A review of pesticide effects on sensitive receptors: pollinators (draft)

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INTRODUCTION

Pollinators, in particular bees, are critically important in sustaining biodiversity by providing essential pollination for a wide range of crops and nature plants. They contribute to human wealth and wellbeing directly through the production of nutritious food, honey and other feed supplies such as: pollen, wax for food processing, propolis in food technology, and royal jelly as a dietary supplement and ingredient in food. United Nations (FAO) estimated that of the 70% crop species that provide 90% of food worldwide are pollinated by bees.

Before the turn of this century, beekeepers around the world have been reporting the ongoing weakening of honeybees (*Apis mellifera*) health and subsequently the increasing colony losses. However, it was not until the abrupt emergence of colony collapse disorder (CCD) in the United States in 2006 (vanEngelsdorp et al. 2007, 2008) that has raised the concern of losing this important perennial pollinator on the global scale. A recent United Nations report highlighted the persistence of CCD worldwide (United Nations 2011) and called for changes in honeybee colony management in order to save this important insect. CCD is a symptomatic disease and commonly characterized by the sudden disappearance of adult honeybees from hives containing adequate food (e.g. honey, nectar, and pollen). It is generally agreed that some losses of bee colonies during winter is common in apiculture, however, never in the history of beekeeping has the losses of honeybee hives occurred in such magnitude, over such a widely distributed geographic area, and lasting for many years.

In light of the important ecological and economic value of pollinators, there is a need to take immediate action to identify man-made factors associated with the declining numbers of pollinators in order to sustain crop production and environmental conservation. While the prevailing opinions in U.S. suggest the linkage of CCD to multi-factorial causes including pathogen infestation, beekeeping practices (including malnutrition), and pesticide exposure in general (Cox-Foster et al., 2007; Blanchard et al., 2008; Higes et al., 2008; vanEngelsdorp et al., 2009; Alaux et al., 2010; de Miranda et al., 2010; Williams et al., 2010; Di Prisco et al., 2011; Vidau et al., 2011; USDA 2013), recent scientific findings linking declines of bee colonies with exposure to pesticides, in particular to the systemic neonicotinoid insecticides, appear to be gaining traction (Maini et al., 2010; Pareja et al., 2011; Lu et al., 2012; Farooqui 2013; Takashi 2013), and have led to new regulatory control in the European Union (Erickson 2012).

In this report, we provide a summary review of the effects of pesticides on pollinators' health from a list of papers published in peer-review scientific journals. We used the following procedures to identify relevant papers. We first conducted a literature search on PubMed using

the combined key words of “pollinators and pesticides”, which yields 70 papers, as of January 31st, 2014. We supplemented the literature listed in PubMed by a cross-reference check with the Report titled *“Existing Scientific Evidence of the Effects of Neonicotinoid Pesticides on Bees”* (Grimm et al. 2012). This report was a result of a request made by European Parliament in preparation to fulfill their regulatory mandate on the issue of protecting pollinators among their membership nations. This cross-reference check yielded additional 47 papers to the final list. We then excluded papers from this summary review report if; a) papers do not contain either pesticide exposure or toxicological endpoint data in associated with pollinators, b) papers only included flies or beetles as the study insects, c) papers reported the use of pesticides that are not registered to be used in the United States, and d) papers were not written in English. At the end, we have identified 30 papers, as listed in Tables 1-4 that are relevant to examine the effects of pesticide exposure on the health honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*).

PESTICIDE EXPOSURE ASSESSMENT IN BEES

It is well documented in the literature that bees are constantly being exposed to a very long list of pesticides. Those pesticides that are either brought back by bees from the outside foraging environment or applied by the beekeepers for treating infectious diseases could inadvertently harm the health of bees and the whole colony. A 2010 study published by Mullin et al. has demonstrated the magnitude of pesticide contamination in bee hives, and would help to differentiate the sources of pesticides found in the hives. They have analyzed hundreds of pollen, wax, foundation, and immature (brood) and adult bee samples for approximately 120 pesticides. Those “convenient” samples were collected as part of different studies and epidemiological surveys to investigate possible threats of pesticides to colony health, specifically CCD. Unfortunately, Mullin et al. did not include data showing the comparison of pesticide residues in hives with and without CCD symptoms were made.

Regardless, Mullin et al. have shown that hives treated with common miticides, such as fluvalinate, coumaphos, and amitraz, are often detected with much higher levels of residues inside the hives. The finding of 98% of comb and foundation wax samples contained up to 204 and 94 ppm of fluvalinate and coumaphos, respectively, is very alarming comparing to the national average of up to 12ppb of coumaphos and fluvalinate in the survey of US honey. Accordingly, the persistent exposure to those three pesticides has led to the development of resistance by *Varroa* mites in bees. The huge concentration gap of fluvalinate and coumaphos between honey and comb/wax samples has three implications. First, it indicates the excessive

use of both pesticides by beekeepers over the years, probably for battling the worsening *Varroa* mite infestation. Second, the intention of applying miticides to control or prevent pathogen infestation in hives is not only counter-effective but could lead to a more serious mite infestation problem in the future as well, because of the resistant development by those pathogens. Last, the high levels of fluvalinate and coumaphos residues found in the hives could no doubt put additional pressure on bees' health. Mullin et al. stated in the paper that fluvalinate has long been considered a relatively "safe" pesticide for honey bees at the LD_{50} level of of 65.85 µg/bee. However, US EPA in 1995 reported the LD_{50} of fluvalinate as 0.2 µg/bee, a 330-fold increase of its acute toxicity in bees. Those implications highlight the extreme challenge for the survivals of bees because of the extensive exposure to various agrochemicals and the worsening mite infestation problem. Chauzat et al. (2006) also reported coumaphos and fluvalinate residues were the most commonly detected pesticide residues inside the hives with average concentrations of 925 and 487 ppb, respectively.

Pesticide residue measured in pollen samples might be a more realistic matrix for assessing pesticide exposure in bees during foraging activities. Also, data from pollen samples could help us to establish the field-realistic pesticide exposure levels encountered by bees. Besides high levels of fluvalinate and coumaphos, Mullin et al. found approximately 100 pesticides in the stored pollen samples, including systemic pesticides (concentrations in ppb of low to high), such as azoxystrobin (1-107), trifloxystrobin (1-264), propiconazole (3-361), thiacloprid (2-115), acetamiprid (14-134), and imidacloprid (6-206). A comparable study published by Krupke et al. (2012) also demonstrated that bees living and foraging near agricultural fields, specifically corn field, are exposed to pesticides in several ways throughout the foraging seasons in Indiana. During spring, extremely high levels of clothianidin and thiamethoxam were found in planter exhaust material produced during the planting of neonicotinoids-treated maize seeds. When maize plants reached anthesis, maize pollen from treated seed was found to contain clothianidin and other pesticides; and those contaminated pollen is readily available for honey bees to collect. Krupke et al. showed that 3 of 20 and 10 of 20 pollen samples collected directly from bees using a pollen trap contained thiamethoxam and clothianidin, respectively. Fungicides were also frequently detected: azoxystrobin and propiconazole were found in all pollen samples, while trifloxystrobin was found in 12 of the 20 samples analyzed. Concentrations (µg/g) of thiamethoxam, clothianidin, trifloxystrobin, azoxystrobin and propiconazole in pollen collected from returning bees of hives placed adjacent to maize fields planted with treated seeds ranged from non-detected to 7.4, non-detected to 88, non-detected to 9.8, 4.3 to 66, and 3.2 to 23.8, respectively. Bernal et al. (2010) reported more

than 30% of stored pollen samples contained multiple pesticides that their concentrations ranging from low ppb to low ppm levels.

The concern of pesticide contamination is not limited to pollen or nectar that bees have access to. While foraging for pollen and nectar, bees often look for water on the ground puddles. One accessible and alternative source of water for bees is the leaf guttation drops. Girolami et al. (2009) showed that by growing corns from neonicotinoid-coated seeds coated with 4 different neonicotinoids at the rage of 0.5-1.25 mg/seed, they reported the leaf guttation drops germinated from those seeds containing neonicotinoids at the ppm levels, with maximum concentrations of up to 100 ppm for thiamethoxam and clothianidin, and up to 200 ppm for imidacloprid. Those levels were approximately 5-6 orders of magnitude higher than concentrations found in pollen or nectar, and so acutely toxic that bees were found dead after minutes of consuming those guttation drops. Girolami et al. raised the concern of the contamination of a source of water for bees, and likely for other pollinators, by neonicotinoids at the levels as shown in their study. By taking into account the persistence of those dangerously high levels of neonicotinoids and the wide planting of those neonicotinoids-coated seeds, Girolami et al. stated that this is a threatening scenario for bees and other pollinators, and does not comply with an ecologically acceptable situation.

It is conceivably difficult to compare pesticide levels in samples collected from bees and their hives across studies because many factors would affect the final concentrations in those samples. Therefore, the attempt to quantitatively assess the “field-realistic” pesticide exposures in bees is a foreseeable challenging task. If the field-realistic levels for a certain pesticide that bees would encounter in the environment were to be existed, it is likely to encompass a very wide range of concentrations. The data presented in the above studies would support this conclusion. While the interest of this work is to identify the risk of the declining bee population associated with pesticide exposures, regardless of the levels, this review will focus on sub-lethal exposure to pesticides that are commonly present in bees’ foraging environment.

THE ASSOCIATION OF PESTICIDE EXPSOURE AND ADVERSE HEALTH OUTCOMES IN HONEYBEES (*Apis mellifera*)

The majority of literature linking the adverse health effects of pesticides to honey bee did not exist until 2011, several years after the emergence of CCD. It might signal the ignorance of pesticide exposure and adverse effects in honeybees in the research and regulatory communities. An earlier study published by Mayes et al. (2003) have shown that spinosad, an insecticide derived from the bacterial species Saccharopolyspora spinosa, has low risk to adult

honeybees and has little or no effect on hive activity and brood development. Spinosad residues that have been allowed to dry in all experimental conditions for 3 hr were not acutely harmful to honeybees when low-volume and ultralow-volume sprays are used. No studies linking pesticide exposure to adverse health effects in honeybees were published until 2009.

Brood development and adult bee longevity

Wu et al. (2011) had shown that worker bees reared in brood comb containing high levels of many pesticides experienced multiple health effects of reduced adult longevity, increased brood mortality, delayed larval development, and higher fecundity of *Varroa* mites. Delayed development was observed in the early stages (day 4 and 8) of worker bee development that leads to reduced adult longevity by 4 days in bees exposed to pesticides in during development. As observed by the authors that pesticide residue migrated from comb containing high pesticide residues to the control combs after multiple brood cycles causing higher brood mortality and delayed adult emergence in bees reared in those control comb. Subsequently, survivability increased in bees reared in treatment comb after multiple brood cycles when pesticide residues had been reduced in treatment combs due to the migration into untreated control combs. Medrzycki *et al.* (2010) demonstrated a link between the quality of the brood rearing environment and both the reduction in longevity and the susceptibility to insecticides in adult honeybees emerging from their larvae. They reported that by lowering the brood rearing temperature 2°C from the optimal 35°C, it strongly affected adult honeybees' mortality and their susceptibility to dimethoate, an organophosphate insecticide.

Since it is well known that the physiology of adult honeybees can be affected by the health of their larvae and/or pupae, it implies that less than optimal brood rearing environment, such as temperature inside the hive and pesticides, could deteriorate the health of adult bees starting in the larval stage.

Foraging Difficulty

Henry et al. (2012) tested the hypothesis that a sub-lethal exposure to thiamethoxam indirectly increases hive death rate through homing failure in foraging honey bees. They simulated daily intoxication events that bees would have received by a field-realistic, sub-lethal dose of 0.07ppb of thiamethoxam (a real dose of 1.34 ng in a 20-ml sucrose solution). Bees were then released away from their colony with a microchip glued on their thorax so they can be monitoring by a radiofrequency identification (RFID) readers placed at the hive entrance. Mortality due to post-exposure homing failure was then derived from the proportion of non-

returning foragers and corrected by data from non-treated bees for other causes of homing failure in treated foragers—such as natural mortality, predation, or handling stress. The results demonstrated substantial mortality due to postexposure homing failure with the proportion of treated bees returning to the colony being significantly lower than that of control foragers ($p<0.05$). It is estimated that 10 to 32% of thiamethoxam treated bees would have failed to return to their colonies when foraging in treated crops on a daily basis.

Schneider et al. (2013) used the similar RFID technique to monitor the foraging behavior of honeybees after the treatment of sub-lethal doses of imidacloprid (0.15–6 ng/bee) and clothianidin (0.05–2 ng/bee) under field-like circumstances.They found both imidacloprid and clothianidin could lead to a significant reduction of foraging activity and to longer foraging flights at doses of >0.5 ng/bee (0.02ppb assuming each bee weight 30mg) for clothianidin and >1.5 ng/bee (0.06ppb) imidacloprid during the first three hours after treatment. In the trials conducted with imidacloprid at 3ng and clothianidin at 2ng, only 25% and 21% of bees returned to the hives during a 3-hour observation period immediately after treatment, respectively. Conversely, almost all bees in the control groups and groups treated with lower doses returned. Among the bees that were not returned, they observed reduced mobility, followed by a phase of motionlessness with occasional trembling and cleaning movements, moving around with an awkwardly arched abdomen, or sometimes followed by a phase of turning upside down and lying on the back with paddling leg movements.

Results from both studies using the same tool consistently demonstrated the abnormal foraging activities, or homing difficulties, in bees exposed to field-realistic levels of thiamethosam, imidacloprid, or cloathinidan, the 3 most commonly used neonicotinoid insecticides in the world. We can assume with a great confidence that bees that do not return to their hives within the three-hour period after leaving would not be able to survive, and are most likely died.

Cognition/Neurological Impairment

Sub-lethal exposure to neonicotinoids has been shown to disrupt honeybee learning and behavior, such as the abnormal foraging activities described previously, the neurological mechanism of these effects is not yet known. Palmer et al. (2013) have shown that using recordings from mushroom body Kenyon cells (KC) in acutely isolated honeybee brain, imidacloprid (50nM–10μM), clothianidin (200 nM), and the oxon metabolite of organophosphate miticide coumaphos (50nM–1μM), can cause a depolarization-block of neuronal firing and inhibit nicotinic responses. These effects are observed at the concentrations (50nM-10 μM) that are

encountered by foraging honeybees and within the hive, and are additive with combined application. Those new findings provided a neuronal mechanism that may account for the cognitive impairments caused by neonicotinoids and miticides commonly used in hives. It also demonstrate the cumulative effects on targeted cholinergic inhibition caused by multiple pesticides that bees are exposed to, and therefore will cause enhanced toxicity to bees.

Very similar finding and conclusion of exposure to field-realistic concentrations (10 and 100nM) of imidacloprid, coumaphos, and their combination impaired olfactory learning and memory formation in honeybees was made by Williamson and Wright (2013). In this experiment, Williamson and Wright (2013) combined imidacloprid, a neonicotinoid pesticide, with coumaphos, an acetylcholinesterase (AChE) inhibitor, to simulate the situation where honeybees are exposed to pesticides in food and to miticides applied within the colony. They found that either imidacloprid or coumaphos has specific cholinergic effects on learning or memory. Bees exposed to imidacloprid were less likely to form a long-term memory, whereas bees exposed to coumaphos were only less likely to respond during the short-term memory test. When bees exposed to the combination of these two pesticides, the additive responses were observed. The results from this study have demonstrated that exposure to sub-lethal doses of combined cholinergic imidacloprid and coumaphos significantly impairs important behaviors involved in foraging, implying that pollinator population decline could be the result of a failure of neural function of bees exposed to pesticides in agricultural landscapes.

Results from both studies also consistently demonstrated the impairment of neurophysiological functions in bees when they exposed to sub-lethal levels of imidacloprid, a very common neonicotinoids found in the foraging environment, and coumaphos, a miticide commonly used by beekeepers. Subsequently, the impaired neurophysiological functions lead to learning, behavior, and foraging problems in bees.

Immune Suppression

Vidau et al. (2011) reported a synergistic effect of Nosema ceranae infection and sub-lethal insecticide exposure on honeybee mortality in a laboratory incubator setting. Honeybees were experimentally infected with spores of N. ceranae in the lab and then exposed to fipronil at 1ppb, thiacloprid at 5.1ppm, or untreated. They found exposures to fipronil and thiacloprid had no effect on the mortality of uninfected honeybees compared to the untreated control group over the duration of experiments. However, honeybees infected with N. ceranae and then exposed to insecticides died significantly earlier than bees only infected with N. ceranae but no pesticide treatment.

The finding of synergistic effect as reported by Vidau et al. (2011) can be explained by Alaux et al. (2010) in which they showed the interaction between the microsporidia Nosema and imidacloprid significantly increased susceptibility of the colony to pathogens. Alaux et al. demonstrated that by quantifying the strength of immunity at both the individual and social levels, the activity of glucose oxidase, enabling bees to sterilize colony and brood food, was significantly decreased only by the combination of both factors compared with control, Nosema or imidacloprid groups. The doses of imidacloprid used in this study ranged from 0.7 to 70ppb.

The interaction of Nosema infection and sub-lethal neonicotinoids exposure on honeybee was further validated in a study conducted by Pettis et al. (2012). They exposed honeybee colonies during three brood generations to imidacloprid at 5 and 20ppb mixed in the protein patties, and then subsequently challenged newly emerged bees with the gut parasite, Nosema spp. They found Nosema infections increased significantly in the bees from pesticide-treated hives when compared to bees from control hives demonstrating an indirect effect of pesticides on pathogen growth in honey bees. In addition to the interaction with imidacloprid, Pettis et al. (2013) found that fungicide exposure could also increase the probably of Nosema infection in bees consumed pollen with a higher fungicide loads. This finding is not consistent to the prior knowledge among beekeepers and bee researchers that fungicides are typically seen as fairly safe for honey bees. They used pollen traps to collect pollen pellets from bee's corbiculae before entering their hives. They detected 35 different pesticides in the sampled pollen, and found high fungicide loads. Azoxystrobin, a systemic fungicide, is the most commonly detected fungicide in their pollen samples with mean and the maximum concentrations of 60 and 332ppb, respectively. The insecticides esfenvalerate (216ppb) and phosmet (14,700ppb) were at the concentrations higher than their median lethal dose in at least one pollen sample. Those pollen data are useful as the supplement to those reported by Mullin et al. (2010) and Krupke et al. (2012).

The increasing prevalence of N. ceranae in honeybee colonies combined with the ubiquitous presence of multiple pesticides in pollen that worker bees collected from their foraging environment, the finding of synergistic effects of pesticides on bees that are infected with Nosema appears to contribute to the declining numbers of honeybee colony.

Colony Collapse Disorder (CCD)

Although numerous papers that are previous discussed in this review have linked sub-lethal pesticide exposures, along with its synergistic effect on Nosema infection, to CCD in their studies, none of them has demonstrated the exact post-mortem observations that are consistent

to CCD. CCD is commonly characterized by the sudden disappearance of worker bees from hives containing adequate food and various stages of brood in winter. This *in situ* study conducted by Lu et al. (2012) was aimed to replicate CCD based on a plausible mechanistic hypothesis in which the occurrence of CCD. They used a replicated split-plot design consisting of 4 independent apiary sites, and each apiary consisted of 4 different imidacloprid-treated hives and a control hive. The dosages used in this study (20, 40, 200, and 400ppb of imidacloprid in ½ gal. of high fructose corn syrup, HFCS) were determined to reflect imidacloprid levels reported in the environment previously. All hives were healthy and had no diseases of symptoms of parasitism during the 13-week dosing regime, and were alive 12 weeks afterward. However, 15 of 16 imidacloprid-treated hives (94%) were dead across 4 apiaries 23 weeks post imidacloprid dosing. Dead hives were remarkably empty except for stores of food and some pollen left, a close resemblance of CCD. The survival of the control hives managed alongside with the pesticide-treated hives unequivocally augments the conclusion that is sub-lethal imidacloprid exposure via HFCS intake caused CCD after several brood generations.

THE ASSOCIATION OF PESTICIDE EXPSOURE AND ADVERSE HEALTH OUTCOMES IN BUMBLEBEES (*Bombus spp.*)

Acute Toxicity/Direct Contact

Because the widespread use of pesticides in agricultural fields, Scott-Dupree et al. (2009) conducted a laboratory-based toxicological study to determine the acute contact toxicity of 5 common insecticides, imidacloprid, clothianidin, deltamethrin, spinosad, and novaluron on bumble bees [Bombus impatiens (Cresson)], alfalfa leafcutting bees [Megachile rotundata (F.)], and Osmia lignaria Cresson. They found clothianidin and imidacloprid are highly toxic to all three species, followed by deltamethrin and spinosad, and novaluron is non-toxic. Although they found bumblebees were generally more tolerant to pesticide toxicity by direct contact, this result is not consistent. To establish whether imidacloprid, a systemic neonicotinoid and insect neurotoxin, harms individual bees when ingested at environmentally realistic levels, Cresswell et al. (2012) exposed adult worker bumblebees to dietary imidacloprid in feeder syrup at dosages between 0.08 and 125ppb. They found bumblebees progressively developed over time a dose-dependent reduction in feeding rate with declines of 10-30% 10ppb, but neither their locomotory activity nor longevity varied with diet.

The results from the acute toxicological testing of various pesticides in bumblebees are not in consistent to those with honeybees for reasons that needed to be elucidated.

Colony Vitality/Brood Development

Gels et al. (2002) reported the effects of imidacloprid, chlorpyrifos, carbaryl, and cyfluthrin on native pollinators, specifically bumble bees after the applications on turf where they forage on the weed flowering. This is the earliest study aiming to quantify the toxicity of pesticides in pollinators. They measured colony vitality including numbers of brood, workers, and honey pots, and weights of queens, workers, and whole colonies with hives after a period of 14-30 days post-application. They found non-irrigated, or dry residues for all the test pesticides were detrimental to colony vitality for bumblebees, whereas toxicity to bumble bee colonies was abated when the field is irrigated followed by pesticide application. Regardless the methods of application, Gels et al. found that foraging workers did not avoid pesticide-treated field. Similar to the findings published by Mayer et al. (2003), Morandin et al. (2205) demonstrated minimal adverse health effects, including adult mortality, brood development, weights of emerging bees and foraging efficiency of adults, of spinosad insecticide to bumble bee colonies at the concentrations of 0.2-0.8 mg/kg. At more realistic concentrations there were potentially important sub-lethal effects. However, they found adult worker bees exposed to spinosad during larval development at 0.8 mg kg(-1) were slower foragers than bees from low or no spinosad treated colonies.

Whitehorn et al. (2012) conducted a study to simulate the likely effects in wild bumble bee colony to imidacloprid present on the flowers of imidacloprid-treated rapeseed. Colonies received either control, low (0.7-6 µg/kg), or high (1.4-12 µg/kg) for 14 days before they were placed in the field, where they were left to forage independently for a period of 6 weeks. They found bumblebees in imidaclprid-treated colonies gained significantly less weights and produced less numbers of queens than those in the control colonies. Laycock and Cresswell (2012), however, provided a rather conflict results of imidacloprid's effects on brood development in bumblebees. They assessed the amount of brood (number of eggs and larvae) using a pulsed exposure regime in which bees received imidacloprid doses up to 98 µg/kg 14 days (on dose) followed by 14 days 'off dose' in small experimental colonies consisting a queen and four adult workers. They found a dose-dependent repression of brood production with productivity decrease during the "on-dose" period, followed by a dose-dependent recuperation during the "off-dose" period. In continuing this work, Laycock et al. (2013) examined the effects of another neonicotinoids, thiamethoxam's effects on bumblebees to a range of dosages up to 98 µg/kg in syrup for 17 days. They showed that bumblebee workers survival was shortened by fewer days and the production of brood (eggs and larvae) and consumption of syrup and pollen

in microcolonies were significantly reduced by thiamethoxam at the two highest concentrations, 39 and 98 µg/kg, whereas no detectable effects of thiamethoxam at levels between 1 and 11 µg/kg. By comparison to previously published data, they concluced that brood production in worker bumble bees is more sensitive to imidacloprid than thiamethoxam.

Finally, Smagghe et al. (2013) demonstrated an exposure-route dependent toxicity of chlorantraniliprole, an insecticide, in bumblebee workers and their offspring. They showed that while a risk assessment test demonstrated that contact and pollen exposure at 0.4ppm level had no effect on bumblebee worker survival, oral exposure via sugar water caused both acute and chronic toxicity. The most significant sub-lethal effect was on reproduction in colonies orally exposed to pollen treated with chlorantraniliprole.

Foraging Impairment

Gill et al. (2012) showed that chronic exposure of bumblebees to these two insecticides at levels close to field-level exposure impairs natural foraging behavior and leading to significant reductions in brood development and colony success. They have demonstrated that sub-lethal exposure to imidacloprid at 10ppb level causes impairment to pollen foraging efficiency, leading to increased colony demand for food as shown by increased worker recruitment to forage. Consequently, it appeared to affect brood development due to a higher number of workers undertaking foraging. This resulted in reduced worker production, which can only exacerbate the problem of having an impaired colony workforce. These findings show a mechanistic explanation to link effects on individual worker behavior and colony queen production, as a result of neonicotinoid exposure. Moreover, exposure to a second pesticide λ-cyhalothrin (pyrethroid) applied at label guideline concentration for crop use caused additional worker mortality in this study highlighting a synergistic risk. In this study, colonies exposed to combined imidacloprid and λ-cyhalothrin were consistently negatively affected in all measures of worker behavior, suffered the highest overall worker losses.

Feltham et al. (2014) reported a consistent finding of Gill et al. (2012) on the impairment of pollen collection efficiency as a result of imidacloprid exposure in bumblebees. They used the RFID technology to determine whether bumblebee workers's foraging efficiency could be reduced by exposure to imidacloprid at the field-realistic levels (0.7 ppb in sugar water and 6 ppb in pollen). They found imidacloprid-treated bees brought back pollen less often than control bees (40% vs. 63 % of trips, respectively) and where pollen was collected, treated bees brought back 31% less pollen per hour than controls. However, the nectar foraging efficiency of bees treated with imidacloprid was not significantly different than that of control bees.

The consistent findings reported by Gill et al. (2012) and Feltham et al. (2014) provide an unequivocal evidence of foraging impairment caused by sub-lethal levels of imidacloprid. The synergistic effects caused by neonicotinoids and other pesticides not only are common for bees foraging in the environment, but also increase the propensity of colonies to fail. This finding is also true in honeybees.

THE ASSOCIATION OF PESTICIDE EXPSOURE AND ADVERSE HEALTH OUTCOMES IN OTHER BEES (*Hymenoptera: Apidae: Meliponinae, Osmia lignaria*)

Abbott et al. (2008) examined the lethal and sub-lethal effects of imidacloprid on and clothianidin on Osmia lignaria (Cresson) and Megachile rotundata (F.) (Hymenoptera: Megachilidae), respectively, by exposing their larvae to control, low (3 or 6 ppb), intermediate (30 ppb), or high (300 ppb) doses in pollen. They found no lethal effects for imidacloprid or clothianidin on O. lignaria and M. rotundata and minor sub-lethal effects on larval development for O. lignaria, with greater developmental time at the intermediate (30 ppb) and high doses (300 ppb) of imidacloprid. Tomé et al. (2012) studied native stingless bees (Hymenoptera: Apidae: Meliponinae) which are key pollinators in neotropical areas and threatened with extinction due to deforestation and pesticide use. They assessed the effects of imidacloprid ingestion by stingless bee larvae on their survival, development, neuromorphology and adult walking behavior. Survival rates above 50% were only observed at insecticide doses lower than 0.0056 µg active ingredient (a.i.)/bee. Although no sub-lethal effect on body mass or developmental time was observed in the surviving insects, they found imidacloprid negatively affects the development of mushroom bodies in the brain and impairs the walking behavior of newly emerged adult workers. These findings demonstrate the lethal effects of imidacloprid on native stingless bees and provide evidence of novel serious sublethal effects that may compromise colony survival.

Both Rossi et al. (2013) and Catae et al. (2014) showed the effects of neonicotinoids, imidacloprid and thiamethoxam, in the non-target organs of Africanized Apis mellifera. Catae et al. examined the midgut and Malpighian tubule cells of Africanized Apis mellifera in the newly emerged workers in which they were exposed to a diet containing a sub-lethal dose of 0.0428 ng a.i./L until 8 days. They found thiamethoxam is cytotoxic to midgut in which the damage was more evident in bees exposed to thiamethoxam on the first day. However the damage was repaired on the eighth day. On the other hand, the Malpighian tubules showed pronounced alterations on the eighth day of exposure. This study, along with Rossi et al., demonstrates that

the continuous exposure to a sub-lethal dose of thiamethoxam can impair organs that are used to metabolize thiamethoxam, and very likely other pesticides.

POLICY IMPLICATION SYNTHESIS

The ecological and economic importance of pollinators deserves a thorough evaluation on the causes of recent steep declining of their populations. The weight-of-evidence of this review clearly highlights bees' susceptibility to insecticides, specifically the neonicotinoids, and the synergistic effects to diseases that are commonly present in bee colonies. One important aspect of assessing and managing the risks posed by insecticides to bees is the chronic effects induced by exposures at the sub-lethal levels. Majority of literature published after 2009 directly or indirectly imply the adverse health effects associated with sub-lethal exposure to pesticides. Different to the consequence of lethal level exposure, many outcomes, including abnormal foraging activities, impaired brood development, neurological or cognitive effects, and CCD, could result from chronic sub-lethal exposure to pesticides in bees. Therefore, it is a very challenging task to protect pollinators from exposing to lethal and sub-lethal levels of pesticides.

While it is relatively straightforward to define the sub-lethal levels (the dosage would not kill bees right away), it might be problematic to establish the field-realistic exposure levels for pesticides. As many investigators claimed that their dosages used in the experiments are field-realistic exposure levels, those levels actually encompass a wide range of concentrations for each individual pesticide. Establishing the field-realistic exposure levels may not be possible and relevant to the paradigm since so many factors would affect or modify the levels of pesticides in the foraging environment where bees would encounter. For instance, imidacloprid levels in pollen collected from imidacloprid-treated corn seeds would be several orders of magnitude higher than pollen from dandelion flowers in which the main source of imidacloprid residue is from soil. Therefore, the field-realistic levels have very little significance to the risk management of protecting pollinators.

It should be mindful when interprets the outcomes cross bee species, and to other pollinators. Honeybee (*Apis mellifear*), perhaps, is a very unique insect among the pollinator family mainly because they are perennial social insects. In other words, research findings obtained from bumblebees may not be directly applied to honeybees, or vice verse. Social bee colonies depend on the collective performance of numerous individual workers. So while field-level pesticide concentrations can have a sub-lethal effect at the individual bees (such as foraging difficulty, cognition impairment, etc.), it is not known whether it could result in a severe cumulative effect at the colony level, until the emergence of CCD in 2006. The abatement of hives by adult honeybees during the winter months seems to suggest a detrimental effect of sub-lethal pesticide exposures in the colonies. The rising awareness of protecting honeybees

and other pollinators worldwide is directly related to the emergence of honeybee colony collapse disorder, or CCD. The lack of recognition of the association with pesticides (specifically neonicotinoids) in the US, either deliberately or irrationally, may put additional pressure on the declining honeybee population. There are sufficient evidences in the literature that the detrimental effects of neonicotinoids not only affect the survival of honeybees, but all pollinators and wildlife animals as well. The recent regulatory control at EU on certain uses of neonicotinoids in agricultural crops is the first step toward protecting bee populations. More efforts are needed to prevent further losses of pollinator populations.

The extreme acute lethal toxicity of neonicotinoids is well known to USEPA, and therefore the continuing use of neonicotinoids, even when used in a manner consistent with label instructions, poses significant risks to bees, other natural pollinators, and wild birds. The recent loss of more than 50,000 bumblebees in Oregon as a result of tree spraying with the neonicotinoid dinotefuran by licensed applicators highlights this concern (http://www.foxnews.com/us/2013/06/28/memorial-to-honor-50000-bumble-bees-that-died-in-oregon-parking-lot/). Research into the sub-lethal toxicity of neonicotinoids, although not required by EPA during the registration, is most relevant to CCD and may pose the biggest risk to pollinators and wild birds. Since the neonicotinoids are systemic insecticides – infiltrating the entire plant including the pollen and nectar -- their spread cannot be contained or prevented even with the incorporation of best management practices. No matter how neonicotinoids are used, via seed treatment, soil drench, tree injection, or foliar application, they will be absorbed, translocated, and spread within and beyond the crops. With much longer half-lives than other insecticides, neonicotinoids have become ubiquitous and persist in the environment, accumulating in plants, soils, and water systems. Considering neonicotinoids' extended half-lives and the systemic property, it appears there is no safe level. This statement is in consistent with data presented in the literature review.

Since there are well-documented threats to bees, other invertebrates, and birds, it would be a missed opportunity not to take positive steps to intervene the deteriorating health of pollinators. Unfortunately, the federal regulatory agencies, namely USDA and US EPA only muddy the waters by focusing on complexities like land-use policies that are beyond any agency's jurisdiction. This is asking for policy stagnation and for an ongoing depletion of pollinator populations. The implementation of a sound pesticide risk management program at the state government is urgently needed so pollinators will not come in contact with those lethally dangerous neonicotinoid insecticides.

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