.5 ppb (SEM ± 7.2 ppb). Mullin et al., (2010) found no residues of clothianidin in their samples, therefore I used the concentration of 0.0114 ppm, as reported by EFSA, (2012). In reviewing the research articles and determining probable versus improbable, I also considered how the bees were exposed. For example, if bees were exposed to a probable dose but they were fed that probable dose consistently for an extended period, the study would be considered improbable. Further, if a study sprayed outside, I considered the maximum application rates for the different compounds as reported by the EFSA, (2012). Maximum application rates were as follows: imidacloprid = 350 g a.i/ha (grams of active ingredient/hectare), thiacloprid = 360 g a.i/ha, acetamiprid = 250 g a.i/ha, and thiamethoxam and clothianidin = 150 g a.i/ha. If the spray concentration used in the studies exceeded these concentrations, then exposures were considered improbable.

I considered 11 different categories of endpoint measures referred to in articles: residue analyses, longevity, acute mortality, learning, brood, foraging characteristics, overall colony health, biochemical, molecular, non-*Apis* endpoints, and other endpoints on honey bees. Residue analysis studies include those that took samples such as honey, wax, pollen, bee tissue, etc. to determine levels of pesticide within the samples. Studies were put into the longevity category if bees were subjected to a treatment and their survival was monitored for more than four days. This differed from the acute mortality category, which considered studies that measured mortality of bees 24-96 h after a treatment. Studies that examined the sugar response threshold or learning experiments

such as the proboscis extension reflex (PER) were placed in the learning category. If a study examined how brood responded to treatments (i.e., developmentally, their survival, or their activity), it was placed in the brood category. The foraging characteristics category included studies examining honey bee flight path, ability, or length, as well as foraging efficiency, ability, and homing ability. Overall colony health encapsulated studies that looked at many endpoints to quantify the health of the colony, including at least two of the following endpoints: colony weight, honey yield, brood area, healthy queens, worker numbers, worker mortality. Studies that were placed in the biochemical category included any study that examined proteins or enzymes or used chemical methods such as liquid or gas chromatography, mass spectrophotometry, radioanalysis, electrophysiology, or immunohistochemistry. Another category named molecular considered any study that examined changes in gene expression. The category named "non-*Apis*" comprises of studies that have a component where the primary focus is a plant or plant substance (guttation fluids, pollen, nectar) but also examines how the bees are indirectly affected. For example, one study used treated cantaloupe seeds and once the plant bloomed, researchers recorded how many bees were seen foraging at specific flowers (Elzen et al., 2004). The final category incorporates all other studies with direct endpoints on honey bees that did not fit into any other category. This included studies that examined things such as apoptosis on honey bee brain cells, assessing pollen and/or nectar consumption, assessing honey bee weight, looking at specific aspects of honey bee metabolism, locomotory activity, developmental rates, and viral loads. It is important to note that in many cases one study examined multiple endpoints and therefore the same study could be present in multiple categories.

3.2.3 Statistical Analyses

A one-way PERMANOVA (Permutation Multivariate Analysis of Variance; PRIMER version 6; McArdle and Anderson, 2001) was used to assess if there were differences between year categories regarding all literature variables examined. Each literature variable (i.e. the different exposure scenarios, different methodological approaches, different endpoints, different result types) was considered a dependent variable. A SIMPER test (Similarity Percentages; Clarke, 1993; Clarke and Ainsworth, 1993) was then done to determine what literature variables were contributing most to significant differences detected by PERMANOVA between year categories. It was also used to determine what literature variables were most prominent within each year category. The SIMPER calculates how much each literature variable contributions to the average similarity of samples (papers) within each year category, and how much each literature variable contributions to the average dissimilarity between all pairs of groups (comparing year categories to one another; Clarke et al., 2014)). For both PERMANOVA and SIMPER tests, Year Category was a fixed factor with four levels (2000-2004, 2005-2009, 2010-2014, and 2015-2019). Replication came from the number of studies used (73). These analyses were conducted by first constructing a resemblance matrix for the dataset, using Bray-Curtis similarity. In the SIMPER, within-group similarity is calculated using Bray-Curtis similarity matrix (Clarke et al., 2014). The main difference when calculating between-group dissimilarity is the use of the Bray-Curtis dissimilarity matrix, rather than the similarity matrix (Clarke et al., 2014), and then the contribution of each variable is calculated in descending order.

A SIMPER test was also done to determine specifically, which endpoints contributed to the dissimilarity between year categories, complemented with a visual representation using a non-metric multi-dimensional scaling (nMDS) plot, constructed using a resemblance matrix with a dummy variable of 0.01 to account for studies that had zeros for reach response variable (endpoints), and use of the Bray-Curtis coefficient (Clarke et al., 2006; Ricotta and Podani, 2017).

3.3 Results and Discussion

3.3.1 General Patterns in the Literature on Honey Bee Toxicology

The search term "pollinator" yielded a total of 15,845 results from 1950–2019, with the number of publications more than doubling each decade, reaching a high of 9,864 publications between 2010 and 2019 (Table 3.1). When the term "pollinator" was combined with keywords such as "insecticide" or "pesticide", few results emerged until the 1990s where the first notable number of studies were published (Table 3.1). Between 1990 and 2009, a total of 103 papers were published that contained keywords "pollinator" and either "pesticide" or "insecticide", and then from 2010–2019 a total of 1,135 papers were published.

Similar trends were obtained when using the search term "*Apis*", where the overall total number of publications between 1950 and 2019 was 24,305 (Table 3.2). When searching "*Apis*", the number of publications nearly doubles each decade until the 2000s when publications increase drastically, with 83% of all publications being published after 2000, and 71% of those being published between 2010 and 2019 (Table 3.2). When searching keywords "pesticide" and "insecticide" combined with "*Apis*", I found the first publication including both "*Apis*" and "insecticide" was in the 1950s and

Table 3.1. Number of citations pulled from the Web of Science database for the term pollinator with pesticide related terms. Totals represent the total number of citations for each of the years indicated and total number of citations throughout the years for the individual search terms.

Search terms	***Citations per years indicated***							
	1950-1959	1960-1969	1970-1979	1980-1989	1990-1999	2000-2009	2010-2019	***Totals***
Pollinator	2	23	74	250	1692	3940	9864	**15845**
Pollinator AND pesticide	0	0	1	0	14	46	653	**714**
Pollinator AND insecticide	0	0	1	6	20	32	482	**541**
Pollinator AND organochlorine	0	0	0	0	0	0	2	**2**
Pollinator AND (organophosphorous OR organophosphate)	0	0	0	0	0	1	18	**19**
Pollinator AND carbamate	0	0	0	0	0	0	6	**6**
Pollinator AND pyrethroid	0	0	0	1	0	0	37	**38**
Pollinator AND neonicotinoid	0	0	0	0	0	1	370	**371**
Pollinator AND acute toxicity	0	0	0	0	1	3	29	**33**
Pollinator AND acute exposure	0	0	0	0	0	0	13	**13**
Pollinator AND chronic toxicity	0	0	0	0	0	0	13	**13**
Pollinator AND chronic exposure	0	0	0	0	0	0	55	**55**
Pollinator AND sublethal AND toxicity	0	0	0	0	1	5	67	**73**
Pollinator AND sublethal AND exposure	0	0	0	0	0	4	114	**118**
Pollinator AND (hormesis OR hormoligosis)	0	0	0	0	0	0	7	**7**
Pollinator AND risk assessment	0	0	0	0	4	12	174	**190**
Pollinator AND hazard	0	0	0	0	3	12	49	**64**
Totals	**2**	**23**	**76**	**257**	**1735**	**4056**	**11953**	**18102**

Table 3.2. Number of citations pulled from Web of Science database for the term *Apis* along with pesticide related terms. Totals represent the total number of citations for each of the years indicated and total number of citations throughout the years for the individual search terms.

Search terms	***Citations per years indicated***							
	1950-1959	1960-1969	1970-1979	1980-1989	1990-1999	2000-2009	2010-2019	***Totals***
Apis	68	137	420	726	2750	5776	14428	**24305**
Apis AND pesticide	0	0	2	1	32	104	974	**1113**
Apis AND insecticide	1	2	3	6	39	146	748	**945**
Apis AND organochlorine	0	0	0	0	0	2	10	**12**
Apis AND (organophosphorous OR organophosphate)	0	0	0	0	9	18	66	**93**
Apis AND carbamate	0	0	0	0	5	11	14	**30**
Apis AND pyrethroid	0	0	0	2	15	39	123	**179**
Apis AND neonicotinoid	0	0	0	0	1	25	489	**515**
Apis AND acute toxicity	0	0	2	0	8	13	53	**76**
Apis AND acute exposure	0	0	0	0	0	0	20	**20**
Apis AND chronic toxicity	0	0	0	0	1	5	82	**88**
Apis AND chronic exposure	0	0	0	0	1	5	82	**88**
Apis AND sublethal AND toxicity	0	0	0	0	1	19	123	**143**
Apis AND sublethal AND exposure	0	0	0	1	6	16	213	**236**
Apis AND (hormesis OR hormoligosis)	0	0	0	0	0	0	8	**8**
Apis AND risk assessment	0	0	0	0	7	43	264	**314**
Apis AND hazard	0	0	1	1	12	36	123	**173**
Totals	**69**	**139**	**427**	**736**	**2868**	**6179**	**17433**	**27851**

publications including these two keywords increased gradually until 2009. From 2010 to 2019, 748 papers were published with these two keywords, making up 79% of all the papers published since 1950. The first three papers that included "*Apis*" and "pesticide" were published between 1970 and 1979 and publications considerably increased from the 1990s onward, reaching a cumulative total of 1,113. A similar pattern can be seen in most of the keyword searches for both "pollinator" and "*Apis*", where a small number, or no publications are seen until the 2000s and then most research for most keywords emerges between 2010 and 2019 (Tables 3.1 & 3.2). This spike in the literature could, in part, have been due to the increased number of reports of large-scale colony die offs in eastern North America, beginning in late 2006. These die offs were termed colony collapse disorder (CCD) and were characterized by the majority of the female worker bees disappearing, leaving behind, food stores (honey), brood, the queen, and a few workers (vanEngelsdorp et al., 2009). This event was alarming to many, and caused an increased interest for scientists, beekeepers, politicians, and the general public due to the economic, agricultural, and ecosystem services managed honey bees provide (Aizen and Harder, 2009). While this was devastating at the time, CCD has not been reported in many years and is considered a distinct phenomenon. It is now known that honey bee colony losses can be caused by an accumulation of different stressors, including pathogens, parasites, environmental stressors, and beekeeping management stresses (Johnson, 2010). These factors may have contributed to CCD but are not exclusively linked to it.

For the search of different insecticide classes, for both "pollinator" and "*Apis*", neonicotinoids showed the highest number of studies with 371 and 515, respectively. The fewest number of publications were on organochlorines, followed by carbamates,

organophosphates, pyrethroids, and then neonicotinoids (Tables 3.1 & 3.2). The number of publications that include these insecticide classes reflects the order in which they were discovered and used over time. Insecticide classes that have developed more recently have more publications, this is reflective of increased research on bees and pesticides across decades (Tables 3.1 & 3.2).

The first publication to include both "pollinator" and "neonicotinoid" was published between 1990 and 1999 (Table 3.1). This makes sense as imidacloprid was first introduced in 1991. It was not until the early 2000s that its use became widespread and by 2006 the neonicotinoid family represented nearly 17% of the global insecticide market and accounted for worldwide annual sales of around USD 1.56 billion (Jeschke and Nauen, 2008). In 2008, neonicotinoids represented the fastest growing class of insecticides released onto the market since pyrethroids (Nauen and Bretschneider, 2002). This is due to their effectiveness, versatility, and affordability. Neonicotinoids are agonists of nicotinic acetylcholine receptors (nAChRs; Goulson, 2013). This mode of action does not bind strongly to vertebrate receptors but aggressively binds to the nAChRs in insects, making it widely sought after in insect pest management (Tomizawa and Casida, 2005). However, neonicotinoids came under scrutiny around 2011 because pesticide laden dust was being emitted by pneumatic seeders, and this was being linked to honey bee die offs (Marzaro et al., 2011; Tapparo et al., 2012). Considering how important and somewhat controversial neonicotinoids became after 2010, it is not surprising that the number of publications including the keywords "neonicotinoid" and "pollinator" or "*Apis*" skyrocketed in this decade (Table 3.1 & 3.2).

Toxicological effects may be referred to as acute (effects resulting in a short period of time from short-term exposure), chronic (effects resulting from long-term exposure), lethal (exposure results in mortality), or sublethal (exposure resulting in measurable changes but does not cause mortality). I found a total of 96 papers that included the words "acute" and "*Apis*", the first being published in the 1970s. Guedes et al. (2016) reports that acute toxicity was the most common scenario studied in early toxicology research. I found that for both "pollinator" and "*Apis*" the term "acute" had the lowest number of publications, while "chronic" showed more publications, and "sublethal" produced the most publications (Table 3.1 & 3.2). This is likely a combination of two things. Firstly, the search term "acute" may not have produced as many results because not all scientists use this term when conducting and published LD_{50} experiments. Secondly, when different pesticides are being tested on insects, it is rare that studies test the chemical on beneficial insects to begin with. They are usually tested on the target pest species and thus would not have appeared in my search. Studies with pesticides are often done on pest species because that is the point of developing them, to prevent pests. Thus, focus on beneficial insects has shifted towards chronic and sublethal effects, as seen in my data set (Table 3.1 & 3.2). The focus on chronic and sublethal effects with regard to pollinators makes sense because such exposures often occur in the field (Chauzat et al., 2006; Nguyen et al., 2009). The number of studies that examined sublethal exposure was 570 (191 for "pollinator" and 379 for "*Apis"*; Table 3.1 & 3.2), which is more than the number of chronic and acute studies combined. Most sublethal studies were published between 2010 and 2019 (Table 3.1 & 3.2), possibly reflecting a shift in emphasis from classical acute mortality laboratory studies estimating median

lethal dose (LD_{50}) or lethal concentration (LC_{50}) to a focus on sublethal effects following exposure. However, there may be some overlap in the number of studies reported as acute and chronic are not necessarily different from sublethal. There can be evidence of sublethal toxicity with acute or chronic exposure, and acute and chronic effects can be a result of sublethal exposure. Therefore, the three terms are not mutually exclusive.

3.3.2 In-depth Examination of Literature on Honey Bees and Neonicotinoids

3.3.2.1 Overview of Patterns

The literature search yielded 73 useable papers. There was an overall increase in the number of papers published between 2010 and 2019 (Figure 3.1; Tables 3.1 & 3.2). Studies from 1990s were not included in this part of the review as the honey bee-neonicotinoid debate only began after the 2000s. Most studies on *Apis*/pollinators and neonicotinoids were done in the laboratory and published between 2015 and 2019 (Figure 3.1). There was a >3-fold increase in the number of lab studies after 2015 and there was no notable change in the number of field studies across years (Figure 3.1). Field studies are generally logistically more difficult, more labour intensive, more expensive, and come with more uncertainty and therefore less common than laboratory studies. However, the lack of field studies should be considered as testing in the field could be considered the most probable experimental scenario.

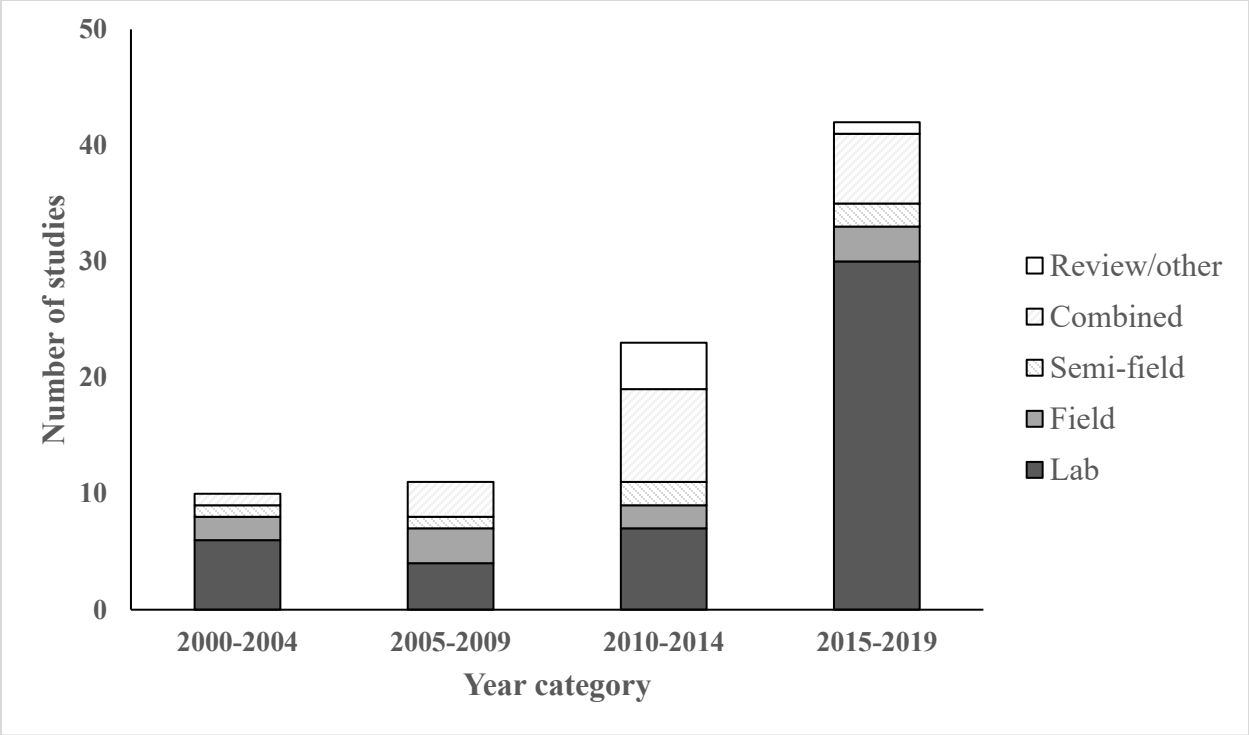

Figure 3.1. Number of studies per study type (Review/other, combined, semi-field, field, and lab) per year category. Studies pulled and pooled from three peer-reviewed journals: Pest Management Science, Journal of Economic Entomology, and PLoS One.

Reports of stimulatory effects were uncommon, with only three such articles published between 2000-2005, and six published within 2015-2019 (Figure 3.2A). Of the studies that produced stimulatory effects, only four explicitly mention stimulatory effects from neonicotinoid exposure in the title or abstract of the paper, and each of those discuss the hormesis phenomenon (Démares et al., 2016; Dickel et al., 2018; Ma et al., 2019; Wong et al., 2018). In other instances reported results of stimulation or hormesis were not discussed, possibly due to a lack of awareness or interest in the hormesis phenomenon, even though this has been reported in bees (Suchail, et al., 2004; Zhu et al., 2017a). The most common endpoint that showed stimulatory effects was longevity, however the number of studies for each endpoint only varied from 1 to 4 (Table 3.3).

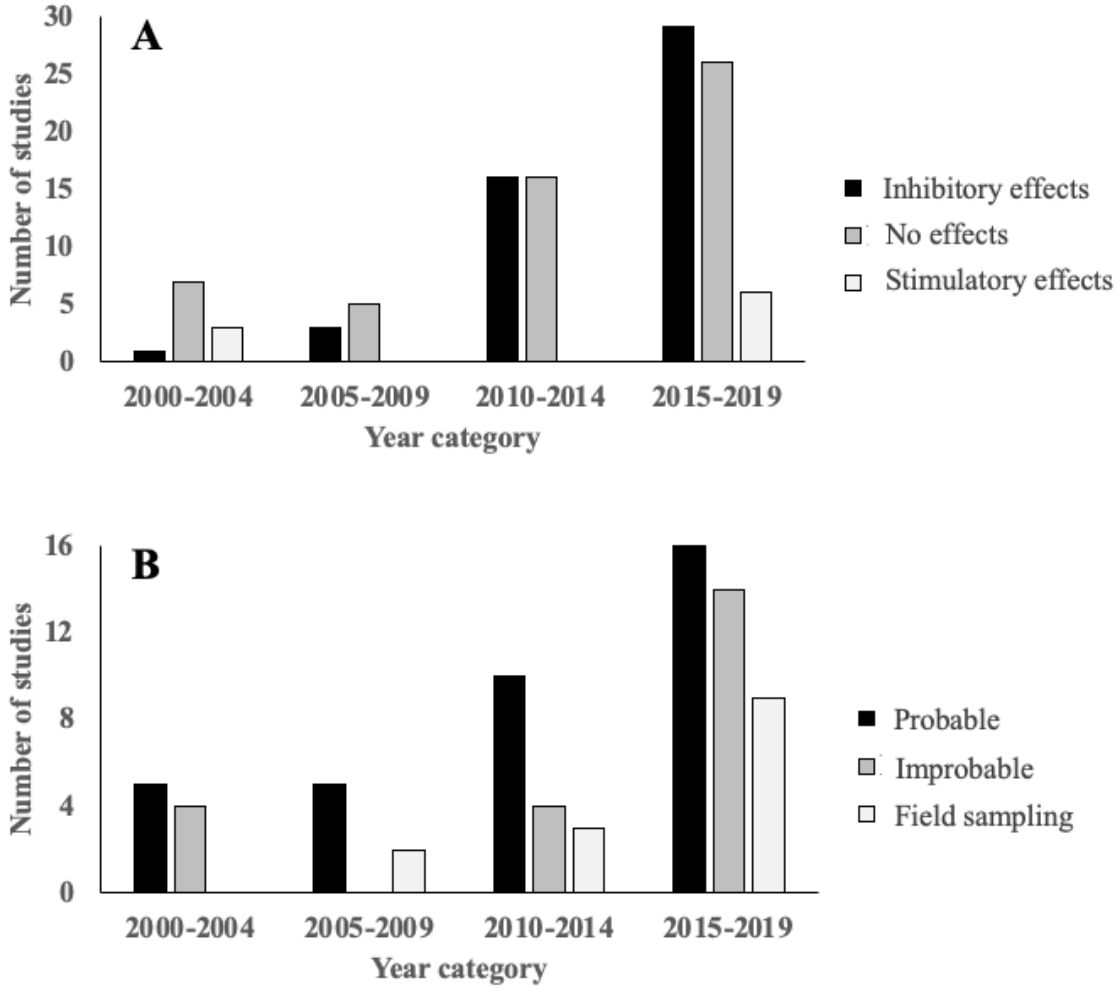

Figure 3.2. **A)** Number of studies with inhibitory, no, and stimulatory effects separated by year category. **B)** Number of studies with probable or improbable exposure scenarios, and those that were considered field sampling studies, separated by year category. Studies pulled and pooled from three peer-reviewed journals: Pest Management Science, Journal of Economic Entomology, and PLoS One.

Table 3.3. Number of studies per endpoint examined as well as per type of effect (i.e. inhibitory, stimulatory, or none). Studies pulled from three peer-reviewed journals: Pest Management Science, Journal of Economic Entomology, and PLoS One. Note that there is overlap in the number of studies because one study may have found both stimulatory and no effect for different endpoints or the same endpoint, and so this study would be under both stimulatory and none.

	Endpoint occurrences										
Effect type observed	Longevity	Learning	Brood	Foraging characteristics	Colony health	Mortality	Other	Non-*Apis*	Biochemical	Molecular	***Totals***
Inhibitory effects	11	5	3	7	6	9	10	4	3	13	**71**
Stimulatory effects	4	3	0	2	1	1	1	1	2	2	**17**
No effects	11	6	5	4	11	10	10	7	8	8	**80**
Totals	**26**	**14**	**8**	**13**	**18**	**20**	**21**	**12**	**13**	**23**	**168**

The number of studies that showed inhibitory effects increased steadily from 2000 to 2019, with a total of 49 studies showing inhibitory effects (Figure 3.3A). Among these studies, there were 71 endpoints examined (Table 3.3). The most common endpoint among these studies that showed inhibitory effects was molecular, and the least common were brood and biochemical (Table 3.3). In examining the molecular and biochemical endpoints, if there was an upregulation of a gene or enzyme, this would be considered a stimulatory response. Conversely, if a gene or enzyme was downregulated, it was considered an inhibitory response. The most common endpoint that produced no effects as a result was colony health and longevity (11 studies each), followed by mortality and "other" (10 studies each; Table 3.3).

More studies examining residue levels within hives began in the second-year category and increased as time progressed, these were deemed "field sampling studies" (Figure 3.3B). Many of these studies sampled multiple apiaries in a region to determine the background levels of pesticide exposure occurring within managed apiaries (Alburaki et al., 2017; Bernal et al., 2010; Böhme et al., 2018; Chauzat et al., 2006; Mullin et al., 2010; Nai et al., 2017; Nguyen et al., 2009; Roszko et al., 2016). The increase in this method after 2005 could be because researchers wanted to get a more accurate representation of how prominent neonicotinoids were in natural hives. Specifically, after the neonicotinoid-honey bee controversy began in the late 2000s it was of interest to quantify the doses that honey bees would be exposed to in the field.

3.3.2.2 Patterns Over Time

The number of studies on bees and neonicotinoids has unsurprisingly increased over the years (Figure 3.1). Using my full set of literature (dependent) variables consisting of

study types, exposure scenarios, endpoints, and result types, the PERMANOVA analysis detected a significant difference in honey bee toxicological research amongst year categories (p=0.007; Table 3.4). The "no effects" variable was prominent (top three contributors) in all year categories, and probable exposure scenarios were present in all year categories (Table 3.5). These results show that although experimental methods and research objectives have shifted through the years, use of probable exposure scenarios in honey bee experiments have been constant. It also shows that studies reporting neonicotinoids have no effects on honey bees have been constant. However, in the later year categories (2010-2014 & 2015-2019), inhibitory effects were also prominent, suggesting many studies also reported inhibition in response to neonicotinoids (Table 3.5). These results are not surprising as the controversy surrounding neonicotinoids and honey bees has been based on these topics. Considering there are similar number of studies reporting no and inhibitory effects throughout the years (Tables 3.3 & 3.5), my data reflects the reason behind the controversy.

Table 3.4. PERMANOVA results investigating whether honey bee toxicology research published in three focus journals (Pest Management Science, Journal of Economic Entomology, and PLoS One) varied significantly between year categories (2000-2004, 2005-2009, 2010-2014, and 2015-2019).

Source of variation	df	MS	Pseudo-F	Unique permutations	p
Year category	3	4283.2	2.28	998	**0.007**
Residual	69	1871.3			
Total	72				

Table 3.5. SIMPER results reporting average within-category similarity for different literature variables across four year categories (2000-2004, 2005-2009, 2010-2014, and 2015-2019). The overall average similarity for a year category is for the assemblage of literature variables, whereas the average similarity within the table is for a given literature variable. Sim/SD is the ratio of the average similarity to standard deviation of the similarities for each literature variable; a ratio ≥ 1 indicates a consistent contribution, whereas a ratio < 1 indicates variability in the contribution. The contribution of that variable to the overall average similarity is indicated next. The cumulative contribution simply accumulates the contribution values. Only the top five contributors are included in this table. Between category dissimilarity percentages found in Appendix B.

Year category 1 (2000-2004)

Overall similarity (%) = 46.38

Literature variable	Average similarity (%)	Sim/SD	Contribution (%)	Cumulative contribution (%)
No effects	15.08	5.18	32.50	32.50
Lab studies	9.93	1.49	21.41	53.91
Probable	7.28	0.90	15.70	69.61
Improbable	3.96	0.62	8.53	78.15
Endpoint "Other on bees"	2.39	0.40	5.16	83.30

Year category 2 (2005-2009)

Overall similarity (%) = 39.85

Literature variable	Average similarity (%)	Sim/SD	Contribution (%)	Cumulative contribution (%)
Probable	7.91	0.90	19.85	19.85
No effects	7.59	0.91	19.06	38.91
Endpoint "Residue analyses"	7.59	0.91	19.06	57.97
Lab studies	4.30	0.61	10.80	68.76
Inhibitory effects	2.70	0.40	6.77	75.53

Year category 3 (2010-2014)

Overall similarity (%) = 36.11

Literature variable	Average similarity (%)	Sim/SD	Contribution (%)	Cumulative contribution (%)
No effects	11.27	1.10	31.21	31.21
Inhibitory effects	9.36	0.98	25.91	57.11
Probable	3.61	0.52	9.98	67.10
Combined studies	2.38	0.39	6.59	73.68
Endpoint "Residue analyses"	2.01	0.33	5.56	79.25

Year category 4 (2015-2019)

Overall similarity (%) = 42.92

Literature variable	Average similarity (%)	Sim/SD	Contribution (%)	Cumulative contribution (%)
Lab studies	11.05	1.24	25.75	25.75
Inhibitory effects	10.02	1.15	23.35	49.10
No effects	8.25	0.91	19.21	68.31
Probable	3.07	0.45	7.15	75.46
Improbable	2.07	0.38	4.81	80.27

The second SIMPER analysis only considered the different endpoints of the studies (Figure 3.3; Table 3.6), as opposed to all literature variables (Table 3.5). The 'other on bees' was the most commonly studied endpoint in the first-year category (2000-2004; contributed 31% similarity; Table 3.6). This endpoint housed all the least studied endpoints, which included examining apoptosis on honey bee brain cells, assessing pollen and/or nectar consumption, assessing honey bee weight, looking at specific aspects of honey bee metabolism, locomotory activity, developmental rates, and viral loads (Charreton et al., 2015; Coulon et al., 2019; Cresswell et al., 2014; Dai et al., 2019; Dai et al., 2016; Fischer et al., 2014; Guseman et al., 2016; Hardstone and Scott, 2010; Nauen et al., 2001; Sgolastra et al., 2017; Suchail et al., 2004a, 2004b; Tan et al., 2014; Wu et al., 2015; Yao et al., 2018). Had these endpoints been their own "variables", the similarity within this category may have been lower. This makes sense because honey bee toxicology and neonicotinoid research started to increase and broaden in scope in the early 2000s.

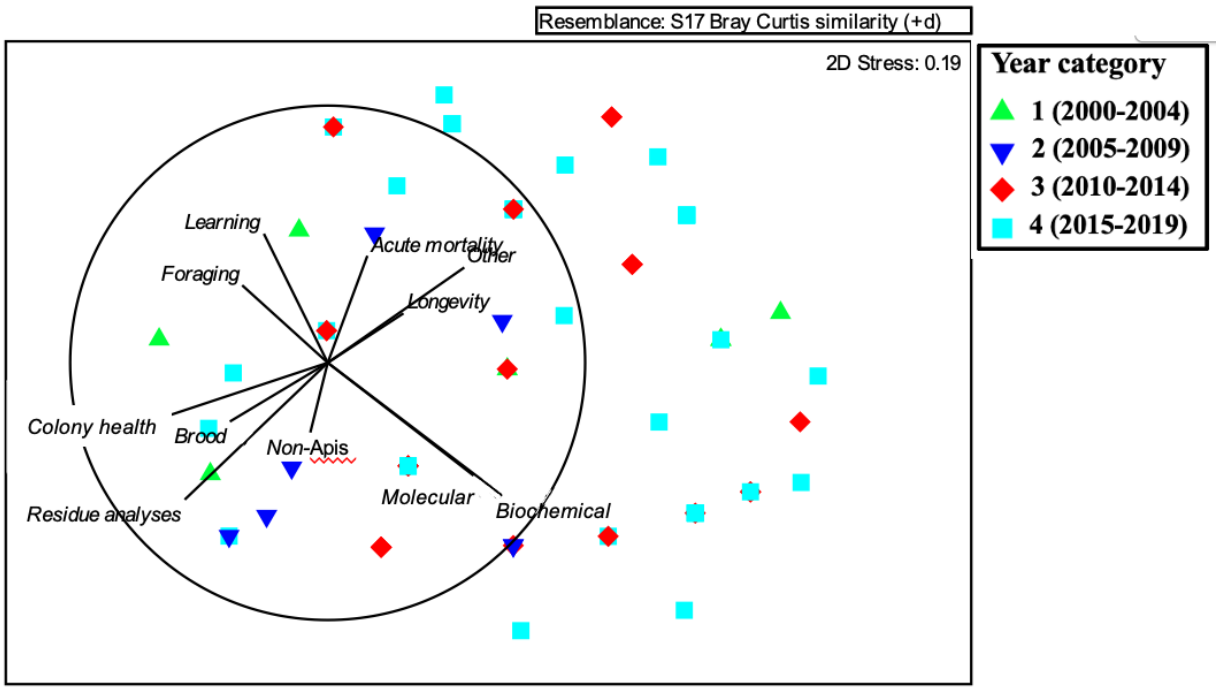

Figure 3.3. Non-metric multidimensional scaling (nMDS) plot of the different studies included in the analysis (see Figure 3.1) and focused on the endpoints (see Table 3.3). A symbol represents a study, and the different symbols represent different year categories. The distance between symbols represents the difference in endpoints between year categories. The vector overlay within the MDS plot represents correlations between the endpoints and MDS axes. The vector of each endpoint shows the direction of increased presence across the nMDS plot, and the circle is the maximum possible length of a vector. 2D stress < 0.2 indicates a good two-dimensional representation of higher dimensional trends (Clarke, 1993)

Table 3.6. SIMPER results reporting average within-category similarity for different endpoints examined across four year categories (2000-2004, 2005-2009, 2010-2014, and 2015-2019). The overall average similarity for a year category is for the assemblage of literature variables, whereas the average similarity within the table is for a given literature variable. Sim/SD is the ratio of the average similarity to standard deviation of the similarities for each literature variable; a ratio ≥ 1 indicates a consistent contribution, whereas a ratio < 1 indicates variability in the contribution. The contribution of that variable to the overall average similarity is indicated next. The cumulative contribution simply accumulates the contribution values. Only the top five contributors are included in this table apart from year category 2, which only had four contributors. Between category dissimilarity percentages found in Appendix C.

Year category 1 (2000-2004)

Overall similarity (%) = 19.95

Endpoint	Average similarity (%)	Sim/SD	Contribution (%)	Cumulative contribution (%)
Other on bees	6.19	0.40	31.02	31.02
Biochemical	6.19	0.40	31.02	62.05
Non-*Apis*	1.90	0.22	9.55	71.59
Acute mortality	1.59	0.22	7.95	79.55
Residue analyses	1.36	0.22	6.82	86.36

Year category 2 (2005-2009)

Overall similarity (%) = 29.16

Endpoint	Average similarity (%)	Sim/SD	Contribution (%)	Cumulative contribution (%)
Residue analyses	18.98	0.90	65.09	65.09
Colony health	4.85	0.39	16.64	81.73
Biochemical	2.38	0.22	8.16	89.89
Longevity	1.59	0.22	5.44	95.33

Year category 3 (2010-2014)

Overall similarity (%) = 16.90

Endpoint	Average similarity (%)	Sim/SD	Contribution (%)	Cumulative contribution (%)
Residue analyses	6.67	0.32	39.46	39.46
Biochemical	2.76	0.27	16.36	55.82
Non-*Apis*	2.70	0.22	15.97	71.79
Other on bees	1.98	0.17	11.74	83.53
Molecular	0.97	0.17	5.74	89.28

Year category 4 (2015-2019)

Overall similarity (%) = 18.55

Endpoint	Average similarity (%)	Sim/SD	Contribution (%)	Cumulative contribution (%)
Biochemical	4.44	0.34	23.92	23.92
Longevity	3.28	0.26	17.66	41.58
Acute mortality	2.57	0.19	13.88	55.46
Residue analyses	2.55	0.19	13.73	69.10
Molecular	2.39	0.26	12.88	82.07

In the second-year category (2005-2009) residue analysis was the most common endpoint examined (contributed 65.1% to its similarity; Table 3.6). Residue analysis is important in toxicology research to quantify exposure, and in such instances is ideally incorporated in field surveys with colony assessments after exposure to assess neonicotinoid residues in honey, beeswax, dead bees, pollen, and nectar (Chauzat et al., 2006; Cutler and Scott-Dupree, 2007; Faucon et al., 2005; Nguyen et al., 2009). Brunet et al. (2005) also incorporated residue analysis in their assessment of the metabolic fate of the neonicotinoid acetamiprid within the honey bee midgut, rectum, and hemolymph. In 2010-2014 residue analyses continued as the most studied endpoint (contributed to 39.5% of within-category similarity; Table 3.6). Thus, residue analyses were the most studied endpoint from 2005 until 2014, although they were most prominent in year category two.

Biochemical endpoints were the most studied endpoints in 2015-2019 (contributed 23.9%; Table 3.6). Just over one third of the studies that fell into the biochemical category also had a component that included a molecular NGS method. This research ranged from examining changes in enzyme, protein, and gene expression in full adult or pupal honey bees, to looking only at small samples of tissue such as the fat body, rectum, brain tissue, etc. (Shi et al., 2017; Wessler et al., 2016; Wu et al., 2015; Yao et al., 2018; Zhu et al., 2017a; Zhu et al., 2017b). In other studies, queens were sacrificed and their spermatheca contents used to estimate genetic diversity (Forfert et al., 2017; Tesovnik et al., 2017). Molecular endpoints were less common between 2015-2019 (contributed 12.8%; Table 3.6). Development of high throughput next-generation sequencing (NGS) had become readily available at an affordable cost by 2009 (Wang et

al., 2009) and thus I expected it to be more prevalent in the fourth year category (2015-2019).

To visualize these trends in endpoints within- and between- year categories, an nMDS plot was used. A resemblance matrix is used to construct nMDS plots. To construct this matrix, I used a dummy variable of 0.01 to account for studies that had zeros for reach response variable (endpoints), and the Bray-Curtis coefficient was used (Clarke et al., 2006). Considering my data are presence and absence (1's and 0's, respectively), some more explanations are required. I initially used the Jaccard coefficient and the Sorensen coefficient, which are typically used to measure similarity for presence-absence data (Ivchenko and Honov, 1998). Note the Sorensen and Bray-Curtis coefficients are closely related (Ricotta and Podani, 2017), and so Bray-Curtis also works well on presence-absence data and enabled me to conduct SIMPER tests. A SIMPER test is like a post-hoc test; it shows what variables are causing the differences between groups. More specifically, the SIMPER test identifies the literature variables contributing most to the within-group similarities and to the between-group dissimilarities. If a variable shows high similarity or dissimilarity it means that it is the variable that is contributing to the similarity or differences within or between groups.

Year categories 2000-2004 and 2005-2009 show minimal spread, suggesting they are more similar to one another, and fewer endpoints were examined (Figure 3.3). Years 2010-2014 and 2015-2019 show a greater amount of spread (Figure 3.3), suggesting more endpoints were studied over time. A vector overlay is used to explain what endpoints were driving these differences (visualization of the SIMPER). For example, as stated above, in year category two residue analysis was the most studied endpoint and this is

evident in Figure 3.3 as the vector for residue analyses is longer and lands right next to three points from year category two.

3.3.3 Conclusion

In conclusion, my analysis shows how the focus of honey bee toxicological research has shifted over the years. Research surrounding pollinators and *Apis* species in relation to pesticides began to expand after 2000 and by 2010 the number of studies in all areas sharply increased, with thousands of studies being published between 2010-2019. Neonicotinoid pesticides were the most common pesticide studied. The number of studies examining sublethal effects surpassed those examining acute or chronic. Few studies examined hormesis or reported stimulatory effects, and this is an area that should be studied more intently, testing *a priori* hypothesis of the phenomenon. Most studies used laboratory methods and there were fewer field studies. Use of probable exposure scenarios was fortunately common, and results that found no effects of neonicotinoids on honey bees were common throughout the time span I examined. This could suggest that when realistic doses of neonicotinoids are used, it is likely no effects will be seen. However, there was a similar number of studies that reported inhibitory effects on bees (Table 3.3) and the SIMPER analysis showed that in the late year categories (2010-2014 & 2015-2019) inhibitory effects were also prominent. Therefore, the controversy surrounding the ban on neonicotinoids is reflected in my data. Considering probable exposure scenarios are a constant throughout the literature and there are studies reporting both inhibitory and no effects on honey bees, it is difficult to determine if there is a correct answer. Expanding and applying hormesis and stimulation to the field of pollinator toxicology could provide some impactful insights on the pollinator-

neonicotinoid debate. Of the endpoints that I identified in the literature, the most prominent across year categories were residue analyses and biochemical endpoints, with residue analyses becoming less common in the later years and biochemical becoming more common. Many other endpoints were present and contributed relatively equally throughout the years, indicating many of the same endpoints were being studied throughout all year categories.

In this chapter I was able to examine how honey bee toxicology literature has developed and changed over the years, while identifying some knowledge gaps. Compiling this data allows honey bee toxicologists to see what methods have been used, the type of experimental doses and conditions that have been used, the different effects that have been reported, and what endpoints have been widely examined. This information could help scientists in this field better understand what research has already been conducted and what should be conducted. It furthers the understanding of how we look at honey bee toxicology.

Chapter 4: Discussion

4.1 Overview of Context

Pollination is an essential agricultural ecosystem service and production practice that is provided by insects, particularly bees (Jaffé et al., 2009). Bees show a great amount of diversity and are key pollinators of natural plants and crops (Klein et al., 2007). Both native and managed bees pollinate most wild and agricultural plants, thereby increasing genetic diversity and crop yields (Klatt et al., 2013; Klein et al., 2007). For many cropping systems, the contribution of non-*Apis* pollinators is unclear or insufficient, and therefore commercially maintained honey bees are often used by default (Frier et al., 2016). This is because of their versatility, large number of individuals in colonies, and ability to pollinate many types of plants (Abrol, 2012). Honey bee colonies, whose development and maintenance are intimately associated with environmental resources, can frequently be exposed to many pesticide active ingredients (Mullin et al., 2010). Pesticides are widely used to supress pests that attack and reduce yields of agricultural crops. They have been considered the most influential tool in pest management since the 1940s, when their use became widespread (Guedes et al., 2016). As honey bees and pesticides are both important for modern agriculture, we must understand how pesticides affect honey bees.

How the scientific community has studied the intersection of pollinators and pesticides has shifted over the years. As different questions emerge, different methods to test the effects of pesticides on beneficial insects have been developed. Initially, assessing the acute toxicity of pesticides relied largely on determining an acute median lethal dose or concentration (Desneux et al., 2007). A shift occurred and more studies examining

sublethal and chronic exposure emerged and continue to emerge. Among these are studies examining sublethal effects and hormesis. Hormesis is a biphasic dose response whereby exposure to low doses of a stressor can stimulate biological processes (Cutler and Rix, 2015). Hormesis has been demonstrated in many organisms, in response to numerous stressors (Calabrese, 2013; Calabrese and Blain, 2011; Campos et al., 2019; Cutler and Rix, 2015; Cutler et al., 2022; Simmons and Angelini, 2017; Wong et al., 2018; Guedes et al., 2022a). This phenomenon has had a "slow start" but in the past two decades has become more appreciated in all disciplines, including among entomologists and pollinator specialists (Calabrese, 2005b; Calabrese 2016; Cutler, 2013; Cutler et al., 2022). The occurrence of hormesis in bees after exposure to pesticides can be significant in how scientists study stress responses in bees and could alter the way risk assessments are completed.

4.2 Contributions of my Thesis, Conclusions, and Considerations for Future Study

In this thesis, I examined whether or not exposure to low doses of a neonicotinoid insecticide, imidacloprid, would increase longevity of honey bees. I did not find evidence to suggest that hormesis is present in honey bees with regard to longevity. I also reviewed how honey bee toxicology research has developed and changed over the decades. I found that the majority of toxicology research surrounding pollinators and *Apis* species, alike, was published after 2010. The pesticide that showed the most citations in relation to pollinators was neonicotinoids. Sublethal exposure/toxicity produced the most citations, as opposed to acute or chronic exposures/toxicity. With regard to pollinators and neonicotinoids, since 2000, the number of studies that use probable exposure scenarios was fairly consistent throughout the years. Studies that found neonicotinoids to have no

and negative effects on honey bees was another consistent pattern over the time span I examined. Of the different endpoints I examined, residue analyses were present in all years, and biochemical endpoints became more prominent in later years (2015-2019). There is a lack of research on hormesis regarding pollinators and neonicotinoids. The occurrence and significance of insecticide-induced hormesis in bees has recently been discussed (Cutler et al., 2022; Cutler and Rix, 2015), but some authors who inadvertently find hormesis in bees are clearly still not familiar with the phenomenon (Catae et al., 2019; Schmuck et al., 2001; Suchail et al., 2004a; Zhu et al., 2017a).

My experimental study in Chapter 2 aimed to further understand and explain the relationship between honey bees and neonicotinoids, by attempting to examine hormesis. Although hormesis was not present in my study I do believe hormesis can occur in bees. Hormesis in response to only neonicotinoids has been observed in *A. mellifera* (Alberoni et al., 2021; Colin et al., 2019; Faucon et al., 2005; Kessler et al., 2015; Ma et al., 2019; Tosi et al., 2016). Of these, imidacloprid was mostly associated with an increase in brood production (Alberoni et al., 2021; Colin et al., 2019; Faucon et al., 2005). However, increased longevity has been seen as a hormetic response in *A. mellifera* (Bounias et al., 1995; Cutler et al., 2022; Dickel et al., 2018; Köhler et al., 2012a; Wong et al., 2018). None of these studies showed an increase in longevity solely in response to a neonicotinoid, and only one involve imidacloprid. Bounias et al., (1995) was not associated with neonicotinoids, rather cupric salts. After exposure to nicotine, Köhler et al., (2012a) only showed an increase in longevity in bees that had been deemed “weak bees”. The others two studies showed an increase in longevity after or co-exposure with a neonicotinoid and another stressor (Dickel et al., 2018; Wong et al., 2018). Therefore, in

A. mellifera, I believe hormesis with respect to longevity may be better observed using a combination of stressors. Should my experiment be repeated, I would not use imidacloprid alone to examine longevity in bees. I believe co-exposure with another stressor could induce a hormetic response in honey bee longevity.

In Chapter 3 the number of citations that included sublethal effects on pollinators and/or *Apis* species since 2010 is large, 570 (191 for pollinator and 379 for *Apis*). This is more than both the "acute" and "chronic" citations combined. Again, most of these "sublethal" studies occurred in the most recent decade (2010-2019). In combing through the literature, it is easy to see that most studies examining sublethal effects consider these effects to all be harmful. This focus on harmful sublethal effects was initially important; we needed to understand if sublethal doses were causing real harm to honey bees. Yet now that there are so many studies surrounding these effects, we can see that there are many factors that can contribute to honey bee colony, and individual success. Beekeeping practices, parasites, environmental fluctuations, genetic diversity, etc. all contribute to honey bee diversity, success, and resilience. Honey bees are extremely versatile and incredible organisms that make up a superorganism but just like most living things, they respond to things based on their genetics and environment. Of course, if bees are exposed to unrealistically high doses of pesticides, they may be adversely affected. If farmers follow the directions surrounding pesticide application, residues in hives should remain low and colonies will often not be affected (Chauzat et al., 2006; Cutler et al., 2014; Cutler and Scott-Dupree, 2007; Faucon et al., 2005; Hardstone and Scott, 2010; Nguyen et al., 2009; Traver et al., 2018). Furthermore, it seems obvious that there are so many variables at play, and because of this any research surrounding sublethal effects on bees

can seemingly yield any result. Therefore, is there a point to examining all of these intricacies so closely? Of course, fully understanding the tangled web of honey bee toxicology response is interesting but will there ever be a clear cut answer? I believe that hormesis is the answer. That if more focus is given to this dose-response model, and more scientists are aware of it, it could paint a clearer picture of how honey bees respond to stressors and could be an integral part of risk assessments.

Considering the search methods used in Chapter 3 of my thesis, for the first objective, I did not examine each paper in depth and I only used the classes of pesticides in my search (i.e. not specific compound names such as imidacloprid, for example). I also may have missed certain publications in my counts because of the terms used (i.e. not all scientists use the same terminology). Therefore, had I used a more comprehensive list of search terms, I may have seen slightly different results. This may have allowed me to better represent the shift of focus from acute mortality to sublethal responses, as outlined in the literature (Desneux et al., 2007; Guedes et al., 2016).

Future work should include more studies examining hormesis and should shift away from this sharp focus on harmful sublethal effects. By testing different hormesis hypotheses in bees, bi-phasic dose-response models could be generated that enable predictions of stimulatory effects on endpoints related to survival, learning, and reproduction (Cutler et al., 2022). Examining synergistic effects between different chemicals could also be beneficial, as bees may be exposed to many stressors in the field. More field studies should also be conducted to get a better understanding of how honey bees react in their natural environments. Further, examining hormesis at the colony level would be of interest as honey bees are social organisms. A more comprehensive

understanding of the eco-evolutionary effects of hormesis and how exposed honey bees may subsequently effect the ecosystem around them would also be important (Guedes et al., 2022b). Hormesis is commonly seen after exposure to a previous stressor (Calabrese et al., 2013). Therefore, exploring co-exposure scenarios with neonicotinoids and other stressor is of interest (e.g. physical, nutritional, other phytochemicals, etc.).

For those involved in the pesticide-pollinator debate, an increase in interest and application of hormesis could provide some impactful insights. Many pesticides are certainly toxic to pollinators, but the possibility of hormetic responses in bees has been overlooked in the literature. Neonicotinoids are widely used pesticides that have been at the centre of the pesticide-pollinator debate for years. They have also been shown to cause stimulatory effects in honey bees. By studying hormesis using well designed experiments, new insights into how bees adapt to exposure to neonicotinoids could be revealed. Not only would it provide integral information for risk assessments, but it could shift agricultural pest management practices and provide scientists, legislators, and the public with a more well-rounded picture of honey bee toxicology.

References

Abrol, D. P. (2012). Pollination Biology: Biodiversity Conservation and Agricultural Production. Springer Dordrecht Heidelberg. London, NY.

Agathokleous, E., & Calabrese, E. J. (2019). Hormesis: the dose response for the 21st century: The future has arrived. *Toxicology*, *425*, 152249.

Aguilar, R., Ashworth, L., Galetto, L., & Aizen, M. A. (2006). Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters*, 968–980.

Aizen, M. A., & Harder, L. D. (2009). The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Current Biology*, *19*, 915–918.

Alberoni, D., Favaro, R., Baffoni, L., Angeli, S., & Di Gioia, D. (2021). Neonicotinoids in the agroecosystem: In-field long-term assessment on honeybee colony strength and microbiome. *Science of the Total Environment*, *762*, 144116.

Alburaki, M., Steckel, S. J., Williams, M. T., Skinner, J. A., Tarpy, D. R., Meikle, W. G., Adamczyk, J., & Stewart, S. D. (2017). Agricultural Landscape and Pesticide Effects on Honey Bee (Hymenoptera: Apidae) Biological Traits. *Journal of Economic Entomology*, *110*, 835–847.

Aufauvre, J., Misme-Aucouturier, B., Vigues, B., Texier, C., Delbac, F., & Blot, N. (2014). Transcriptome analyses of the honey bee response to *Nosems ceranae* and insecticides. *PLoS One, 9*, e91686

Ayyanath, M. M. (2013). *Effect of sublethal concentrations of imidacloprid and precocene on green peach aphid,* Myzus Persicae *(Sulzer) (Hemiptera: Aphididae): a study of hormesis at the gene, individual and population level*. MSc thesis. Dalhousie University, Halifax, Nova Scotia.

Badji, C. A., Guedes, R. N. C., Silva, A. A., Corrêa, A. S., Queiroz, M. E. L., & Michereff-Filho, M. (2007). Non-target impact of deltamethrin on soil arthropods of maize fields under conventional and no-tillage cultivation. *Journal of Applied Entomology*, *131*, 50–58.

Barascou, L., Sene, D., Barraud, A., Michez, D., Lefebvre, V., Medrzycki, P., Di Prisco, G., Strobl, V., Yañez, O., Neumann, P., Le Conte, Y., & Alaux, C. (2021). Pollen nutrition fosters honeybee tolerance to pesticides. *Royal Society Open Science*, *8, 210818*.

Barbosa, W. F., Smagghe, G., & Guedes, R. N. C. (2015a). Pesticides and reduced-risk insecticides, native bees and pantropical stingless bees: pitfalls and perspectives. *Pest Management Science*, *71*, 1049–1053.

Barbosa, W. F., De Meyer, L., Guedes, R. N. C., & Smagghe, G. (2015b). Lethal and sublethal effects of azadirachtin on the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). *Ecotoxicology*, *24*, 130–142.

Bass, C., & Field, L. M. (2018). Neonicotinoids. *Current Biology*, *28*(14), R772–R773.

Bernal, J., Garrido-Bailón, E., Del Nozal, M. J., González-Porto, A. V., Martín-Hernández, R., Diego, J. C., Jiménez, J. J., Bernal, J. L., & Higes, M. (2010). Overview of pesticide residues in stored pollen and their potential effect on bee colony (*Apis mellifera*) losses in Spain. *Journal of Economic Entomology*, *103*, 1964–1971.

Biodiversity Act, 2016. Loi n° 2016-1087 du 8 août 2016 pour la reconquête de la biodiversité, de la nature et des paysages. https://www.legifrance.gouv.fr/eli/loi/2016/8/8/2016-1087/jo/texte

Blacquière, T., Smagghe, G., Van Gestel, C. A. M., & Mommaerts, V. (2012). Neonicotinoids in bees: A review on concentrations, side-effects and risk assessment. *Ecotoxicology*, *21*, 973–992.

Böhme, F., Bischoff, G., Zebitz, C. P. W., Rosenkranz, P., & Wallner, K. (2018). Pesticide residue survey of pollen loads collected by honeybees (*Apis mellifera*) in daily intervals at three agricultural sites in South Germany. *PLoS ONE*, *13*, 1–22.

Bounias, M., Navonenectoux, M., & Popeskovic, D. S. (1995). Toxicology of cupric salts in honeybees. *Ecotoxicology and Environmental Safety*, *31*, 127–132.

Brann, J. L. (1956). Apparatus for application of insecticides. *Annual Review of Entomology*, *1*, 241–260.

Brunet, J. L., Badiou, A., & Belzunces, L. P. (2005). In vivo metabolic fate of [14C]-acetamiprid in six biological compartments of the honeybee, *Apis mellifera* L. *Pest Management Science*, *61*, 742–748.

Calabrese, E. J. (2018). Originator of the hormesis concept:Rudolf Virchow or Hugo Schulz. *Human and Experimental Toxicology, 37*, 889-890.

Calabrese, E. J. (2016). The emergence of the dose-response concept in biology and medicine. *International Journal of Molecular Sciences*, *17*.

Calabrese, E. J. (2013). Origin of the linearity no threshold (LNT) dose-response concept. *Archives of Toxicology, 87*, 1621-1633.

Calabrese, E. J. (2010). Hormesis is central to toxicology, pharmacology and risk assessment. *Human and Experimental Toxicology*, *29*, 249–261.

Calabrese, E. J. (2005a). Paradigm lost, paradigm found: the re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. *Environmental Pollution*, *138*, 379–412.

Calabrese, E. J. (2005b). Toxicological awakenings: The rebirth of hormesis as a central pillar of toxicology. *Toxicology and Applied Pharmacology*, *204*, 1–8.

Calabrese, E. J., & Agathokleous, E. (2019). Building biological sheilds via hormesis. *Trends in Pharmacological Sciences, 40*, 8-10.

Calabrese, E. J., Bachmann, K. A., Bailer, A. J., Bolger, P. M., Borak, J., Cai, L., Cedergreen, N., Cherian, M. G., Chiueh, C. C., Clarkson, T. W., Cook, R. R., Diamond, D. M., Doolittle, D. J., Dorato, M. A., Duke, S. O., Feinendegen, L., Gardner, D. E., Hart, R. W., Hastings, K. L., … Mattson, M. P. (2007). Biological stress response terminology: Integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. *Toxicology and Applied Pharmacology*, *222*, 122–128.

Calabrese, E. J., & Baldwin, L. A. (2002). Defining hormesis. *Human and Experimental Toxicology*, *21*, 91–97.

Calabrese, E. J., & Baldwin, L. A. (2003). The hormetic dose-response model is more common than the threshold model in toxicology. *Toxicological Sciences*, *71*, 246–250.

Calabrese, E. J, & Baldwin, L. A. (2003). Toxicology rethinks its central belief. *Nature*, *421*, 691–692.

Calabrese, E. J., & Blain, R. B. (2011). The hormesis database: The occurrence of hormetic dose responses in the toxicological literature. *Regulatory Toxicology and Pharmacology*, *61*, 73–81

Calabrese, E. J., Iavicoli, I., & Calabrese, V. (2013). Hormesis: Its impact on medicine and health. *Human and Experimental Toxicology*, *32*, 120–152.

Campos, S. O., Santana, I. V., Silva, C., Santos-Amaya, O. F., Guedes, R. N. C., & Pereira, E. J. G. (2019). Bt-induced hormesis in Bt-resistant insects: theoretical possibility or factual concern? *Ecotoxicology and Environmental Safety*, *183*, 109577.

Carson, R. (1962). Silent Spring. Houghton Mifflin. Boston, MA.

Cao, Y., Yang, H., Li, J., Wang, C., Li, C., & Gao, Y. (2019). Sublethal effects of imidacloprid on the population development of western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Insects*, *10*, 1–10.

Casida, J. E. (2018). Neonicotinoids and other insect nicotinic receptor competitive modulators: progress and prospects. *Annual Review of Entomology*, *63*, 125–144.

Casida, J. E., & Durkin, K. A. (2013). Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annual Review of Entomology*, *58*, 99–117.

Catae, A. F., da Silva Menegasso, A. R., Pratavieira, M., Palma, M. S., Malaspina, O., & Roat, T. C. (2019). MALDI-imaging analyses of honeybee brains exposed to a neonicotinoid insecticide. *Pest Management Science*, *75*, 607–615.

Cervantes, L., & López-Martínez, G. (2022). Anoxia hormesis following overwintering diapause boosts bee survivorship and adult performance. *Science of the Total Environment*, *802*, 149934.

Charreton, M., Decourtye, A., Henry, M., Rodet, G., Sandoz, J. C., Charnet, P., & Collet, C. (2015). A locomotor deficit induced by sublethal doses of pyrethroid and neonicotinoid insecticides in the honeybee *Apis mellifera*. *PLoS ONE*, *10*, 1–15.

Chauzat, M. P., Faucon, J. P., Martel, A. C., Lachaize, J., Cougoule, N., & Aubert, M. (2006). A survey of pesticide residues in pollen loads collected by honey bees in France. *Journal of Economic Entomology*, *99*, 253–262.

Chmiel, J. A., Daisley, B. A., Pitek, A. P., Thompson, G. J., & Reid, G. (2020). Understanding the effects of sublethal pesticide exposure on honey bees: a role for probiotics as mediators of environmental stress. *Frontiers in Ecology and Evolution*, *8*, 1–19.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology*, *18*, 117–143.

Clarke, K. R., & Ainsworth, M. (1993). A method of linking multivariate community structure to environmental variables. *Marine Ecology Progress Series*, *92*, 205–219.

Clarke, K. R, Gorley, R. N., Somerfield, P., & Warwick, R. M. (2014). Change in marine communities: an approach to statistical analysis and interpretation. In *PRIMER-E: Plymouth*.

Clarke, K. R., Somerfield, P. J., & Chapman, M. G. (2006). On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology*, *330*, 55–80.

Colin, M. E., Bonmatin, J. M., Moineau, I., Gaimon, C., Brun, S., & Vermandere, J. P. (2004). A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. *Archives of Environmental Contamination and Toxicology*, *47*, 387–395.

Colin, T., Meikle, W. G., Paten, A. M., & Barron, A. B. (2019). Long-term dynamics of honey bee colonies following exposure to chemical stress. *Science of the Total Environment*, *677*, 660–670.

Cordeiro, E. M. G., de Moura, I. L. T., Fadini, M. A. M., & Guedes, R. N. C. (2013). Beyond selectivity: are behavioral avoidance and hormesis likely causes of pyrethroid-induced outbreaks of the southern red mite *Oligonychus ilicis*? *Chemosphere*, *93*, 1111–1116.

Coulon, M., Schurr, F., Martel, A. C., Cougoule, N., Bégaud, A., Mangoni, P., Prisco, G. D., Dalmon, A., Alaux, C., Ribière-Chabert, M., Le Conte, Y., Thiéry, R., & Dubois, E. (2019). Influence of chronic exposure to thiamethoxam and chronic bee paralysis virus on winter honey bees. *PLoS ONE*, *14*, 1–20.

Cox, C. (1987). Threshold dose-response models in toxicology. *Biometrics*, *43*, 511–523.

Cressey, D. (2017). Neonics vs bees. *Nature*, *551*, 156–158.

Cresswell, J. E., Robert, F. X. L., Florance, H., & Smirnoff, N. (2014). Clearance of ingested neonicotinoid pesticide (imidacloprid) in honey bees (Apis mellifera) and bumblebees (*Bombus terrestris*). *Pest Management Science*, *70*, 332–337.

Croft, B. A., & Brown, A. W. (1975). Responses of arthropod natural enemies to insecticides. *Annual Review of Entomology*, *20*, 285–335.

Crone, M. K., & Grozinger, C. M. (2021). Pollen protein and lipid content influence resilience to insecticides in honey bees (*Apis mellifera*). *Journal of Experimental Biology*, *224*, 1-11.

Cutler, G. C. (2013). Insects, insecticides and hormesis: evidence and considerations for study. *Dose-Response*, *11*, 154–177.

Cutler, G. C., Amichot, M., Benelli, G., Guedes, R. N. C., Qu, Y., Rix, R. R., Ullah, F., & Desneux, N. (2022). Hormesis and insects: Effects and interactions in agroecosystems. *Science of The Total Environment*, *825*, 153899.

Cutler, G. C., & Guedes, R. N. C. (2017). Occurrence and significance of insecticide-induced hormesis in insects. *ACS Symposium Series*, *1249*, 101–119.

Cutler, G. C., Ramanaidu, K., Astatkie, T., & Isman, M. B. (2009). Green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), reproduction during exposure to sublethal concentrations of imidacloprid and azadirachtin. *Pest Management Science*, *65*, 205–209.

Cutler, G. C., & Rix, R. R. (2015). Can poisons stimulate bees? Appreciating the potential of hormesis in bee-pesticide research. *Pest Management Science*, *71*, 1368–1370

Cutler, G. C., Scott-Dupree, C. D., Sultan, M., McFarlane, A. D., & Brewer, L. (2014). A large-scale field study examining effects of exposure to clothianidin seed-treated canola on honey bee colony health development, and overwintering success. *PeerJ*, *2014*, 1–23.

Cutler, G. C., & Scott-Dupree, C. D. (2007). Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. *Journal of Economic Entomology*, *100*, 765–772.

Dai, P., Jack, C. J., Mortensen, A. N., Bustamante, T. A., Bloomquist, J. R., & Ellis, J. D. (2019). Chronic toxicity of clothianidin, imidacloprid, chlorpyrifos, and dimethoate to *Apis mellifera* L. larvae reared in vitro. *Pest Management Science*, *75*, 29–36.

Dai, P. L., Jia, H. R., Geng, L. L., & Diao, Q. Y. (2016). Bt toxin Cry1Ie causes no negative effects on survival, pollen consumption, or olfactory learning in worker honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, *109*, 1028–1033.

de Castro, A. A., Poderoso, J. C. M., Ribeiro, R. C., Legaspi, J. C., Serrão, J. E., & Zanuncio, J. C. (2015). Demographic parameters of the insecticide-exposed predator *Podisus nigrispinus*: implications for IPM. *BioControl*, *60*, 231–239.

Decourtye, A., Armengaud, C., Renou, M., Devillers, J., Cluzeau, S., Gauthier, M., & Pham-Delègue, M. H. (2004). Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). *Pesticide Biochemistry and Physiology*, *78*, 83–92.

Decourtye, A., Lacassie, E., & Pham-Delégue, M. H. (2003). Learning performances of honeybees (*Apis mellifera* L) are differentially affected by imidacloprid according to the season. *Pest Management Science*, *59*, 269–278.

Déglise, P., Grünewald, B., & Gauthier, M. (2002). The insecticide imidacloprid is a partial agonist of the nicotinoic receptor of honeybee Kenyon cells. *Neuroscience Letters, 321*, 13-16.

Démares, F. J., Crous, K. L., Pirk, C. W. W., Nicolson, S. W., & Human, H. (2016). Sucrose sensitivity of honey bees is differently affected by dietary protein and a neonicotinoid pesticide. *PLoS ONE*, *11*, 1–17.

Desneux, N., Decourtye, A., & Delpuech, J.-M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, *52*, 81–106.

Desneux, N., Fauvergue, X., Dechaume-Moncharmont, F. X., Kerhoas, L., Ballanger, Y., & Kaiser, L. (2005). *Diaeretiella rapae* limits *Myzus persicae* populations after applications of deltamethrin in oilseed rape. *Journal of Economic Entomology*, *98*, 9–17.

Dickel, F., Münch, D., Amdam, G. V., Mappes, J., & Freitak, D. (2018). Increased survival of honeybees in the laboratory after simultaneous exposure to low doses of pesticides and bacteria. *PLoS ONE*, *13*, 1–18.

Dorneles, A. L., de Souza Rosa, A., & Blochtein, B. (2017). Toxicity of organophosphorus pesticides to the stingless bees *Scaptotrigona bipunctata* and *Tetragonisca fiebrigi*. *Apidologie*, *48*, 612–620.

Ebdai, R., Gary, N., & Lorenzen, K. (1980). Effects of carbon dioxide and low temperature narcosis on honey bees, *Apis mellifera*. *Environmental Entomology*, *9*, 144–147.

EFSA. (2018). Q&A: conclusions on neonicotinoids 2018. Press release.

EFSA. (2012). Statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe. *EFSA Journal*, *10*, 1–27.

Elekonich, M. M., & Roberts, S. P. (2005). Honey bees as a model for understanding mechanisms of life history transitions. *Comparative Biochemistry and Physiology*, *141*, 362–371.

Elzen, P. J., Elzen, G. W., & Lester, G. E. (2004). Compatibility of an organically based insect control program with honey bee (Hymenoptera: Apidae) pollination in cantaloupes. *Journal of Economic Entomology*, *97*, 1513–1516.

European Union. (2013). Commission implementing regulation (EU) No 485/2013. *Official Journal of the European Union*.

Faucon, J. P., Aurières, C., Drajnudel, P., Mathieu, L., Ribière, M., Martel, A. C., Zeggane, S., Chauzat, M. P., & Aubert, M. F. A. (2005). Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. *Pest Management Science*, *61*, 111–125.

Fischer, J., Müller, T., Spatz, A. K., Greggers, U., Grünewald, B., & Menzel, R. (2014). Neonicotinoids interfere with specific components of navigation in honeybees. *PLoS ONE*, *9,* e91364.

Forfert, N., Troxler, A., Retschnig, G., Gauthier, L., Straub, L., Moritz, R. F. A., Neumann, P., & Williams, G. R. (2017). Neonicotinoid pesticides can reduce honeybee colony genetic diversity. *PLoS ONE*, *12*, 1–15.

Frost, E. H., Shutler, D., & Hillier, N. K. (2011). Effects of cold immobilization and recovery period on honeybee learning, memory, and responsiveness to sucrose. *Journal of Insect Physiology*, *57*, 1385–1390.

Gallai, N., Salles, J. M., Settele, J., & Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, *68*, 810–821.

Gill, R. J., Ramos-Rodriguez, O., & Raine, N. E. (2012). Combined pesticide exposure severely affects individual-and colony-level traits in bees. *Nature*, *491*, 105–108.

Goel, M. K., Khanna, P., & Kishore, J. (2010). Understanding survival analysis: Kaplan-Meier estimate. *International Journal of Ayurveda Research*, *1*, 212–216.

Goñalons, C. M., & Farina, W. M. (2015). Effects of sublethal doses of imidacloprid on young adult honeybee behaviour. *PLoS ONE*, *10*, 1–15.

Goulson, D. (2013). An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology*, *50*, 977–987.

Government of Canada. 2022. Pest Control Products Act. *Minister of Justice.* S.C. 2002, ch 28.

Gregorc, A., Alburaki, M., Rinderer, N., Sampson, B., Knight, P., Karim, S., & Adamczyk, J. (2018). Effects of coumaphos and imidacloprid on honey bee (Hymenoptera: Apidae) lifespan and antioxidant gene regulations in laboratory experiments. *Scientific Reports*, *8*, 1–13.

Guedes, N. M. P., Tolledo, J., Corrêa, A. S., & Guedes, R. N. C. (2010). Insecticide-induced hormesis in an insecticide-resistant strain of the maize weevil, *Sitophilus zeamais*. *Journal of Applied Entomology*, *134*, 142–148.

Guedes, R. N. C., Benelli, G., & Agathokleous, E. (2022a). Arthropod outbreaks, stressors and sublethal stress. *Current Opinion in Environmental Science & Health*, *28*, 100371.

Guedes, R. N. C., Rix, R. R., & Cutler, G. C. (2022b). Pesticide-induced hormesis in arthropods: Towards biological systems. *Current Opinion in Toxicology*, *29*, 43–50.

Guedes, R. N. C., Smagghe, G., Stark, J. D., & Desneux, N. (2016). Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. *Annual Review of Entomology*, *61*, 43–62.

Guedes, R. N. C., Walse, S. S., & Throne, J. E. (2017). Sublethal exposure, insecticide resistance, and community stress. *Current Opinion in Insect Science*, *21*, 47–53.

Gunther, F. A., & Blinn, R. C. (1956). Persisting insecticide residues in plant materials. *Annual Review of Entomology*, *1*, 167–180.

Guseman, A. J., Miller, K., Kunkle, G., Dively, G. P., Pettis, J. S., Evans, J. D., VanEngelsdorp, D., & Hawthorne, D. J. (2016). Multi-drug resistance transporters and a mechanism-based strategy for assessing risks of pesticide combinations to honey bees. *PLoS ONE*, *11*, 1–16.

Haddi, K., Mendonça, L. P., Dos Santos, M. F., Guedes, R. N. C., & Oliveira, E. E. (2015). Metabolic and behavioral mechanisms of indoxacarb resistance in *Sitophilus zeamais* (Coleoptera: Curculionidae). *Journal of Economic Entomology*, *108*, 362–369.

Hardstone, M. C., & Scott, J. G. (2010). Is *Apis mellifera* more sensitive to insecticides than other insects? *Pest Management Science*, *66*, 1171–1180.

Haynes, K. F. (1988). Sublethal effects of neurotoxic insecticides on insect behaviour. *Annual Review of Entomology*, *33*, 149–168.

Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J., Aupinel, P., Aptel, J., Tchamitchian, S., & Decourtye, A. (2012). A common pesticide decreases foraging success and survival in honey bees. *Science*, *336*, 3–5.

Henry, M., Cerrutti, N., Aupinel, P., Decourtye, A., Gayrard, M., Odoux, J. F., Pissard, A., Rüger, C., & Bretagnolle, V. (2015). Reconciling laboratory and field assessments of neonicotinoid toxicity to honeybees. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 1–8.

Henschler, D. (2006). The origin of hormesis: historical background and driving forces. *Human and Experimental Toxicology*, *25*, 347–351.

Hercus, M. J., Loeschcke, V., & Rattan, S. I. S. (2003). Lifespan extension of *Drosophila melanogaster* through hormesis by repeated mild heat stress. *Biogerontology*, *4*, 149–156.

Hoskins, W. M., & Gordon, H. T. (1956). Arthropod resistcance to chemicals. *Annual Review of Entomology*, *1*, 89–122.

Huang, S. K., Csaki, T., Doublet, V., Dussaubat, C., Evans, J. D., Gajda, A. M., Gregorc, A., Hamilton, M. C., Kamler, M., Lecocq, A., Muz, M. N., Neumann, P., Özkirim, A., Schiesser, A., Sohr, A. R., Tanner, G., Tozkar, C. Ö., Williams, G. R., Wu, L., … Chen, Y. P. (2014). Evaluation of cage designs and feeding regimes for honey bee (Hymenoptera: Apidae) laboratory experiments. *Journal of Economic Entomology*, *107*, 54–62.

Human, H., Dietemann, V., Köhler, A., Pirk, C. W. W., Strauss, U., Brodschneider, R., Tanner, G., Williams, G. R., Dively, G., Ellis, J. D., Forsgren, E., Fries, I., Hatjina, F., Hu, F.-L., Zheng, H.-Q., Jaffé, R., Jensen, A. B., Magyar, J. P., Özkýrým, A., … Wilde, J. (2013). Miscellaneous standard methods for *Apis mellifera* research. *Journal of Apicultural Research*, *52*, 1–56.

Ivchenko, I., & Honov, S. A. (1998). On the Jaccard similarity test. *Journal of Mathematical Sciences. 88*, 789-794.

Jactel, H., Verheggen, F., Thiéry, D., Escobar-Gutiérrez, A. J., Gachet, E., & Desneux, N. (2019). Alternatives to neonicotinoids. *Environment International*, *129*, 423–429.

Jaffé, R., Dietemann, V., Crewe, R. M., & Moritz, R. F. A. (2009). Temporal variation in the genetic structure of a drone congregation area: an insight into the population dynamics of wild African honeybees (*Apis mellifera scutellata*). *Molecular Ecology*, *18*, 1511–1522.

Jeschke, P., & Nauen. (2008). Neonicotinoids - from zero to hero in insecticide chemistry. *Pest Management Science*, *63*, 1084–1098.

Johnson, R. (2010). Honey bee colony collapse disorder. *Congressional Research Service*, 7-5700, 69–92.

Johnson, R. M., Mao, W., Pollock, H. S., Niu, G., Schuler, M. A., & Berenbaum, M. R. (2012). Ecologically appropriate xenobiotics induce cytochrome P450s in *Apis mellifera*. *PLoS ONE*, *7*, 1–10.

Kearns, C. (1956). The mode of action of insecticides. *Annual Review of Entomology*, *1*(1), 123–148.

Kenna, D., Cooley, H., Pretelli, I., Ramos Rodrigues, A., Gill, S. D., & Gill, R. J. (2019). Pesticide exposure affects flight dynamics and reduces flight endurance in bumblebees. *Ecology and Evolution*, *9*, 5637–5650.

Kessler, S. C., Tiedeken, E. J., Simcock, K. L., Derveau, S., Mitchell, J., Softley, S., Stout, J. C., & Wright, G. A. (2015). Bees prefer foods containing neonicotinoid pesticides. *Nature*, *521*, 74–76.

Kevan, P. G., & Phillips, T. P. (2001). The economic impacts of pollinator declines: an approach to assessing the consequences. *Conservation Ecology*, *5*, 8.

Klatt, B., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., & Tscharntke, T. (2013). Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 1–7.

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 303–313.

Knapp, J. L., Phillips, B. B., Clements, J., Shaw, R. F., & Osborne, J. L. (2021). Socio-psychological factors, beyond knowledge, predict people's engagement in pollinator conservation. *People and Nature*, *3*, 204–220.

Köhler, A., Pirk, C. W. W., & Nicolson, S. W. (2012a). Honeybees and nectar nicotine: deterrence and reduced survival versus potential health benefits. *Journal of Insect Physiology*, *58*, 286–292.

Köhler, A., Pirk, C. W. W., & Nicolson, S. W. (2012b). Simultaneous stressors: Interactive effects of an immune challenge and dietary toxin can be detrimental to honeybees. *Journal of Insect Physiology*, *58*, 918–923.

Laycock, I., Lenthall, K. M., Barratt, A. T., & Cresswell, J. E. (2012). Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). *Ecotoxicology*, *21*, 1937–1945.

Liao, L. H., Pearlstein, D. J., Wu, W. Y., Kelley, A. G., Montag, W. M., Hsieh, E. M., & Berenbaum, M. R. (2020). Increase in longevity and amelioration of pesticide toxicity by natural levels of dietary phytochemicals in the honey bee, *Apis mellifera*. *PLoS ONE*, *15*, 1–18.

Liao, L. H., Wu, W. Y., & Berenbaum, M. R. (2017). Impacts of dietary phytochemicals in the presence and absence of pesticides on longevity of honey bees (*Apis mellifera*). *Insects*, *8*, 1-13.

Longley, M., & Jepson, P. C. (1996). The influence of insecticide residues on primary parasitoid and hyperparasitoid foraging behaviour in the laboratory. *Entomologia Experimentalis et Applicata*, *81*, 259–269.

Lunardi, J. S., Zaluski, R., & Orsi, R. O. (2017). Evaluation of motor changes and toxicity of insecticides Fipronil and imidacloprid in Africanized Honey bees (Hymenoptera: Apidae). *Sociobiology*, *64*, 50–56.

Lundin, O., Rundlöf, M., Smith, H. G., Fries, I., & Bommarco, R. (2015). Neonicotinoid insecticides and their impacts on bees: a systematic review of research approaches and identification of knowledge gaps. *PLoS ONE*, *10*, 1–20.

Ma, C., Zhang, Y., Sun, J., Imran, M., Yang, H., Wu, J., Zou, Y., Li-Byarlay, H., & Luo, S. (2019). Impact of acute oral exposure to thiamethoxam on the homing, flight, learning acquisition and short-term retention of *Apis cerana*. *Pest Management Science*, *75*, 2975–2980.

Martin, H. (1956). The chemisrty of insecticides. *Annual Review of Entomology*, *1*, 149–166.

Marzaro, M., Vivan, L., Targa, A., Mazzon, L., Mori, N., Greatti, M., Toffolo, E. P., di Bernardo, A., Giorio, C., Marton, D., Tapparo, A., & Girolami, V. (2011). Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat. *Bulletin of Insectology*, *64*, 119–126.

Matsuda, K., Buckingham, S. D., Kleier, D., Rauh, J. J., Grauso, M., & Sattelle, D. B. (2001). Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends in Pharmacological Sciences*, *22*, 573–580.

McArdle, B. H., & Anderson, M. J. (2001). Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology*, *82*, 290–297.

Medrzycki, P., Giffard, H., Aupinel, P., Belzunces, L. P., Chauzat, M.-P., Claßen, C., Colin, M. E., Dupont, T., Girolami, V., Johnson, R., Le Conte, Y., Lückmann, J., Marzaro, M., Pistorius, J., Porrini, C., Schur, A., Sgolastra, F., Delso, N. S., van der Steen, J. J. M., … Vidau, C. (2013). Standard methods for toxicology research in Apis mellifera . *Journal of Apicultural Research*, *52*, 1–60.

Meikle, W. G., Colin, T., Adamczyk, J. J., Weiss, M., & Barron, A. B. (2022). Traces of a neonicotinoid pesticide stimulate different honey bee colony activities, but do not increase colony size or longevity. *Ecotoxicology and Environmental Safety*, *231*, 113202.

Millar, N. S., & Denholm, I. (2007). Nicotinic acetylcholine receptors: targets for commercially important insecticides. *Invertebrate Neuroscience*, *7*, 53–66.

Moffat, C., Buckland, S. T., Samson, A. J., McArthur, R., Chamosa Pino, V., Bollan, K. A., Huang, J. T. J., & Connolly, C. N. (2016). Neonicotinoids target distinct nicotinic acetylcholine receptors and neurons, leading to differential risks to bumblebees. *Scientific Reports*, *6*, 1–10.

Mokkapati, J. S., Bednarska, A. J., & Laskowski, R. (2021). The development of the solitary bee *Osmia bicornis* is affected by some insecticide agrochemicals at environmentally relevant concentrations. *Science of the Total Environment*, *775*, 145588.

Morse, J. G. (1998). Agricultural implications of pesticide-induced hormesis of insects and mites. *Human and Experimental Toxicology*, *17*, 266–269.

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., VanEngelsdorp, D., & Pettis, J. S. (2010). High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLoS ONE*, *5*, e9754.

Mulvey, J., & Cresswell, J. E. (2020). Time-dependent effects on bumble bees of dietary exposures to farmland insecticides (imidacloprid, thiamethoxam and fipronil). *Pest Management Science*, *76*, 2846–2853.

Nai, Y. S., Chen, T. Y., Chen, Y. C., Chen, C. T., Chen, B. Y., & Chen, Y. W. (2017). Revealing pesticide residues under high pesticide stress in Taiwan's agricultural environment probed by fresh honey bee (Hymenoptera: Apidae) pollen. *Journal of Economic Entomology*, *110*, 1947–1958.

Nauen, R., & Bretschneider, T. (2002). New modes of action of insecticides. *Pesticide Outlook*, *13*, 241–245.

Nauen, R., Ebbinghaus-Kintscher, U., & Schmuck, R. (2001). Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). *Pest Management Science*, *57*, 577–586.

Nguyen, B., Saegerman, C., Pirard, C., Mignon, J., Widart, J., Thirionet, B., Verheggen, F., Berkvens, D., Pauw, E., & Haubruge, E. (2009). Does imidacloprid seed-treated maize have an impact on honey bee mortality? *Journal of Economic Entomology*, *102*, 616–623.

Palmer-Young, E. C., Tozkar, C. O., Schwarz, R. S., Chen, Y., Irwin, R. E., Adler, L. S., & Evans, J. D. (2017). Nectar and pollen phytochemicals stimulate honey bee (Hymenoptera: Apidae) immunity to viral infection. *Journal of Economic Entomology*, *110*, 1959–1972.

Paris, L., Peghaire, E., Moné, A., Diogon, M., Debroas, D., Delbac, F., & El Alaoui, H. (2020). Honeybee gut microbiota dysbiosis in pesticide/parasite co-exposures is mainly induced by *Nosema ceranae*. *Journal of Invertebrate Pathology*, *172*, 107348.

Parsons, P. A. (2001). The hormetic zone: an ecological and evolutionary perspective based upon habitat characteristics and fitness selection. *The Quarterly Review of Biology*, *76*, 459–467.

Peterson, E. M., Green, F. B., & Smith, P. N. (2021). Toxic responses of blue orchard mason bees (*Osmia lignaria*) following contact exposure to neonicotinoids, macrocyclic lactones, and pyrethroids. *Ecotoxicology and Environmental Safety*, *208*, 111681.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution*, *25*, 345–353.

Ramanaidu, K., & Cutler, G. C. (2013). Different toxic and hormetic responses of *Bombus impatiens* to *Beauveria bassiana*, *Bacillus subtilis* and spirotetramat. *Pest Management Science*, *69*, 949–954.

Ramirez-Romero, R., Desneux, N., Decourtye, A., Chaffiol, A., & Pham-Delègue, M. H. (2008). Does Cry1Ab protein affect learning performances of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae)? *Ecotoxicology and Environmental Safety*, *70*, 327–333.

Retschnig, G., Williams, G. R., Odemer, R., Boltin, J., Di Poto, C., Mehmann, M. M., Retschnig, P., Winiger, P., Rosenkranz, P., & Neumann, P. (2015). Effects, but no interactions, of ubiquitous pesticide and parasite stressors on honey bee (*Apis mellifera*) lifespan and behaviour in a colony environment. *Environmental Microbiology*, *17*, 4322–4331.

Ricotta, C., & Podani, J. (2017). On some properties of the Bray-Curtis dissimilarity and their ecological meaning. *Ecological Complexity*, *31*, 201–205.

Ripper, W. E. (1956). Effect of pesticides on balance of arthropod populations. *Annual Review of Entomology*, *1*, 403–438.

Rix, R. R., & Cutler, G. C. (2022). Review of molecular and biochemical responses during stress induced stimulation and hormesis in insects. *Science of the Total Environment*, *827*, 154085.

Rix, R. R., & Cutler, G. C. (2020). Low doses of a neonicotinoid stimulate reproduction in a beneficial predatory insect. *Journal of Economic Entomology*, *113*, 2179–2186.

Rodney, S., & Purdy, J. (2020). Dietary requirements of individual nectar foragers, and colony-level pollen and nectar consumption: a review to support pesticide exposure assessment for honey bees. *Apidologie*, *51*, 163–179.

Roszko, M. Ł., Kamińska, M., Szymczyk, K., & Jędrzejczak, R. (2016). Levels of selected persistent organic pollutants (PCB, PBDE) and pesticides in honey bee pollen sampled in Poland. *PLoS ONE*, *11*, 1–23.

Saitanis, C. J., & Agathokleous, E. (2019). Stress response and population dynamics: Is Allee effect hormesis? *Science of the Total Environment*, *682*, 623–628.

Santos-Junior, V. C. dos, Martínez, L. C., Plata-Rueda, A., Bozdoğan, H., Zanuncio, J. C., & Serrão, J. E. (2019). Exposure to spinosad induces histopathological and cytotoxic effects on the salivary complex of the non-target predator *Podisus nigrispinus*. *Chemosphere*, *225*, 688–695.

Santos, M. F., Krüger, A. P., Turchen, L. M., Cutler, G. C., Oliveira, E. E., & Guedes, R. N. C. (2018). Non-targeted insecticidal stress in a pest species: insecticides, sexual fitness and hormesis in the Neotropical brown stink bug *Euschistus heros*. *Annals of Applied Biology*, *172*, 375–383.

SAS Institute Inc. 2014. SAS statistical software. Version 9.4 Cary, NC.

Schmuck, R., Nauen, R., & Ebbinghaus-Kintscher, U. (2003). Effects of imidacloprid and common plant metabolites of imidacloprid in the honeybee: toxicological and biochemical considerations. *Bulletin of Insectology*, *56*, 27–34.

Schmuck, R., Schning, R., Stork, A., & Schramel, O. (2001). Risk posed to honeybees (*Apis mellifera* L, Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Management Science*, *57*, 225–238.

Schneider, C. W., Tautz, J., Grünewald, B., & Fuchs, S. (2012). RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PLoS ONE*, *7*, 1–9.

Serrão, J. E., Plata-Rueda, A., Martínez, L. C., & Zanuncio, J. C. (2022). Side-effects of pesticides on non-target insects in agriculture: a mini-review. *Science of Nature*, *109*, 1–11.

Sgolastra, F., Medrzycki, P., Bortolotti, L., Renzi, M. T., Tosi, S., Bogo, G., Teper, D., Porrini, C., Molowny-Horas, R., & Bosch, J. (2017). Synergistic mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species. *Pest Management Science*, *73*, 1236–1243.

Shi, J., Liao, C., Wang, Z., Zeng, Z., & Wu, X. (2019). Effects of sublethal acetamiprid doses on the lifespan and memory-related characteristics of honey bee (*Apis mellifera*) workers. *Apidologie*, *50*, 553–563.

Shi, T. F., Wang, Y. F., Liu, F., Qi, L., & Yu, L. S. (2017). Sublethal effects of the neonicotinoid insecticide thiamethoxam on the transcriptome of the honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, *110*, 2283–2289.

Simmons, W. R., & Angelini, D. R. (2017). Chronic exposure to a neonicotinoid increases expression of antimicrobial peptide genes in the bumblebee *Bombus impatiens*. *Scientific Reports*, *7*, 44773, 1-9.

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs, C., Furlan, L., Gibbons, D. W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C. H., Liess, M., Long, E., Mcfield, M., Mineau, P., Mitchell, E. A., Morrissey, C. A., … Wiemers, M. (2015). Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research*, *22*, 5–34.

Signh, S. R., Walters, K. F. A., Port, G. R., & Northing, P. (2004) Consumption rates and predatory activity of adult and fourth instar larvae of the seven spot ladybird, *Coccinella septempunctata* (L.), following contact with dimethoate residue and contaminated prey in laboratory arenas. *Biological Control, 30,* 127-133.

Southam, C., & Ehrlich, J. (1943). Effects of extrct of Western red-cedar heartwood on certain wood-decaying fungi in culture. *Phytopathology*, *33*, 517–524.

Sparks, T. C., Crossthwaite, A. J., Nauen, R., Banba, S., Cordova, D., Earley, F., Ebbinghaus-Kintscher, U., Fujioka, S., Hirao, A., Karmon, D., Kennedy, R., Nakao, T., Popham, H. J. R., Salgado, V., Watson, G. B., Wedel, B. J., & Wessels, F. J. (2020). Insecticides, biologics and nematicides: updates to IRAC's mode of action classification - a tool for resistance management. *Pesticide Biochemistry and Physiology*, *167*, 104587.

Stanley, D. A., & Raine, N. E. (2016). Chronic exposure to a neonicotinoid pesticide alters the interactions between bumblebees and wild plants. *Functional Ecology*, *30*, 1132–1139.

Stanley, D. A., Russell, A. L., Morrison, S. J., Rogers, C., & Raine, N. E. (2016). Investigating the impacts of field-realistic exposure to a neonicotinoid pesticide on bumblebee foraging, homing ability and colony growth. *Journal of Applied Ecology*, *53*, 1440–1449.

Stanley, D. A., Smith, K. E., & Raine, N. E. (2015). Bumblebee learning and memory is impaired by chronic exposure to a neonicotinoid pesticide. *Scientific Reports*, *5*, 1–10.

Stark, J. D., & Banks, J. E. (2003). Population-level effects of pesticides and other toxicants on arthropods. *Annual Review of Entomology*, *48*, 505–519.

Stejskalová, M., Konradyová, V., & Kazda, J. (2021). The influence of pesticides repellency used in oilseed rape (*Brassica napus* subsp. napus) on the preference by bees (*Apis mellifera* L.). *Journal of Apicultural Research*, *60*, 270–276.

Strobl, V., Camenzind, D., Minnameyer, A., Walker, S., Eyer, M., Neumann, P., & Straub, L. (2020). Positive correlation between pesticide consumption and longevity in solitary bees: Are we overlooking fitness trade-offs? *Insects*, *11*, 1–12.

Suchail, S., De Sousa, G., Rahmani, R., & Belzunces, L. P. (2004a). In vivo distribution and metabolisation of 14C-imidacloprid in different compartments of *Apis mellifera* L. *Pest Management Science*, *60*, 1056–1062.

Suchail, S., Debrauwer, L., & Belzunces, L. P. (2004b). Metabolism of imidacloprid in *Apis mellifera*. *Pest Management Science*, *60*, 291–296.

Suchail, S., Guez, D., & Belzunces, L. P. (2001). Discrepancy between acute and chronic toxicity Induced by imidacloprid and Its metabolites in *Apis mellifera*. *Environmental Toxicology and Chemistry*, *20*, 2482–2486.

Tan, K., Chen, W., Dong, S., Liu, X., Wang, Y., & Nieh, J. C. (2014). Imidacloprid alters foraging and decreases bee avoidance of predators. *PLoS ONE*, *9*, 3–10.

Tapparo, A., Marton, D., Giorio, C., Zanella, A., Soldà, L., Marzaro, M., Vivan, L., & Girolami, V. (2012). Assessment of the environmental exposure of honeybees to particulate matter containing neonicotinoid insecticides coming from corn coated seeds. *Environmental Science and Technology*, *46*, 2592–2599.

Tesovnik, T., Cizelj, I., Zorc, M., Čitar, M., Božič, J., Glavan, G., & Narat, M. (2017). Immune related gene expression in worker honey bee (*Apis mellifera carnica*) pupae exposed to neonicotinoid thiamethoxam and Varroa mites (*Varroa destructor*). *PLoS ONE*, *12*, 1–16.

Thany, S. H., & Gauthier, M. (2005). Nicotine injected into the antennal lobes induces a rapid modulation of sucrose threshold and improves short-term memory in the honeybee *Apis mellifera*. *Brain Research*, *1039*, 216–219.

Tomé, H. V. V., Barbosa, W. F., Martins, G. F., & Guedes, R. N. C. (2015). Spinosad in the native stingless bee *Melipona quadrifasciata*: Regrettable non-target toxicity of a bioinsecticide. *Chemosphere*, *124*, 103–109.

Tomizawa, M., & Casida, J. E. (2005). Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annual Review of Pharmacology and Toxicology*, *45*, 247–268.

Tosi, S., Burgio, G., & Nieh, J. C. (2017). A common neonicotinoid pesticide, thiamethoxam, impairs honey bee flight ability. *Scientific Reports*, *7*, 1–8.

Tosi, S., Démares, F. J., Nicolson, S. W., Medrzycki, P., Pirk, C. W. W., & Human, H. (2016). Effects of a neonicotinoid pesticide on thermoregulation of African honey bees (*Apis mellifera scutellata*). *Journal of Insect Physiology*, *93–94*, 56–63.

Traver, B. E., Feazel-Orr, H. K., Catalfamo, K. M., Brewster, C. C., & Fell, R. D. (2018). Seasonal effects and the impact of in-hive pesticide treatments on parasite, pathogens, and health of honey bees. *Journal of Economic Entomology*, *111*, 517–527.

Tsvetkov, N., Samson-Robert, O., Sood, K., Patel, H. S., Malena, D. A., Gajiwala, P. H., Maciukiewicz, P., Fournier, V., & Zayed, A. (2017). Chronic exposure to neonicotinoids reduces honey bee health near corn crops. *Science*, *356*, 1395–1397.

USEPA. (2012). *White Paper in Support of the Proposed Risk Assessment Process for Bees*.

van der Steen, J. J. M., Cornelissen, B., Donders, J., Blacquière, T., & van Dooremalen, C. (2012). How honey bees of successive age classes are distributed over a one storey, ten frames hive. *Journal of Apicultural Research*, *51*, 174–178.

Vanbergen, A. J., Garratt, M. P., Vanbergen, A. J., Baude, M., Biesmeijer, J. C., Britton, N. F., Brown, M. J. F., Brown, M., Bryden, J., Budge, G. E., Bull, J. C., Carvell, C., Challinor, A. J., Connolly, C. N., Evans, D. J., Feil, E. J., Garratt, M. P., Greco, M. K., Heard, M. S., … Wright, G. A. (2013). Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment*, *11*, 251–259.

vanEngelsdorp, D., Evans, J. D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B. K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Underwood, R., Tarpy, D. R., & Pettis, J. S. (2009). Colony collapse disorder: a descriptive study. *PLoS ONE*, *4*, e6481.

Vargas-Hernandez, M., Macias-Bobadilla, I., Guevara-Gonzalez, R. G., Romero-Gomez, S. de J., Rico-Garcia, E., Ocampo-Velazquez, R. V., Alvarez-Arquieta, L. de L., & Torres-Pacheco, I. (2017). Plant hormesis management with biostimulants of biotic origin in agriculture. *Frontiers in Plant Science*, *8*, 1762.

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*, *10*, 57–63.

Watson, G. B., Siebert, M. W., Wang, N. X., Loso, M. R., & Sparks, T. C. (2021). Sulfoxaflor - A sulfoximine insecticide: Review and analysis of mode of action, resistance and cross-resistance. *Pesticide Biochemistry and Physiology, 178,* 104924

Wessler, I., Gärtner, H. A., Michel-Schmidt, R., Brochhausen, C., Schmitz, L., Anspach, L., Grünewald, B., & Kirkpatrick, C. J. (2016). Honeybees produce millimolar concentrations of non-neuronal acetylcholine for breeding: possible adverse effects of neonicotinoids. *PLoS ONE*, *11*, 1–19.

Whitehorn, P. R., Wallace, C., & Vallejo-Marin, M. (2017). Neonicotinoid pesticide limits improvement in buzz pollination by bumblebees. *Scientific Reports*, *7*, 1–8.

Williams, G. R., Doublet, V., Medrzycki, P., Eisenhardt, D., Tanner, G., Brodschneider, R., Neumann, P., Shutler, D., Murray, T. E., McMahon, D. P., Alaux, C., Costa, C., Kuhn, R., Pernal, S. F., Natsopoulou, M. E., Csáki, T., Fries, I., van der Steen, J. J. M., Paxton, R. J., & Oliver, R. (2013). Standard methods for maintaining adult Apis mellifera in cages under *in vitro* laboratory conditions. *Journal of Apicultural Research*, *52*, 1–36.

Williams, P., & Osborne, J. (2009). Bumblebee vulnerability conservaton world-wide. *Apidologie*, *40*, 367–387.

Williamson, S. M., Moffat, C., Gomersall, M. A. E., Saranzewa, N., Connolly, C. N., & Wright, G. A. (2013). Exposure to acetylcholinesterase inhibitors alters the physiology and motor function of honeybees. *Frontiers in Physiology*, *4*, 1–10.

Wilson, J. S., Forister, M. L., & Carril, O. M. (2017). Interest exceeds understanding in public support of bee conservation. *Frontiers in Ecology and the Environment*, *15*, 460–466.

Winston, M. L. (1987) The Biology of the Honey Bee. Harvard University Press, Cambridge, MA

Wojda, I., Kowalski, P., & Jakubowicz, T. (2009). Humoral immune response of *Galleria mellonella* larvae after infection by *Beauveria bassiana* under optimal and heat-shock conditions. *Journal of Insect Physiology*, *55*, 525–531.

Wong, M. J., Liao, L. H., & Berenbaum, M. R. (2018). Biphasic concentration-dependent interaction between imidacloprid and dietary phytochemicals in honey bees (*Apis mellifera*). *PLoS ONE*, *13*, 1–15.

Wood, T. J., & Goulson, D. (2017). The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environmental Science and Pollution Research*, *24*, 17285–17325.

Wright, G. A., Baker, D. D., Palmer, M. J., Stabler, D., Mustard, J. A., Power, E. F., Borland, A. M., & Stevenson, P. C. (2013). Caffeine in floral nectar enhances a pollinator's memory of reward. *Science*, *339*, 1202–1204.

Wu, Y. Y., Zhou, T., Wang, Q., Dai, P. L., Xu, S. F., Jia, H. R., & Wang, X. (2015). Programmed cell death in the honey bee (*Apis mellifera*) (Hymenoptera: Apidae) worker brain induced by imidacloprid. *Journal of Economic Entomology*, *108*, 1486–1494.

Yang, E. C., Chuang, Y. C., Chen, Y. L., & Chang, L. H. (2008). Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *Journal of Economic Entomology*, *101*, 1743–1748.

Yao, J., Zhu, Y. C., & Adamczyk, J. (2018). Responses of honey bees to lethal and sublethal doses of formulated clothianidin alone and mixtures. *Journal of Economic Entomology*, *111*, 1517–1525.

Zhou, D. R., Eid, R., Miller, K. A., Boucher, E., Mandato, C. A., & Greenwood, M. T. (2019). Intracellular second messengers mediate stress inducible hormesis and programmed cell death: a review. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1866*, 773–792.

Zhu, Y. C., Yao, J., Adamczyk, J., & Luttrell, R. (2017a). Feeding toxicity and impact of imidacloprid formulation and mixtures with six representative pesticides at residue concentrations on honey bee physiology (*Apis mellifera*). *PLoS ONE*, *12*, 1–20.

Zhu, Y. C., Yao, J., Adamczky, A., & Luttrell, R. (2017b). Synergistic toxicity and physiological impact of imidacloprid alone and binary mixtures with seven representative pesticides on honey bee (*Apis mellifera*). *PLoS ONE, 12*, 1-17.

Appendix A

Median time (in days) required for 50% of the population to die (TL_{50} values) for each dose (ng/bee) of imidacloprid used in *Apis mellifera* longevity bioassays.

Dose	TL50	Lower	Upper	Mean	SE
0	26	24	27	25.77	0.455
0.0025	25	24	25	24.22	0.3715
0.025	26	25	26	25.93	0.3314
0.25	24	23	25	23.88	0.367
1	24	23	25	24.22	0.357
2	25	24	26	25.40	0.3724
2.5	26	25	27	25.83	0.3918
25	23	22	23	21.68	0.4027

Appendix B: SIMPER results reporting average between-category dissimilarities for different literature variables between four year categories (2000-2004, 2005-2009, 2010-2014, and 2015-2019). The overall average dissimilarity for a year category is for the assemblage of literature variables, whereas the average dissimilarity within the table is for a given literature variable. Diss/SD is the ratio of the average dissimilarity to standard deviation of the dissimilarities for each literature variable; a ratio ≥ 1 indicates a consistent contribution, whereas a ratio < 1 indicates variability in the contribution. The contribution of that variable to the overall average dissimilarity is indicated next. The cumulative contribution simply accumulates the contribution values.

Year category 1 vs 4

Overall average dissimilarity (%) = 59.65

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Inhibitory	5.66	1.41	9.48	9.48
Probable	4.41	1.03	7.39	16.87
Improbable	4.21	1.01	7.06	23.93
Biochemical	3.99	0.93	6.68	30.62
Other on bees	3.88	0.90	6.51	37.13
Stimulatory	3.40	0.89	5.70	42.83
Acute mortality	3.05	0.76	5.12	47.95
Residue analyses	2.90	0.75	4.86	52.80
Field	2.65	0.65	4.45	57.25
None	2.62	0.66	4.39	61.64
Lab	2.61	0.63	4.37	66.01
Indirect	2.59	0.64	4.34	70.34
Colony health	2.50	0.71	4.19	74.53
Foraging/flight path	2.16	0.66	3.62	78.15
Longevity	2.12	0.59	3.56	81.71
Field sampling studies	2.05	0.54	3.43	85.14
Molecular	2.04	0.58	3.42	88.57
Combined	1.93	0.57	3.23	91.80

Year category 1 vs 2

Overall average dissimilarity (%) = 61.42

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Residue analyses	4.82	1.15	7.85	7.85
Improbable	4.38	1.12	7.14	14.98
Biochemical	3.87	0.91	6.30	21.29
Lab	3.82	0.87	6.21	27.50
Field	3.74	0.89	6.09	33.59
Inhibitory	3.74	0.86	6.08	39.67
Other on bees	3.64	0.84	5.93	45.60
Combined	3.63	0.87	5.90	51.51
Colony health	3.58	0.91	5.83	57.34
Probable	3.30	0.80	5.37	62.70
Stimulatory	3.05	0.84	4.96	67.66
Indirect	2.83	0.69	4.61	72.27
Foraging/flight path	2.66	0.69	4.33	76.60
None	2.49	0.61	4.06	80.65
Brood	2.48	0.71	4.04	84.70
Field sampling studies	2.37	0.61	3.86	88.56
Acute mortality	2.24	0.62	3.65	92.21

Year category 2 v 4

Overall average dissimilarity (%) = 63.81

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Residue analyses	5.34	1.25	8.45	8.45
Probable	4.67	1.04	7.40	15.85
Inhibitory	4.61	1.04	7.30	23.15
Lab	4.21	0.90	6.67	29.81
Combined	4.00	0.89	6.33	36.15
None	3.78	0.83	5.99	42.14
Biochemical	3.78	0.85	5.98	48.12
Field	3.72	0.85	5.88	54.01
Colony health	3.69	0.88	5.85	59.85
Field sampling studies	3.49	0.78	5.52	65.37
Longevity	3.33	0.80	5.27	70.64
Improbable	3.05	0.75	4.83	75.47
Brood	2.44	0.68	3.86	79.32
Molecular	2.17	0.59	3.44	82.76
Acute mortality	1.84	0.51	2.91	85.67
Other on bees	1.77	0.51	2.81	88.48
Foraging/flight path	1.76	0.46	2.79	91.26

Year category 1 v 3

Overall average dissimilarity (%) = 64.46

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Inhibitory	5.57	1.30	8.64	8.64
Lab	5.31	1.22	8.24	16.88
Probable	4.57	0.97	7.09	23.97
Improbable	4.55	1.06	7.06	31.03
Biochemical	4.14	0.91	6.42	37.44
Other on bees	4.08	0.89	6.33	43.78
Residue analyses	3.57	0.84	5.54	49.31
Indirect	3.40	0.76	5.28	54.59
Combined	3.38	0.82	5.25	59.84
Stimulatory	3.24	0.85	5.02	64.86
Field	2.90	0.66	4.50	69.36
Acute mortality	2.74	0.68	4.26	73.61
Foraging/flight path	2.66	0.71	4.12	77.74
Colony health	2.53	0.67	3.93	81.66
None	2.11	0.55	3.27	84.93
Other	1.91	0.47	2.96	87.89
Semi-field	1.66	0.51	2.58	90.47

Year category 2 v 3

Overall average dissimilarity (%) = 63.10

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Residue analyses	5.21	1.11	8.25	8.25
Probable	4.87	0.99	7.71	15.96
Inhibitory	4.81	1.02	7.62	23.58
Lab	4.73	1.02	7.50	31.08
Combined	4.46	0.94	7.07	38.16
Field	3.97	0.85	6.29	44.45
Colony health	3.85	0.86	6.11	50.55
None	3.74	0.77	5.92	56.48
Biochemical	3.69	0.81	5.85	62.32
Field sampling studies	3.27	0.72	5.19	67.51
Indirect	2.90	0.66	4.60	72.11
Longevity	2.85	0.72	4.51	76.62
Foraging/flight path	2.42	0.55	3.83	80.45
Brood	2.21	0.62	3.50	83.95
Other	2.06	0.48	3.26	87.21
Other on bees	1.87	0.47	2.97	90.18

Year category 4 v 3

Overall average dissimilarity (%) = 62.69

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Lab	5.73	1.18	9.15	9.15
Probable	4.64	0.97	7.41	16.55
Biochemical	3.93	0.86	6.26	22.81
Improbable	3.86	0.83	6.15	28.97
None	3.85	0.81	6.15	35.11
Residue analyses	3.84	0.81	6.13	41.24
Combined	3.83	0.83	6.11	47.35
Inhibitory	3.83	0.78	6.11	53.46
Molecular	3.09	0.73	4.94	58.39
Other on bees	3.05	0.67	4.87	63.27
Field sampling studies	3.05	0.66	4.86	68.13
Longevity	2.97	0.69	4.74	72.87
Indirect	2.53	0.59	4.04	76.90
Acute mortality	2.48	0.59	3.95	80.86
Other	2.27	0.50	3.62	84.48
Colony health	1.92	0.49	3.07	87.55
Foraging/flight path	1.58	0.47	2.52	90.07

Appendix C: SIMPER results reporting average between-category dissimilarity for different endpoints examined across four year categories (2000-2004, 2005-2009, 2010-2014, and 2015-2019). The overall average dissimilarity for a year category is for the assemblage of literature variables, whereas the average dissimilarity within the table is for a given literature variable. Diss/SD is the ratio of the average dissimilarity to standard deviation of the dissimilarities for each literature variable; a ratio ≥ 1 indicates a consistent contribution, whereas a ratio < 1 indicates variability in the contribution. The contribution of that variable to the overall average dissimilarity is indicated next. The cumulative contribution simply accumulates the contribution values.

Year category 1 v 2

Overall average dissimilarity (%) = 78.45

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Residue analyses	12.90	1.12	16.44	16.44
Biochemical	10.53	0.88	13.42	29.86
Other on bees	9.71	0.82	12.38	42.24
Colony health	9.36	0.89	11.93	54.17
Indirect	8.20	0.65	10.45	64.63
Foraging/flight path	7.57	0.64	9.65	74.28
Brood	6.37	0.70	8.12	82.40
Acute mortality	5.55	0.62	7.07	89.47
Longevity	5.48	0.59	6.99	96.46

Year category 1 v 4

Overall average dissimilarity (%) = 81.41

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Biochemical	11.40	0.89	14.00	14.00
Other on bees	11.08	0.87	13.61	27.61
Acute mortality	8.71	0.72	10.70	38.31
Residue analyses	8.42	0.70	10.34	48.65
Indirect	8.08	0.59	9.92	58.57
Colony health	6.91	0.69	8.49	67.06
Longevity	6.09	0.57	7.49	74.55
Foraging/flight path	6.00	0.65	7.36	81.91
Learning	5.38	0.53	6.61	88.52
Gene expression	5.21	0.57	6.39	94.91

Year category 2 v 4

Overall average dissimilarity (%) = 81.44

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Residue analyses	15.10	1.16	18.54	18.54
Biochemical	10.61	0.82	13.03	31.56
Colony health	10.00	0.84	12.27	43.84
Longevity	9.54	0.77	11.72	55.56
Brood	6.34	0.67	7.78	63.34
Foraging/flight path	5.95	0.44	7.31	70.64
Acute mortality	5.78	0.48	7.09	77.74
Molecular	5.50	0.57	6.75	84.49
Other on bees	4.69	0.50	5.76	90.24

Year category 1 v 3

Overall average dissimilarity (%) = 78.99

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Biochemical	11.71	0.88	14.82	14.82
Other on bees	11.65	0.85	14.75	29.57
Residue analyses	10.44	0.79	13.22	42.80
Indirect	10.22	0.70	12.94	55.74
Foraging/flight path	7.37	0.70	9.33	65.07
Acute mortality	7.27	0.66	9.20	74.28
Colony health	7.26	0.64	9.19	83.47
Learning	3.90	0.45	4.93	88.40
Molecular	3.59	0.47	4.54	92.94

Year category 2 v 3

Overall average dissimilarity (%) = 77.79

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Residue analyses	14.76	1.04	18.98	18.98
Colony health	10.54	0.81	13.54	32.52
Biochemical	10.42	0.78	13.40	45.92
Indirect	8.32	0.64	10.70	56.62
Longevity	7.77	0.69	9.99	66.61
Foraging/flight path	7.69	0.52	9.88	76.50
Brood	5.60	0.62	7.20	83.70
Other on bees	5.42	0.46	6.97	90.67

Year category 4 v 3

Overall average dissimilarity (%) = 82.99

Literature variable	Average dissimilarity (%)	Diss/SD	Contribution (%)	Cumulative contribution (%)
Residue analyses	12.37	0.76	14.90	14.90
Biochemical	11.07	0.83	13.34	28.24
Other on bees	9.19	0.64	11.08	39.32
Longevity	8.79	0.67	10.59	49.91
Molecular	8.43	0.71	10.16	60.07
Acute mortality	8.17	0.55	9.84	69.91
Indirect	7.43	0.57	8.95	78.86
Colony health	6.08	0.46	7.33	86.18
Learning	4.87	0.43	5.87	92.05