# Pesticides and Honey Bees:
## State of the Science

**MAY 2012**

**PESTICIDE ACTION NETWORK NORTH AMERICA**

## Pesticide Action Network North America

Pesticide Action Network North America (PAN North America) works to replace the use of hazardous pesticides with ecologically sound and socially just alternatives.

As one of five PAN Regional Centers worldwide, we link local and international consumer, labor, health, environment and agriculture groups into an international citizens' action network. This network challenges the global proliferation of pesticides, defends basic rights to health and environmental quality, and works to ensure the transition to a just and viable society.

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## Introduction

Honey bees and other pollinators are dying off at unprecedented rates around the world. First in France, then in the U.S. and elsewhere, colonies have been mysteriously collapsing with adult bees abandoning their hives. In 2006, two years after this phenomenon hit the U.S., it was named "Colony Collapse Disorder," or CCD. Each year since, U.S. beekeepers have reported annual hive losses of 29% - 36%. Commercial beekeepers tell us that their industry, which is the care and cultivation of an indicator species, is on the verge of collapse.

Honey bees pollinate 71 of the 100 most common crops that account for 90% of the world's food supply, making managed honey bees the most economically important pollinator.[i] In the U.S. alone, 2000 data from the U.S. Department of Agriculture (USDA) indicates that this industry was worth more than $15 billion per year twelve years ago.[ii] With the subsequent growth of U.S. agriculture and decline of natural pollinators, commercial beekeeping likely contributes much more than $15 billion to the U.S. economy now.

Claims of imminent food system collapse are not supported by pollination biologists, but without pollination, agriculture quickly becomes less efficient—requiring more land and water to grow the same amount of food—and our diets lose nutritionally vital variety. For instance, most fruits and many nuts rely on the pollination services of bees, including almonds, blueberries, apples and melons. Alfalfa, which is a primary feed source for dairy cows, relies on bees for pollination as well. Altogether, more than one in every three bites of food depends on honey bees for pollination.

While few contest that the recent, dramatic decline of honey bee populations presents serious challenges to an already-stressed food system, the public debate over what lies behind CCD is at this point so polarized and confusing that concerned citizens find it difficult to know how or where to intervene. Indeed, the debate over the causes of CCD has become a case study in public, scientific controversy[iii]. As with other recent matters of public and environmental health, such as the link between tobacco and cancer and anthropogenic climate change, this issue has become characterized by policymaker inaction in the face of irreducibly complex science.

Two increasingly intractable sides have emerged in this controversy: beekeepers and environmental health advocates vs. pesticide companies and the scientists supported by them. While PANNA's position in this line-up is clear enough, we have sought to hew to a commitment to non-partisan, scientifically literate public discourse. We believe that engaged forms of scientific citizenship are a vital part of democratic civic life, and a needed force in environmental decision-making. In cases such as this where the debate has become intractable and position-driven, as is reported by participants and scholars alike, the conversation between experts has clearly broken down. Historically, these kinds of logjams are broken either through concerted public demand, or through a catastrophic focusing or "triggering" event that compels policymaker action—or both in conjunction. With one-third of our bees dying off each winter and wild pollinators facing similarly catastrophic declines, it would seem that we have before us the focusing event. Needed now is public demand for policy action.

"The weight of evidence demonstrates that pesticides are indeed key in explaining honey bee declines, both directly and in tandem with the other two leading factors, pathogens and poor nutrition."

Thus, the purpose of this document is to inform public debate and build national will for policy action on a timeline that will be meaningful to bees and beekeepers. Our consideration of the evidence for the causes of bee decline and CCD is focused on pesticides as one of three leading factors identified by researchers. By our analysis, the weight of evidence demonstrates that pesticides are indeed key in explaining honey bee declines, both directly and in tandem with the other two leading factors, pathogens and poor nutrition. The science supporting this conclusion is presented in this report.

# Overview :: Colony Collapse Disorder in context

Honey bee populations have steadily declined in the U.S. since 1947 at a gradual rate averaging 1% per year[iv]. Steeper declines have been recorded since 1987, but the last four winters have seen extraordinary losses averaging 29 to 36% per year.[v,vi,vii,viii]

**What is Colony Collapse Disorder?**

Colony Collapse Disorder, or CCD, was first described in the U.S. in 2006. Its symptoms are distinct from other loss epidemics and include the following:

- Colonies found suddenly empty of adult bees, leaving their brood unattended
- No sign of dead bees
- No hive pests or food robbers, despite surplus honey and pollen stores
- Common parasites not present at levels thought to cause population decline

Most scientists agree that there is no single cause of CCD. Rather, recent population declines are likely caused by a combination of factors acting in concert to weaken bee colonies to the point of collapse;[ix] and emerging science points specifically to impaired immunity. Lead suspects in this causal complex include: nutritional stress, pathogens and pesticides.

- ***Nutritional stress ::*** Nutritional stress undermines colony health through a variety of mechanisms, including immune system harm and reduction in reproductive viability. One key component of nutritional stress for honey bees includes habitat loss that results in a less varied and therefore less nutritious diet. Habitat loss has been occurring steadily for the last 50 years with measurable effects on bee health. For instance, regional differences in ratios of open to developed land have been traced to higher colony losses. One key driver of recent habitat loss is the increased use of broad-spectrum herbicides that accompanies herbicide-resistant, genetically engineered crops.[x]

- ***Pathogens ::*** Pathogens like parasitic mites, viruses and a gut fungus have garnered the most media attention as causal factors in CCD. Multiple studies have confirmed, however, that there is no single pathogen associated with the disorder. In an analysis of studies published as of early 2009, two leading U.S. researchers noted that "...no single pathogen found in the insects could explain the scale of the disappearance. In other words, the bees were all sick, but each colony seemed to suffer from a different combination of diseases."[xi] Parasitic mites of the genus *Varroa* are the most important pest to honey bees globally[xii] and act as vectors to transmit a number of viruses that significantly weaken colonies. Deformed wing virus and a trio of related paralysis viruses have also emerged as important to colony losses, as has a fungal gut pathogen of the genus *Nosema*. Emerging microbiota research points to the possible disruption of normal, symbiotic bee gut cultures by a combination of stressors resulting in increased susceptibility to pathogens.

- ***Pesticides ::*** Pesticides have been known to cause large-scale bee deaths since the early 1900s, many through direct poisoning during aerial sprays. These types of acute bee die-offs are not at issue in CCD, although they do still happen. Regulations and phase-outs of acutely toxic pesticides have reduced the number of acute poisonings in most of Europe and North America, but bee exposure to multiple pesticides continues. Sub-lethal effects, less studied and understood than acute effects, have become a key concern as systemic neonicotinoid pesticides —present in small amounts throughout plant tissues from seed to harvest—have become an important and rapidly growing segment of the global insecticide market since their introduction in the 1990s.[xiii] Other pesticides of concern include those used by beekeepers to control pathogens, and certain fungicides thought to be safe for bees which have recently been found to act synergistically with some neonicotinoids, increasing the latter pesticides' bee toxicity by 200- to 1,000-fold.[xiv]

The following four studies provide an overview of the factors involved in recent honey bee declines as well as a description of Colony Collapse Disorder.

1) vanEngelsdorp D, Meixner, MD. 2010. **A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them.** J Invertebr Pathology 103: S80-S95.

> This article is the most recent and comprehensive review of historical loss patterns and the data gaps that make these patterns difficult to describe. Researchers reviewed 110 years of census surveys, experimental studies, technical reports and review studies to arrive at an analysis of global population trends and the factors that affect managed honey bee populations. Various data inconsistencies notwithstanding, they determined that populations have increased worldwide over the last 50 years, but severe declines have occurred in the U.S. (61%), Mexico, and Europe (27% continent-wide) in the same time frame. In all of these declines, several interacting factors are likely. These include long-term reductions in bee foraging habitat, changing weather patterns, a contraction of the gene pool, weak queen bees, pathogens, pesticides and socioeconomic factors that affect beekeeping popularity and profitability.

2) vanEngelsdorp D, Evans JD, Saegerman C, Mullin C, Haubruge E, Nguyen BK, et al. 2009. **Colony collapse disorder: A descriptive study.** PLoS ONE 4(8): e6481.

> This study looked at 91 managed honey bee colonies from 13 apiaries in California and Florida to confirm the definition of CCD, identify its potential causes and inform future research. Over 200 variables were quantified and compared between CCD-afflicted colonies and apiaries, and those not afflicted. While 61 of the 200 variables were found frequently enough to make worthwhile comparisons, no single one stood out as being significantly linked to CCD. The comparative results did suggest some important trends: 1) compromised immunity is likely playing a role; and 2) CCD is either a contagious condition or caused by exposure to a common risk factor. Recommendations for future research include: 1) longitudinal studies that monitor parasite, pathogen and pesticide loads while quantifying pesticide tolerance in the study populations; 2) studies on the interactions among pesticides and pathogen loads.

3) vanEngelsdorp D, Speybroeck N, Evans JD, Nguyen BK, Mullin C, Frazier M, et al. 2010. **Weighing risk factors associated with bee colony collapse disorder by classification and regression tree analysis.** J Econ Entomol 103(5): 1517-1523.

> To better understand the relative importance and relationships among different risk factors in explaining CCD, researchers performed a classification and regression tree (CART) analysis on the data set of the above epidemiological study (vanEngelsdorp et al. 2009). This was the first case of CART analysis being used to understand bee pathology. Overall, the results of the analysis provide further evidence for the prevailing consensus that CCD is caused by multiple factors acting together to decrease colony fitness and increase susceptibility to disease. Pesticides accounted for 6 of the 19 variables having greatest discriminatory power. Of note is the fact that, at a level above 66 ppb in developing bees, the *Varroa* miticide, coumaphos, was positively correlated to healthy colonies; this was the most predictive factor between the two populations. Results indicate that pesticides are very likely involved in the CCD causal complex. Among several areas recommended for further study, two were highlighted: the effect of sub-lethal pesticide exposure on pathogen prevalence, and the relationship between varying tolerance to pesticides and colony survival.

# Understanding pesticides as a causal factor in colony collapse

## *Pesticide Prevalence*

Pesticides are a prominent part of the honey bee environment, both in the hive and in the larger environment. This is especially true in the U.S., where 1,200 different pesticide active ingredients are approved and in use in 18,000 different product combinations. By contrast, France and Britain have each registered around 500 and 300, respectively.[xv] At least 143 million of the 442 million acres of U.S. cropland is planted with crops treated with one of three neonicotinoid pesticides known to be highly toxic to bees: clothianidin, imidacloprid and/or thiamethoxam. This is a conservative estimate derived from cross-referencing USDA ARS data, industry reports and available pesticide use data. It does not begin to account for non-agricultural uses. (See appendix A for more complete use data and analysis.)

Multiple surveys in the U.S. and Europe have shown that a mixture of pesticide formulations and types are present in bees, wax, stored food and the pollen and nectar on which bees forage. Field studies have found neonicotinoid pesticides in particular in soil, dust, planter exhaust, water (guttation) droplets exuded by treated plants and on nearby, untreated plants and fields. A cross-section of these studies follows.

1) Krupke C, Hunt G, Eitzer B, Andino G, Given K. 2012. **Multiple routes of pesticide exposure for honey bees living near agricultural fields.** PLoS ONE 7(1).

This field study established that bees near agricultural fields are exposed to a variety of pesticides via multiple routes at harmful levels throughout the foraging period. Pesticides found include the neonicotinoids clothianidin and thiomexotham, atrazine (an herbicide) and fungicides, including one known to synergize with neonicotinoids (propiconazole). Soils, pollen (bee-collected and directly from plants), dandelions, dead and healthy bees, and planter waste products were all examined as potential exposure routes. The authors looked specifically at corn, which occupies more arable land in North America than any other crop—88 million acres. Corn is planted throughout the U.S. Midwest from mid-April through early May when the energetic requirements of bees are increasing rapidly as hives prepare for colony growth, requiring increased foraging. Virtually all corn (excepting the 0.2% cultivated organically) in the U.S. is grown from treated seeds, and this study found that bees forage heavily on corn: corn pollen made up over 50% of the pollen collected by bees, by volume, in 10 of 20 samples. Authors also sampled dandelions, which are a preferred nectar and pollen source during this period. Dandelions in nearby, untreated fields were contaminated with clothianidin. Soil from fields which had not been planted with treated seeds in over two growing seasons tested positive for clothianidin as well, which authors interpreted as a feature of the chemical's persistence and mobility.

Pollen collected from the treated plants was contaminated by clothianidin as expected, but bee-collected pollen samples showed higher levels, indicating additional pathways of exposure. Levels of contamination in bee-collected corn pollen in this study were 10-fold higher than reported from an experiment on clothianidin-treated canola. This is significant because clothianidin was approved for use on corn and canola simultaneously based on the canola field test. The finding that bee-collected pollen contained neonicotinoids is of particular concern because clothianidin is even more toxic when ingested orally by a bee, and because of the potential for harm when

developing bees are exposed to pesticides within the hive through stored pollen. During the period observed, nurse bees were emerging and fed on pollen reserves in the form of royal jelly. Authors calculated that, at the levels observed, a new bee would consume 50% of the oral LD50 during the 10 days it spends as a nurse bee.

In sum, the study established multiple exposure routes, at harmful levels, at a critical time, on the most common crop. The authors' most salient finding, however, is the establishment of a new, especially mobile and toxic exposure route—planter exhaust material. Corn seeds are sown using an automated planting system that relies on air/vacuum mechanisms to space the seeds; in order to keep seeds treated with pesticides from sticking to one another, talc is used. This talc becomes contaminated and is then exhausted during planting, either down with the seed or into the air. Authors found "extremely high" levels of neonicotinoids and fungicides in planter exhaust material.

2) Mullin CA, Frazier M, Frazier J, Ashcraft S, Simonds R, vanEngelsdorp D, et al. 2010. **High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health.** PLoS ONE 5(3): e9754.

Researchers conducted the most extensive North American survey of pesticide residues in managed honey bee colonies to date in 23 states and one Canadian province during the 2007-2008 growing season. They used conventional (gas chromatography-mass spectrometry) and recently developed (liquid chromatography-tandem mass spectrometry) analytical techniques to detect any of 200 pesticides and their metabolites at concentration levels as low as 0.1 ppb in a representative cross-section of bees, pollen and wax. A total of 121 pesticides and metabolites comprising 5,519 total residues were detected and quantified in 887 samples. Wax samples averaged 8, pollen samples averaged 7.1, and bee samples averaged 2.5 different pesticide residues each, with at least two pesticides detected in 92% of all samples analyzed. Pyrethroids, a group of widely used pesticides that are toxic to bees, were the dominant class of insecticides detected in all samples. Nearly half (49.9%) of all samples contained at least one systemic pesticide. Pollen contained high levels of fungicides, which tended to co-occur with low levels of systemic pesticides, implicating possible synergistic effects.

The authors concluded that "The widespread occurrence of multiple residues, some at toxic levels for single compounds, and the lack of any scientific literature on the biological consequences of combinations of pesticides, argues strongly for urgent changes in regulatory policies regarding pesticide registration and monitoring procedures as they relate to pollinator safety. This further calls for emergency funding to address the myriad holes in our scientific understanding of pesticide consequences for pollinators."

3) vanEngelsdorp D, Evans JD, Donovall L, Mullin C, Frazier M, Frazier J, et al. 2009. **"Entombed pollen": a new condition in honey bee colonies associated with increased risk of colony mortality.** J Invertebr Pathol 101(2): 147-149.

Two U.S. longitudinal studies that sought to uncover causes of poor colony health (and CCD in particular), were begun in spring of 2007. During both studies, researchers discovered a

phenomenon known as entombed pollen, in which bees responsible for managing food stores in the hive seal off some pollen with propolis and wax. This behavior is generally used by bees to quarantine microbial threats, such as an invading lizard or mouse that dies inside the hive. Entombed pollen had much higher levels of three specific pesticides (two miticides and one fungicide) than typical pollen, and notably had no detectable microbes. Hives with such pollen in late spring were twice as likely to die in mid-fall as hives with normal pollen. Incidence of entombed pollen was notably greater in reused wax comb regardless of any disinfection treatments applied, which suggests there is a transmittable factor common to both entombing behavior and colony death. Researchers urged further study.

"The presence of entombing is the biggest single predictor of colony loss. It's a defence mechanism that has failed."
- Dr. Jeffrey Pettis, in the *UK Guardian*

4) Bonmatin JM, Marchand PA, Charvet R, Moineau I, Bengsch ER, Colin ME. 2005. **Quantification of imidacloprid uptake in maize crops.** J Agr Food Chem 53: 5336-5341.

Researchers in France conducted a three-year, random-sample field study of environmental contamination by the most prevalent neonicotinoid, imidacloprid, in corn fields with active bee colonies. This study was part of a national research effort seeking to evaluate the environmental risk of imidacloprid to honey bees. Using a recently developed method capable of quantifying imidacloprid at 1 ppb and detecting it at 0.1 ppb (high pressure liquid chromatography–mass tandem spectrometry, HPLC/MS/MS), researchers established that imidacloprid is habitually present in flowering, treated corn at levels known from previous dose studies to induce a variety of harmful effects, including eventual death, for honey bees. Average levels were 6.6 ppb for pollen-producing flowers, 4.1 ppb for stems and leaves, and 2.1 ppb for pollen itself. These levels are similar to those previously studied for sunflower and canola. Imidacloprid-contaminated corn pollen made up 54% of pollen samples collected at hive entrances, reflecting a mixture of pollen sources and with a correspondingly lower average contamination of 0.6 ppb.

## *Neonicotinoids :: Acute, sub-lethal & chronic effects*

Neonicotinoids are a relatively new, and very widely used class of insecticides that work on the central nervous system of sucking insects such as fleas and aphids.[xvi] They were introduced in the 1990s and have since become the fastest-growing class of insecticides in the history of synthetic pesticides. By 2005 neonicotinoids had gained a 16% total market share of the nearly € 8 billion global market, and a near-lock (77%) on the global seed treatment market which itself grew from a niche € 155 million to a € 535 million market.[xvii] Among their approved uses in the U.S. are topical flea treatments for pets, lawn and garden uses, and a variety of agricultural uses including stone fruits, nuts, canola, sunflowers and corn.

Table 1 :: Concentrations of highly toxic neonicotinoid insecticides known to cause harm to honey bees. Sources :: EPA; Aliouane et al. 2009; Iwasa et al. 2004; Krupke 2012.

| | Toxicity to bees | Oral acute toxicity 48-hr LD 50 (a.i./bee) | Contact acute toxicity 48-hr LD 50 (a.i./bee) | Sub-lethal effects range (a.i./bee) | Persistence (half-life in soil) |
|---|---|---|---|---|---|
| Clothianidin | Highly | 2.8 - 3.79 ng/bee | 22-44 ng/bee | 24 ng/bee | 148 – 1,155 days |
| Imidacloprid | Highly | 3.8 ng/bee | 78 ng/bee | 24 ng/bee | 40 - 997 days |
| Thiamethoxam | Highly | 5 ng/bee | 24 ng/bee | 50 ng/bee | 25 - 100 days |
| Dinotefuran | Highly | 7.6 - 23 ng/bee | 24 - 61 ng/bee | Unknown | 138 days |

Neonicotinoids fall into two subclasses: nitroguanidines and cyanoamidines. The nitroguanidines, which are highly acutely toxic to honey bees, include imidacloprid, clothianidin, thiamethoxam and dinotefuran. The cyanoamidines are not as acutely toxic to honey bees and include thiacloprid and acetamiprid. Neonicotinoids are known to persist in soil for years and have the potential to accumulate in soil. These chemicals are also highly water soluble, and are present throughout treated plants from seed to harvest.

To date, most U.S. regulatory decisionmaking addressing the risks posed to honey bees by neonicotinoids has hinged, by default, on the establishment of acute toxicity exposure scenarios without requiring tests for sub-lethal effects.[xviii] The standard laboratory method for assessing pesticide risk is determine the median lethal dose ($LD_{50}$) required to kill half the tested population over a certain timeframe. In the U.S. this protocol remains the primary basis for risk assessment in pesticide registration. However, this approach to risk assessment only takes into account the survival of adult honey bees exposed to pesticides over a short time frame: the typical acute toxicity test is 48 hours although it may be extended to 96 hours. Acute toxicity tests establishing $LD_{50}$ levels on adult honey bees may be particularly ill-suited for the testing of systemic pesticides because of the ways bees are exposed to systemics in the field —in smaller doses, over a long period of time, through a variety of routes. Chronic feeding tests using whole colonies have been recommended as a better way to quantify the effects of systemics.[xix]

"U.S. regulatory decisionmaking ... has hinged, by default, on the establishment of acute toxicity exposure scenarios without requiring tests for sub-lethal effects."

Despite repeated calls for a reevaluation of pesticide testing protocols, regulatory processes in the U.S. and Europe have not been adapted to consider sub-lethal, chronic or synergistic effects of pesticides on pollinators.[xx]

**Key terms for describing pesticide impacts**

- ***Acute toxicity ::*** The acute toxicity of a pesticide to bees (either by contact or ingestion) is quantified by noting the dose at which half of the insects die within a specific time period. This is known dose that is lethal to 50% of the test population or $LD_{50}$. "Acute" and "lethal" are often used synonymously, but a range of effects, such as general agitation, vomiting, wing paralysis, arching of the abdomen similar to a sting reflex and uncoordinated movement, have been observed in association with acute doses.[xxi] The acceptable risk for each pesticide is set by regulatory agencies based on acute toxicity tests and the expected rate and mode of pesticide

application, both of which are determined by the manufacturer. The $LD_{50}$ values of neonicotinoids are low compared to older classes of insecticides; they are considered highly toxic to honey bees.

- ***Sub-lethal toxicity ::*** The sub-lethal toxicity of neonicotinoid pesticides is of particular concern because the most common field exposure scenarios are likely at the sub-lethal rather than acute level. Sub-lethal effects of neonicotinoids on honey bees include behavioral disruptions such as disorientation, reduced foraging, impaired memory and learning, and shifts in communication behaviors. Other important sub-lethal effects might include compromised immunity, delayed development and a host of indirect, potentially cascading effects that impact the hive's ability to sustain itself.

- ***Cumulative and chronic effects ::*** Neonicotinoids function by binding to nicotinic acetylcholine receptors in insects' brains, receptors which are particularly abundant in bees, increasing during development from larval to adult stages.[xxii] This binding leads to an over-accumulation of acetylcholine, resulting in paralysis and death. The most recent scientific observations point to a long-lasting effect in which molecules unbind from receptors, but remain in the bee brain, possibly rebinding multiple times before metabolization occurs.[xxiii] Whether this constitutes effectively irreversible, cumulative toxicity remains unclear; but chronic toxicity effects over time are a likely result.

**Neonicotinoids at-a-glance**

Neonicotinoids are a widely used class of systemic insecticides introduced in the early 1990s that have been of particular interest for their effects on honey bees.

They can be applied as a spray (foliar) or, more commonly, used as systemics. Systemic pesticides are applied as seed coatings or soil drenches and are taken up through the plant's vascular system, and then transmitted to all parts of the plant, including pollen and nectar. Neonicotinoids are very persistent and therefore accumulate over time in the environment.

Most neonicotinoids are classified as acutely toxic to bees. But single, high-dose (i.e. acute) exposures are likely less common than are the chronic, sub-lethal exposure levels faced by bees over time as they forage in the field.

Honey bee colony collapses in France in 1999 (called "mad bee disease") were the first to implicate imidacloprid, the most widely used neonicotinoid, in colony loss. Researchers have since found a range of sub-lethal effects caused by neonicotinoids: altered foraging and feeding behavior, impaired orientation and social communication, undermined immunity and delayed larval development.

Many independent studies in the U.S. and in Europe have shown that small amounts of neonicotinoids—both alone and in combination with other pesticides—can cause impaired communication, disorientation, decreased longevity, suppressed immunity and disruption of brood cycles in honey bees. A selection of these studies follow.

1) Henry, M., Reguin, M., Requier, F., et al. 2012. **A Common Pesticide Decreases Foraging Success and Survival in Honey Bees.** Science (20): 348 - 350.

Researchers attached radio frequency devices (RFIDs) to honey bees to test the impact of sublethal doses of thiamethoxam (a neonicotinoid) on foraging, homing and survival. They then plugged these findings into colony population dynamics models to assess the extent to which "homing failure" may contribute to colony collapse. The study confirms the hypothesis that sublethal, field-realistic doses of thiamethoxam undermine bees' foraging and homing abilities at rates significant enough to increase risk of colony collapse. Intoxicated bees were up to twice as likely as control bees to fail to return home to their hives.

This semi-field study was carried out in an agricultural area in western France and in a suburban area in southern France. Bees were given a field-realistic, sublethal dose of thiamethoxam (1.34 ng in a 20-µl sucrose solution), then released up to 1 km away from their hives with an RFID glued to their thorax. RFID readers were placed at the hive entrance to track the return of tagged bees. "Post-exposure homing failure was then derived from the proportion of non-returning foragers. To discriminate against other potential causes of homing failure in treated foragers (e.g natural mortality, handling stress, predation), control foragers were fed with untreated sucrose and tracked in the same way. The authors conclude that sublethal, commonly encountered doses of thiamethoxam can impact forager survival at sufficient rates to contribute to colony collapse and that this impact increases when homing is more difficult. One consequence of this finding, according to authors, is that "impact studies are likely to severely underestimate sublethal pesticide effects when they are conducted on honey bee colonies placed in the immediate proximity of treated crops."

2) Aliouane Y., El Hassani Athiamethoxam. K., Gary V., Armengaud C., Lambin M., Gauthier M., 2009. **Subchronic exposure of honey bees to sublethal doses of pesticides: effects on behavior.** Environ. Toxicol. Chem. 28(1): 113–122.

In this laboratory study, emergent honey bees received a daily dose of insecticide ranging from 1/5th to 1/500th of the median lethal dose (LD50) of three pesticides over 11 days. Two of these pesticides were neonicotinoids, thiamethoxam and acetamiprid; the other was fipronil. Fipronil is another systemic pesticide that is banned in part of Europe because it is highly toxic to bees. The authors sought to test the effects of sublethal, chronic exposure to these pesticides on honeybee behavior. Fipronil, used at the dose of 0.1 ng/bee, induced mortality of all honey bees after one week of treatment. Fipronil-treated bees also exhibited impaired oflactory memory, and spent more time immobile. Thiamethoxam by contact induced either a significant decrease of olfactory memory 24 h after learning at 0.1 ng/bee or a significant impairment of learning performance with no effect on memory at 1 ng/bee. The experiments with thiamethoxam show that repeated exposure to a dose that has no behavioral effect when applied in acute conditions results in the appearance of some behavioral deficits.

3) Medrzycki P, Montanari R, Bortolotti L, Sabatini AG, Maini S, Porrini C. 2003. **Effects of imidacloprid administered in sub-lethal doses on honey bee behaviour. Laboratory tests.** B Insectol 56(1): 59-62.

In this study, researchers monitored the behavior of adult forager bees in highly controlled, laboratory conditions to observe effects of imidacloprid fed at sub-lethal doses in sugar solution. Four different contamination concentrations were tested: 100 ppb and 500 ppb as a single dose (20 microliters), and each concentration as a continuously available food source for 24 hours. Three replications of the experiment were performed. Researchers concluded that imidacloprid at all doses given caused significant reductions in mobility that lasted for one to several hours, with bees remaining stationary for longer periods as well as moving more slowly. They also noted that bees seemed to lose their communicative ability at all doses, failing to coordinate their activity with other bees. They recommended further study to better understand the length of time that these effects last, and to investigate the effects on social behavior essential to the proper functioning of a hive.

4) Colin ME, et al. 2004. **A method to quantify and analyze the foraging activity of honey bees: relevance to the sub-lethal effects induced by systemic pesticides.** Archives of Environmental Contamination and Toxicology 47: 387-395.

This study investigated the sub-lethal effects of two insecticides in semi-field conditions on the foraging behavior of honey bees. Imidacloprid and fipronil were chosen because both behave

systemically, were recently introduced, considered highly toxic to bees, had shown sub-lethal effects on bees in lab conditions and had been implicated in honey productivity declines in Europe. The primary aim was to address a gap in environmental assessment of systemic pesticides by improving on the methods used to quantify foraging behavior changes. Bee colonies were placed in enclosed tunnels and their feeding behavior video recorded over a period of five days, constituting a cumulative effects study much shorter than a bee or hive lifecycle study would be. With imidacloprid at 6.0 µg/kg, inactive bees—those visiting the feeder, but not feeding—increased over time in relation to active bees. With fipronil at 2.0 µg/kg, most bees stopped coming to the feeder by the last day, and the few that did tended to be inactive. Convulsions and paralysis were also observed in bees feeding on fipronil-contaminated food. Researchers concluded that both insecticides disturb the hive's primary activity, feeding, at sub-lethal levels 70 times below the referenced $LD_{50}$s. They also concluded that their experimental protocol "provided an indispensable interface between controlled conditions in the laboratory and the field," which suggests its adoption in regulatory testing of sub-lethal effects.

5) Decourtye A, Armengaud C, Renou M, Devillers J, Cluzeau S, Gauthier M, et al. 2004. **Imidacloprid impairs memory and brain metabolism in the honeybee (Apis mellifera L.).** Pestic Biochem Phys 78: 83-92.

This laboratory study shows sub-lethal effects at low concentrations of imidacloprid using four different methodologies. Researchers sought to build from prior studies that clarified the role of nicotinic acetylcholine receptors (nAChR) in honey bee learning and memory capacities to investigate the specific effects of the neonicotinoid imidacloprid on those capacities. Bees were conditioned to respond to a specific floral scent in association with food, a behavior that demonstrates associative learning and memory formation. Behavioral results led researchers to conclude that imidacloprid at a dose of 12 ng/bee significantly inhibited associative learning as well as retention of successfully learned associations, whether learned before, during or after exposure. This impairment of retention notably affected medium-term, but not long- or short-term memory, appearing as a temporary amnesia. Metabolic activity in specific parts of the bee brain were also analyzed for changes in response to imidacloprid. These results indicated that at both doses, imidacloprid was acting detrimentally in the parts of the bee brain involved with associative and contextual memory.

6) Yang EC, Chuang YC, Chen YL, Chang LH. 2008. **Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae).** J of Econ Entomol 101(6): 1743-1748.

Researchers conducted a semi-field experiment to follow on results of laboratory studies that had shown a number of detrimental effects from ingestion of imidacloprid-contaminated food. They tested the time intervals between individual worker bee visits to a feeder after ingestion of imidacloprid-contaminated sugar solution at concentrations ranging from 40 µg/L to 6,000 µg/L. Bees showed abnormal foraging behavior beginning at 50 µg/L (41.6 ppb) and worsening with higher contamination. Effects ranged from a statistically significant time delay between feeder visits, to disappearance (from feeder and hive) for a full day without return. At 1600 µg/L, more than 90% of bees went missing for a full day, but all returned the following day. Above this concentration, a portion of bees did not return, and above 800 µg/L, bees that returned from long delays continued to show abnormal foraging behavior. Based on an estimation of average meal size per feeder visit, abnormal foraging behavior began a dose per bee of 1.82-4.33 nanograms. Researchers concluded that abnormal foraging behavior could occur in the field through multiple visits to imidacloprid-contaminated flowers.

## *Synergistic + Combined Effects*

Synergism is a phenomenon in which two or more factors produce a combined effect that is greater than the sum of their separate effects. As investigations into the causes of CCD have continued to point toward multiple factors working in concert to increase bees susceptibility to disease, synergism and combined effects have emerged as a critical area of research.

In 2004, a lab study (see # 1 below) showed that the acute toxicity of two neonicotinoid pesticides on honey bees dramatically increases when combined with either of two common fungicides. Four years after this finding was published, researchers established that these types of combinations are prevalent in bee hives.[xxiv] Between 2010 and 2012, three separate studies demonstrated synergism between the common parasite *Nosema* and pesticide exposure (thiacloprid, imidacloprid and the non-neonicotinoid systemic fipronil). Hives exposed to these pesticides were significantly more susceptible to infection.

## *"Chemical cocktails" :: Fungicides, pyrethroid insecticides, miticides*

Neonicotinoids are but one class of pesticides, honey bees are exposed to dozens of different pesticides on a daily basis (see "Pesticide Prevalence," esp. Mullin 2010). Included among these are a mix, or "chemical cocktail," of insecticides, herbicides and fungicides as well as the miticides used by beekeepers to control pathogens in the hive.

Non-neonicotinoid pesticides of special concern for their impacts on bees, and potential role in the causal complex of CCD are fungicides and pyrethroids—particularly in combination. Fungicides have long been thought to be relatively harmless to bees, but their recent, dramatic uptick in use (especially with corn, beginning around 2007)[xxv] coupled with their synergizing effect on certain insecticides (including neonicotinoids and pyrethroids) have brought renewed attention. Pyrethroids are highly variable in their toxicity to bees, but have come under recent scrutiny both because their high fat solubility means that pyrethroids persist and bioaccumulate in bee wax, and because they are known to synergize with certain fungicides.

As bee detoxification mechanisms are increasingly understood, the potential threats posed by particularly toxic "chemical cocktails" composed of certain fungicides, pyrethroids and neonicotinoids are slowly coming into resolution. Scientists believe that part of bees' increased vulnerability to pesticides comes from their having relatively few genes that encode detoxification enzymes. For example, one such group of enzymes (P450s) mediate detoxification pathways in ways that can be inhibited by certain widely used fungicides (e.g. propiconazole), and are thought to be important for bees' ability to tolerate the common pyrethroid miticide, fluvalinate.[xxvi] Although current data are unclear, another hypothesis as to the physiological mechanisms by which synergistic effects between pathogens and pesticides may operate is that pathogen metabolites may interfere with the detoxification process.[xxvii]

"Honey bees have fewer genes involved in detoxification than other insects."

1) Iwasa T, Motoyama N, Ambrose JT and Roe M. 2004. **Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, Apis mellifera.** Crop Protection 23(5): 371.

> This laboratory study established a synergistic effect between neonicotinoids and fungicides. A neonicotinoid was found to be up to 1,141 times more toxic to bees when combined with a common fungicide. Researchers performed laboratory tests for acute toxicity of several neonicotinoid pesticides and metabolites, both alone and combined with each of several fungicides commonly used in crop production. These tests were for contact toxicity, rather than

oral ingestion. They found that three combinations between a neonicotinoid and a fungicide were highly synergistic. Acetamiprid, a neonicotinoid that is much less acutely toxic than imidacloprid, becomes 244 times more so when combined with the fungicide triflumizole. Thiacloprid, also much less toxic than imidacloprid, becomes 559 times more so when combined with the fungicide propiconazole, and 1,141 times more toxic when combined with triflumizole. Though plants treated with the maximum recommended levels for agricultural use did not exhibit a statistically significant effect on honey bee mortality after three and 24 hours, further study was suggested before reaching conclusion as to in-field, synergistic toxicity.

2) Smodis Skerl MI, Kmecl V, Gregorc A. 2010. **Exposure to pesticides at sublethal level and their distribution within a honey bee (Apis mellifera) colony.** Bulletin of Environmental Contamination and Toxicology 85(2): 125-8.

This study examined the role and prevalence of pesticides in honeybee colonies, both those introduced intentionally by beekeepers to control mites (acaricides) as well as agricultural chemicals found incidentally in the hive. The goal was to determine whether, and if so, how much, pesticides accumulated in the bodies of bees in the hive, in the royal jelly, and in bee larvae as well as how the chemicals are spread throughout the hive. The acaricides introduced into the colony were amitraz, coumaphos and fluvalinate, while the organophosphate diazinon served as a representative agricultural chemical. The acaricides were applied following commonly accepted regimes to treat against the *Varroa destructor*, and the diazinon was introduced in accordance with a treatment plan that would be applicable to an apple orchard. Coumaphos and fluvalinate were the most prevalent throughout the colony. No diazinon was found in the samples tested and amitraz only in amounts below the level of detection. Coumaphos was found in royal jelly from nurse bees, while fluvalinate was found in the bodies of bees and in bee larvae. The presence of fluvalinate throughout the colony, especially in larvae, demonstrate that chemicals in the hive can be transmitted from bee to bee as well as to food and thus to larvae, spreading throughout the entire colony.

3) Dai P-L, Wang Q, Sun J-H, Liu F, Wang X, Wu Y-Y and Zhou T. 2010. **Effects of sub-lethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee Apis mellifera ligustica.** Environmental Toxicology and Chemistry 29: 644–649.

This study examined the sub-lethal effects of two pyrethroid insecticides, bifenthrin and deltamethrin, on honeybee health. The study was performed on colonies in the laboratory that were fed small doses of the chemicals. Effects measured included fecundity, growth and the development of individual bees. The importance of these particular issues for overall health of the colony and general bee population was emphasized. Data was taken over several years and results measured against control colonies not fed the insecticides. The authors found that bifenthrin is "highly toxic" and deltamethrin "moderately toxic" to honey bees. This was based on findings that exposure to the insecticides significantly reduced colony fecundity due to reduced rates of egg laying and impaired ability of the colony to transition to a new queen. Developmental effects on honeybee larvae were also observed. In comparing their findings to pesticide applications in the field, the study's authors note that commonly recommended bifenthrin application rates are higher than the insecticide's $LC_{50}$ for honey bees and deltamethrin application rates are about the same as the $LC_5$.