How Neonicotinoids Can Kill Bees

The Science Behind the Role These Insecticides Play in Harming Bees

2nd Edition; Revised & Expanded

Jennifer Hopwood, Aimee Code, Mace Vaughan, David Biddinger, Matthew Shepherd,
Scott Hoffman Black, Eric Lee-Mäder, and Celeste Mazzacano

XERCES SOCIETY
for Invertebrate Conservation

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The Xerces Society for Invertebrate Conservation

www.xerces.org

The Xerces® Society for Invertebrate Conservation is a nonprofit organization that protects wildlife through the conservation of invertebrates and their habitat. Established in 1971, the Society is at the forefront of invertebrate protection, harnessing the knowledge of scientists and the enthusiasm of citizens to implement conservation programs worldwide. The Society uses advocacy, education, and applied research to promote invertebrate conservation.

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Dedication

We dedicate this report to the memory of Jacqueline Robertson. Jackie was commited to clear communication and to quantifying and understanding the effects of pesticides in order to reduce the risks to beneficial insects, including bees. Jackie's life-long work provided a standard still in place today and helped train generations of graduate students.

Acknowledgements

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This report is a revised and expanded version of the Xerces 2012 report *Are Neonicotinoids Killing Bees?* The title has been changed to reflect the expanded understanding of the risk neonicotinoids pose to bees.

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Executive Summary

Neonicotinoids have been adopted for use on an extensive variety of farm crops as well as ornamental landscape plants. They are the most widely used group of insecticides in the world, and have been for a decade. Developed as alternatives for organophosphate and carbamate insecticides, neonicotinoids are compounds that affect the nervous system of insects, humans, and other animals. Although less acutely toxic to mammals and other vertebrates than older insecticides, neonicotinoids are highly toxic in small quantities to many invertebrates, including beneficial insects such as bees.

The impact of this class of insecticides on pollinating insects such as honey bees and native bees is a cause for concern. Because they are systemic chemicals absorbed into the plant, neonicotinoids can be present in pollen and nectar, making them toxic to pollinators that feed on them. The potentially long-lasting presence of neonicotinoids in plants, although useful from a pest management standpoint, makes it possible for these chemicals to harm pollinators even when the initial application is made weeks before the bloom period. In addition, depending on the compound, rate, and method of application, neonicotinoids can persist in the soil and be continually taken in by plants for a very long periods of time.

Across Europe and North America, a possible link to honey bee die-offs has made neonicotinoids controversial. In December 2013, the European Union significantly limited the use of clothianidin, imiadcloprid, and thiamethoxam on bee-attractive crops. In the United States, Canada, and elsewhere, local, state, and federal decision makers are also taking steps to protect pollinators from neonicotinoids. For example, the U.S. Fish and Wildlife Service phased out all uses of neonicotinoids on National Wildlife Refuges lands starting in January 2016.

This report reviews research on the impact of these pesticides on bees. For a research review on beneficial insects, including those important to biological control, see Hopwood et al. (2013). See Morrissey et al. (2015), Mineau et al. (2013), and Gibbons et al. (2015) for reviews on aquatic invertebrates, birds, and vertebrates, respectively.

The Xerces Society for Invertebrate Conservation also maintains an annotated bibliography of relevant research published since the writing of this report on its web site. That bibliography can be accessed at: www.xerces.org/neonicotinoids-and-bees.

Every year, neonicotinoids are applied to millions of acres of farmland. While there are still much to be learned about the risks of these chemicals, research has demonstrated that they can harm beneficial insects including bees.

Findings

The following findings are divided into three sections. In the first section, we present clearly documented information about neonicotinoid impacts on bees, i.e., facts that are supported by an extensive body of research. (Fully cited evidence for these is detailed in the main body of this report.) The second section covers what can be inferred from the available research. This includes possible effects for which there is currently only limited research or the evidence is not conclusive. In the third section, we identify knowledge gaps in our understanding of pollinator and neonicotinoid interactions. Filling these gaps will allow better-informed decisions about the future use and regulation of these chemicals.

Clearly Documented Facts

Exposure of Bees to Neonicotinoids

- Neonicotinoid residues found in pollen and nectar are consumed by flower-visiting insects such as bees. Residue concentrations can reach levels that cause sublethal effects through a variety of application methods, including use of coated seed, and in some situations can reach lethal levels.
- Neonicotinoids can persist in soil for months or years after a single application. Residues have been found in woody plants up to six years after soil drench application.
- Untreated plants have been found to absorb the residues of some neonicotinoids that persisted in the soil from the previous year.
- Neonicotinoids applied to crops, even as seed coatings, can contaminate adjacent vegetation, including bee-attractive wildflowers.
- Products approved for home and garden use may be applied to plants at rates substantially higher than the maximum label rate approved for agricultural crops.
- Direct contact from foliar applications of the most toxic neonicotinoids has caused bee kills; additionally, foliar residues on plant surfaces may remain lethal to bees for several days.
- Bee kills have been caused by legal applications of neonicotinoids to *Tilia* (linden, basswood). Some of these applications, designed to be uptaken by the trees, occurred weeks to months prior to when bees visited the trees.

Bumble bees and solitary bees respond differently to neonicotinoids than do honey bees. Current regulatory testing in the United States doesn't address these differences even though many crops are pollinated by native bees—such as this bumble bee pollinating peach blossoms (left) and blue orchard bee pollinating almond blossoms (right).

Effects on Honey Bees (*Apis mellifera*)

- Clothianidin, dinotefuran, imidacloprid, and thiamethoxam are highly toxic to honey bees by contact and ingestion.
- Thiacloprid and acetamiprid are moderately toxic to honey bees. (To understand how the EPA defines the levels of toxicity, see EPA Toxicity Classification Scale for Bees on right.)
- Neonicotinoids absorbed by plants are metabolized over time. Some of the resulting breakdown products are also toxic to honey bees, and sometimes even more toxic than the original compound.
- Honey bees exposed to sublethal levels of neonicotinoids can experience problems with flight and navigation, reduced taste sensitivity, and slower learning of new tasks, all of which impact foraging ability and hive productivity.
- Larvae exposed to sublethal doses of imidacloprid in brood food had reduced survival and pupation, altered metabolism, and reduced olfactory response as adults.
- Contaminated talc, abraded seed coating, or dust that becomes airborne during planting of neonicotinoid-coated seed is acutely toxic on contact to honey bees.

EPA Toxicity Classification Scale for Bees

A pesticide's classification is determined by the EPA. As there are generally multiple LD_{50}s for each pesticide, the EPA selects one LD_{50} for contact and one for oral exposure to classify the pesticide's toxicity:

Toxicity Rating		LD_{50}
Highly toxic	(H)	<2 µg
Moderately toxic	(M)	2–10.99 µg
Slightly toxic	(S)	11–100 µg3
Practically non-toxic	(N)	>100 µg4

Sources:

1. EPA 2015b
2. EPA 2012b

Effects on Bumble Bees (*Bombus* spp.)

- Imidacloprid, clothianidin, dinotefuran, and thiamethoxam are highly toxic to bumble bees.
- Exposure to sublethal amounts of neonicotinoids can result in reductions in food consumption, reproduction, worker survival rates, colony survival, and foraging activity.
- Queen production is significantly reduced by sublethal amounts of neonicotinoids, which may lower bumble bee populations because fewer colonies are established the following year.

Effects on Solitary Bees

- Clothianidin and imidacloprid are highly toxic to blue orchard bees (*Osmia lignaria*) and alfalfa leafcutter bees (*Megachile rotundata*).
- Imidacloprid residues on alfalfa foliage increase rates of mortality of alfalfa leafcutter bees and alkali bees (*Nomia melanderi*).
- Blue orchard bee larvae required more time to mature after consuming sublethal levels of imidacloprid in pollen.
- Sublethal amounts of neonicotinoids can have harmful effects on the reproduction of red mason bees (*Osmia bicornis*).

Presence in the Environment

- Tens of millions of acres of neonicotinoid-coated seed is planted annually in the United States and Canada. When applied systemically and taken up by the plant, imidacloprid, thiamethoxam, and clothianidin can have residual activity within plants for months to years.
- Imidacloprid, thiamethoxam, and clothianidin are persistent in soil, with residues present for months to years.
- Neonicotinoids can move into water and have been found in a range of water bodies, where they may persist. Clothianidin has been found in rivers and streams, wetlands, groundwater, and puddles. Imidacloprid has been found in surface water, groundwater, and puddles. Thiamethoxam has been found in waterways, wetlands, groundwater, and puddles, and has also been detected in irrigation water pulled from ground wells. Acetamiprid and dinotefuran have been found in waterways.

Inferences from Research Results

Exposure of Bees to Neonicotinoids

- Application as a seed coating can result in low levels of residues in pollen and nectar that have been linked with sublethal effects in solitary bees.
- Application methods such as foliar sprays, soil drenches, and trunk injections apply a higher dosage per plant than seed coatings and may result in much higher—even lethal—levels of neonicotinoid residues in pollen and nectar.
- Application of neonicotinoids before and during bloom may result in residue levels in pollen and nectar that cause sublethal effects or even mortality.
- Application by soil drench or trunk injection to woody ornamental species may result in residue levels in blossoms that cause lethal and sublethal effects for more than a year after treatment.
- Foliar applications may have shorter residual toxicity in comparison to other application methods such as trunk injection and soil drench.
- Pesticide residues, including from planting coated seeds, have been found in honey bee hives.

Effects on Pollinators

- There is no direct link demonstrated between neonicotinoids and the honey bee syndrome known as colony collapse disorder (CCD). However, recent research suggests that pesticides, including neonicotinoids, may make honey bees more susceptible to parasites and viruses, including the intestinal parasite *Nosema*, which has been implicated as one causative factor in CCD.
- Neonicotinoids may synergistically interact with demethylase inhibitor (DMI) fungicides. DMI fungicides have significantly increased the toxicity of neonicotinoids to honey bees in some laboratory tests. The synergistic effects of these mixtures in field settings using formulated pesticides in water appear to be less dramatic in comparison with the laboratory research.
- Bumble bees and solitary bees can be affected by neonicotinoids at lower concentrations than are honey bees. Currently, evaluation of other pollinators beyond honey bees is extremely limited in EPA's pesticide registration process.

Knowledge Gaps

Exposure of Bees to Neonicotinoids

- What are the conditions under which residue levels both in the plant and soil increase in concentration over time with repeated applications?
- What is the risk posed by neonicotinoid contamination of nontarget plants growing near treated plants?
- What is the risk posed by bees from forage cover crops absorbing neonicotinoids when planted in a rotation with a neonicotinoid-treated row crop?
- How do these chemicals move through the plant? For example, how soon after product application do neonicotinoid residues appear in pollen and nectar, how fast is it metabolized in the plant, and how do levels vary with application method, crop, and specific compound? Do they move in phloem tissue in addition to xylem?
- Is the combined presence of neonicotinoids and their break-down products in pollen or nectar as toxic or more toxic to bees than the individual chemicals?
- Does the movement of neonicotinoids within a plant vary with the type of plant (e.g., herbaceous vs. woody), or by functional group (e.g., forbs vs. legumes vs. grasses)?
- How do residue levels vary in plants grown under differing field conditions (e.g., drought), soil types (e.g., sandy vs. loam), or under variable nutrient levels?

Effects on Pollinators

- What are the acute and chronic contact and ingestion effects of neonicotinoids to bees other than honey bees?
- What is the full extent of the sublethal effects of neonicotinoids on adult bees?
- What is the full extent of effects of neonicotinoids on larval bees?
- Do honey bees experience delayed effects of neonicotinoids during adverse weather conditions (e.g., winter or drought) when stored foods are consumed?
- What is the full extent of effects that soil residues have on ground-nesting bees (which represent approximately 70% of bee species)?
- What effects, if any, do neonicotinoid contaminated plant tissues and mud have on bees that construct nests from these materials?
- How do neonicotinoids affect other pollinators such as butterflies, moths, beetles, flies, and wasps?

For More Information

Ongoing review of research related to neonicotinoids and pollinators has helped the Xerces Society formulate recommendations regarding federal regulation, pesticide risk assessment, areas to advance research, and best management practices to protect pollinators. For guidance specific to pesticides and pollinator habitat go to: www.xerces.org/protect-pollinators-from-pesticides. Furthermore, to access Xerces annotated bibliography of new research released since this report was completed go to: www.xerces.org/neonicotinoids-and-bees.

Mining bees (*Andrena* spp.) on apple (*Malus domestica*) blossoms. Pollinators–mostly bees–are needed for more than ⅔ of the world's crop species. Bee pollinated crops are worth around $20 billion each year in the United States.

1

Introduction

Neonicotinoid insecticides became available for use on farms and in gardens and ornamental landscapes in the mid-1990s. These insecticides provide long-term plant protection from chewing and sap-sucking insects because they are systemic, i.e., they are absorbed by and are incorporated into the tissue of the treated plant.

As a class, neonicotinoids are considered less toxic to mammals than organophosphate and carbamate insecticides that have well documented effects on the nervous system of insects, humans, and other animals. As such, neonicotinoids have gradually replaced other insecticides. Yet, neonicotinoids are highly toxic in very small quantities to most insects and other invertebrates, including bees, which can be exposed in a variety of ways, such as through the consumption of contaminated nectar or pollen. Furthermore, new research has raised issues about broader effects on biodiversity and ecosystem functioning.

There are seven neonicotinoid active ingredients. Only six of these ingredients are found in plant protection products, but there are dozens of such products on the shelves of garden centers and agricultural supply stores. There are also new systemic insecticides, including flupyradifurone and sulfoxaflor, that are chemically very similar to neonicotinoids yet are not classified as neonicotinoids by the U.S. Environmental Protection Agency (EPA). Neonicotinoids may be applied as foliar sprays or soil drenches, through irrigation, or by direct injection, and they are used on field and orchard crops, ornamental plants in nurseries and gardens, and on plants in gardens, streets, and parks. Neonicotinoids are also used as an insecticidal coating on seeds of field crops such as sunflower, soybean, wheat, corn, cotton, and more. As a result, millions of acres of America's farmlands have been treated, as have uncounted parks, gardens, and backyards in the nation's cities and suburbs.

Common on garden center shelves, neonicotinoids can be applied in greater concentrations in gardens than on farms, and with fewer restrictions. These products may not always carry a warning about hazards to bees or other pollinators.

Bees can come into contact with neonicotinoids in numerous ways on farms (above) or urban landscapes (below) and have varying reactions to the chemicals depending on a host of factors.

Neonicotinoids have become the subject of public debate, particularly with regards to their impacts on honey bees. Much has been published about this type of insecticide and many opinions have been voiced. However, opinion sometimes obscures fact, and in the midst of this, at times vigorous discussion, the science underlying the issues has not always been clearly laid out.

In undertaking this review of research, the Xerces Society focused on the interactions between neonicotinoids, plants, and pollinating insects, especially bees. Our intent is to identify the concentrations at which these insecticides may occur in the environment, the ways in which pollinating insects are exposed to neonicotinoids, and how they affect bees. We also include a case study on the use of neonicotinoids in Pennsylvania apple production to explore how applied research undertaken by independent university scientists is used to mitigate risk to pollinators.

Xerces Society scientists, and our colleague at Pennsylvania State University, first published a review of neonicotinoid studies, *Are Neonicotinoids Killing Bees?*, in 2012. There has been a significant amount or research completed and many additional papers published since then. In preparing this updated and revised edition of the 2012 report, we have reviewed all of the available published studies that we are aware of as of April 2015. By the time of publication, many new studies will have been added to this subject. To address this, the Xerces Society is maintaining an annotated bibliography of relevant research published since the writing of this report on its web site. The bibliography will be continuously updated. It can be accessed at www.xerces.org/neonicotinoids-and-bees.

2

The Importance of Bees

Insects are a highly diverse group of animals and are abundant in all terrestrial environments. With more than 90,000 species identified and thousands more undescribed in North America, insects easily outnumber all species of birds, mammals, fish, reptiles, and amphibians combined. Despite this huge diversity, most insects are overlooked, with the notable exception of the relatively small number of species—less than 1% of the total—that are considered pests.

Insects provide a number of valuable ecosystem services, such as pest management, nutrient cycling, and pollination. Pollinators support the reproduction of nearly 85% of the world's flowering plants (Ollerton et al. 2011) and 35% of global crop production (Klein et al. 2007). The great majority of pollinators are insects, including bees, wasps, flies, beetles, ants, butterflies, and moths. There are also a few species of pollinating birds and bats. Bees are considered the most important group of pollinators in temperate climates. There are over 3,500 species of bees in the United States (Ascher and Pickering 2015); almost all of these are native.

The European honey bee (*Apis mellifera*), introduced to the Americas in the 1600s, is the most widely managed crop pollinator in the United States. Studies indicate honey bees are important for more than $15 billion in crop production annually (Morse and Calderone 2000). However, the number of honey bee colonies has been in decline because of disease, parasites, and other factors (National Research Council 2007). Colony collapse disorder (CCD), a syndrome that causes a large-scale loss of European honey bees, has contributed significantly to colony losses since it was first observed in the United States during the winter of 2006–2007. Although reports of CCD have been less prominent in the past few years, beekeepers continue to report over-winter losses greater than the acceptable winter mortality rate (Wilson and vanEngelsdorp 2015).

Native bees are also important crop pollinators. They provide free pollination services, and are often more efficient on an individual bee basis at pollinating particular crops, such as squash, berries, and tree fruits (Tepedino 1981; Bosch and Kemp 2001; Javorek et al. 2002). Native bees are important in the production of an estimated $3 billion worth of crops annually to the United States economy (Losey and Vaughan 2006). Beyond agriculture, pollinators are keystone species in most terrestrial ecosystems: they pollinate the seeds and fruits that feed everything from songbirds to grizzly bears. They form the foundation of a food web that supports a wildlife hunting, fishing, and viewing industry valued at almost $50 billion per year (Losey and Vaughan 2006). Thus, conservation of pollinating insects is critically important to preserving both wider biodiversity and agriculture.

3

What Are Neonicotinoids?

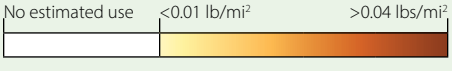
3.1 Introduction

Neonicotinoids are synthetic chemical insecticides that are similar in structure and action to nicotine, a naturally occurring plant compound that was once widely used as an insecticide. They are used to control crop and ornamental plant pests such as aphids or leaf beetles, structural pests like termites, and pests of domesticated animals such as fleas. Six neonicotinoid insecticides are used on crops: imidacloprid, clothianidin, thiamethoxam, dinotefuran, acetamiprid, and thiacloprid (note: the use of thiacloprid has been voluntarily cancelled in the United States but it is still in use in other countries). See Table 3.2 on page 9 for examples of uses and products. A seventh neonicotinoid, nitenpyram, is used to treat for fleas and other external parasites of livestock and pets. Because it is less likely to affect flower-visiting insects, nitenpyram is not discussed further in this report.

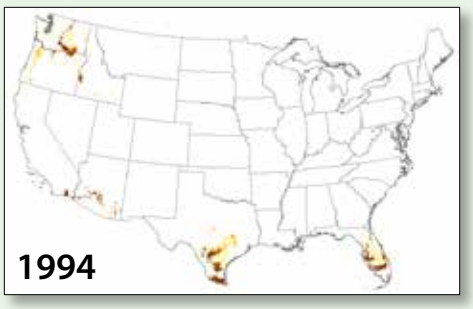
Neonicotinoids are currently the most widely used group of insecticides in the world. They comprise roughly 25% of the agrochemical market, with annual sales worth $1.9 billion (Jeschke et al. 2011). The first neonicotinoid on the world market was imidacloprid (Elbert et al. 2008), which became available in the United States in 1994 and is currently present in over 400 products on the market (Gervais et al. 2010). While comprehensive pesticide use reporting data is not available in the U.S., the state of California tracks pesticide applications within the state. In 2012, imidacloprid was among the most used pesticides in California, applied to approximately 1.3 million acres and roughly 125 crops, in addition to transplants and container plants (CA DPR 2014).

Agricultural use of neonicotinoids has increased substantially in the last ten years (Figures 3.1.1 & 3.1.2). The EPA estimates that over 3.5 million pounds of neonicotinoids were applied to nearly 127 million acres of agricultural crops annually from 2009 to 2011 (EPA 2012a). Research published in 2015 suggests that these and other estimates of neonicotinoid use likely underestimate actual use due to the increasing practice of planting seeds coated with neonicotinoids (Christian Krupke, pers. comm.; Douglas and Tooker 2015). In addition to usage in agriculture, imidacloprid, thiamethoxam, clothianidin, dinotefuran, and acetamiprid are approved for application on ornamental plants like turf grass, garden shrubs, and trees. Consequently, neonicotinoids can be applied in many diverse settings, including farms, gardens, schools, and other public spaces such as parks.

Figure 3.1: Estimated Annual Agricultural Use of Imidacloprid in the United States: 1994–2013



Source: http://water.usgs.gov/nawqa/pnsp/usage/maps/compound_listing.php



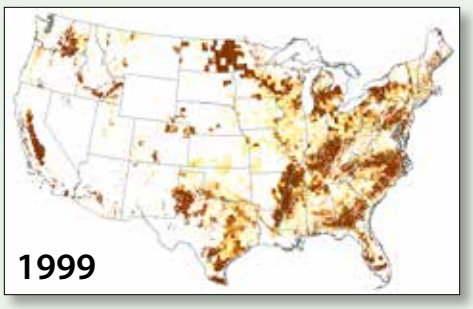
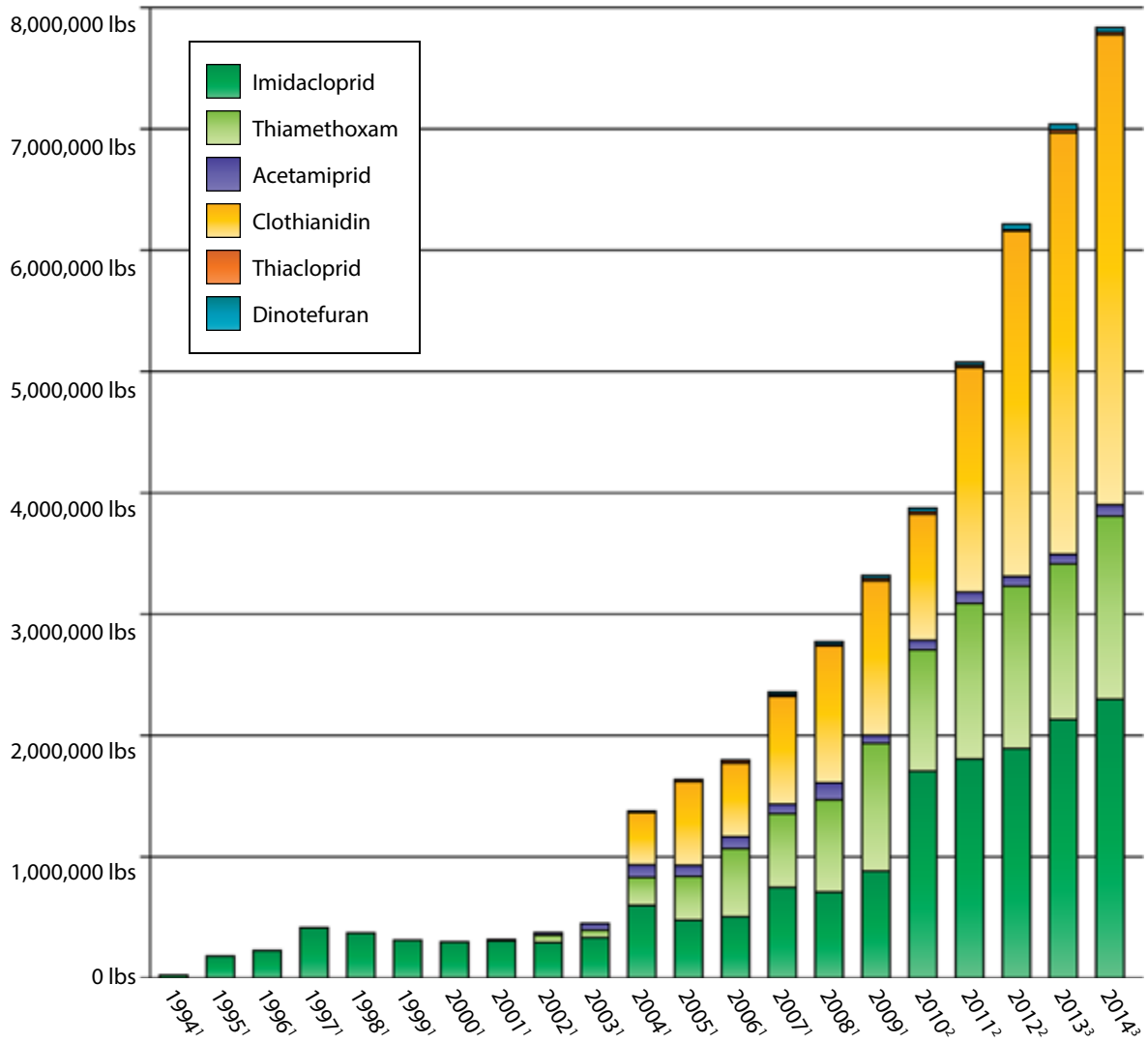


Figure 3.2: Estimated Annual Agricultural Use of Neonicotinoids* in the United States: 1994–2014



Sources:

1. Stone (2013)†: http://pubs.usgs.gov/ds/752/
2. Baker and Stone (2015)†: http://pubs.usgs.gov/ds/0907/
3. Preliminary pesticide use estimates*†: http://water.usgs.gov/nawqa/pnsp/usage/maps/county-level/

* These estimates include coated seed uses

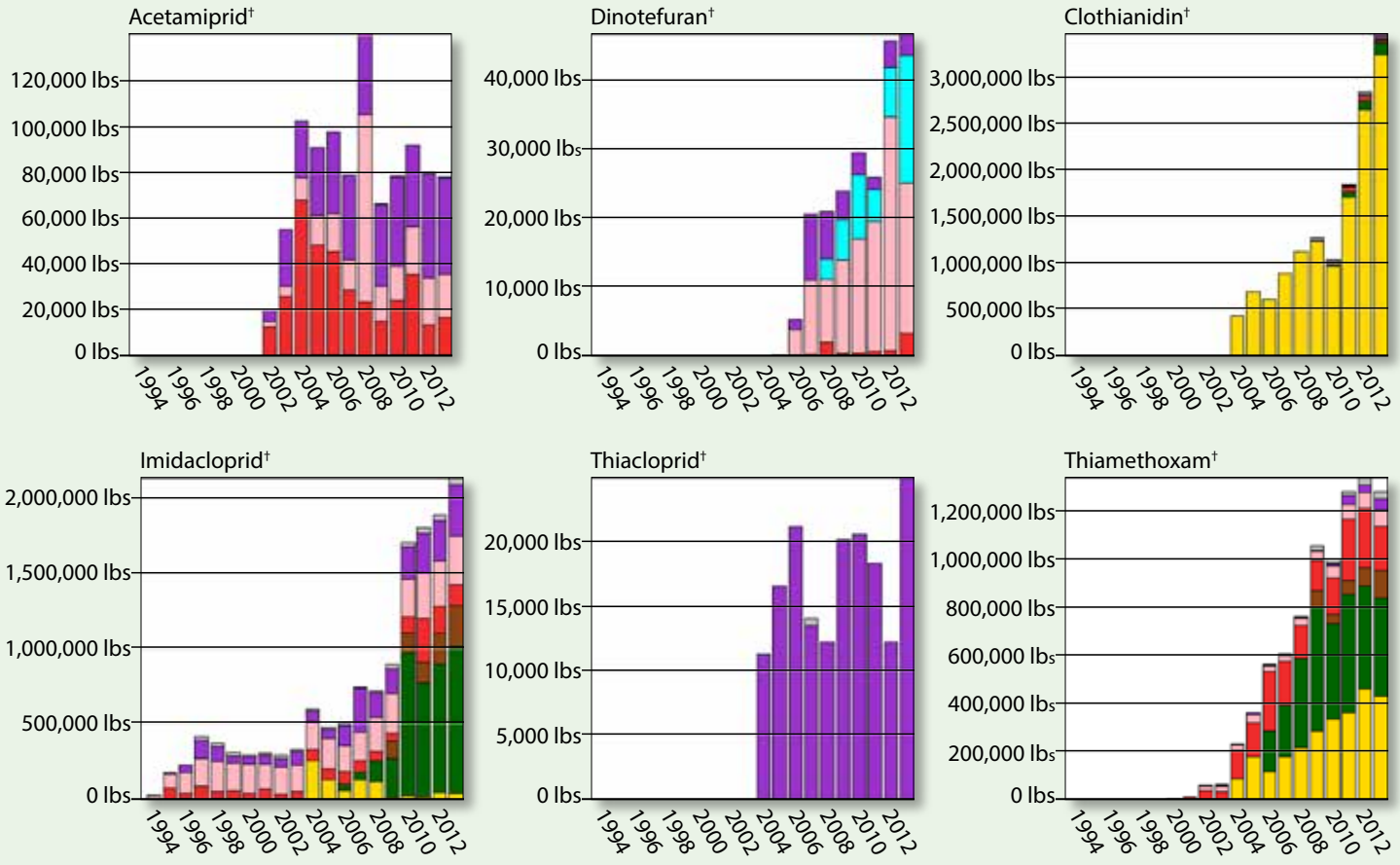
† *Accessed June 24, 2016*

Neonicotinoids paralyze insects by blocking a chemical pathway that transmits nerve impulses in an insect's central nervous system (Tomizawa and Casida 2003). Neonicotinoids are more effective at blocking nerve impulses in insects and other invertebrates than in many other animals. Consequently, they have less direct toxicity to many birds and mammals than the older classes of insecticides they are replacing, including many organophosphate products (Harper et al. 2009; Gervais et al. 2010). Still, new research has raised significant concerns regarding the effects neonicotinoids have on biodiversity and ecosystem functioning (Goulson 2013; Hallmann et al. 2014; Pisa et al. 2014; Chagnon et al. 2015).

Neonicotinoids are systemic insecticides; plants absorb these chemicals (often through roots or leaves) and the vascular tissues transport the chemical into stems, leaves, flowers, and even fruit. This systemic action allows protection of treated plants from boring, sucking, chewing, and root-feeding pests (Jeschke et al. 2011). Neonicotinoids can be applied as seed coatings, soil drenches or granules, foliar sprays, by direct injection into tree trunks, or by chemigation (addition of the insecticide to irrigation water). This variety of application methods, along with their systemic properties and lower toxicity to vertebrates, is one of the primary reasons why these chemicals are so widely used (Elbert et al. 2008).

Figure 3.3: Estimated Use of Neonicotinoids* by Year & Crop: 1994–2013

In addition to increased annual usage of neonicotinoids by weight†, neonicotinoids are being applied to a greater variety of crops. These graphs illustrate the widespread and expanding use of neonicotinoids in different cropping systems.



Sources:

1. Estimated Use of Neonicotinoids: http://water.usgs.gov/nawqa/pnsp/usage/maps/compound_listing.php (*Accessed June 24, 2016.*)

* These estimates‡ include coated seed uses

† Pounds of the **active ingredient (a.i.)** applied

An advantage of neonicotinoids for pest control is that some methods of application (such as trunk injection and basal applications) help to reduce direct contact with nontarget insects during treatment. However, because these chemicals are systemic and absorbed into plant tissues, nontarget insects that rely on nectar, pollen, or other plant resources have increased oral exposure to residues of neonicotinoids and/or their metabolites. Residues have been recorded in pollen (Laurent and Rathahao 2003; Bonmatin et al. 2003, 2005a), nectar (Schmuck et al. 2001; Krischik et al. 2007), and to a much lesser degree, other plant exudates (Girolami et al. 2009). Residues are also found in contaminated dust released from seed planting equipment (Greatti et al. 2006; Krupke et al. 2012; Tapparo et al. 2012) and in non-crop plants growing within or adjacent to treated fields (Krupke et al. 2012; CDRC 2014; Pecenka and Lundgren 2015).

Due to the systemic action of neonicotinoids and their long half-lives, some applications allow for residual activity within the plant for considerable periods of time. Following an application, residue levels in plants decrease, but in some cases can remain at high enough levels to be toxic to pests for months or even years. For example, a single application of thiamethoxam to citrus was enough to suppress pests for five months (Castle et al. 2005) and a single soil application of imidacloprid controlled a wood-boring pest of maple trees for up to four years (Oliver et al. 2010). Imidacloprid residues could be found in rhododendron flowers up to six years after a soil treatment (Doering et al. 2004b). Research out of Pennsylvania State University demonstrates that not all neonicotinoid applications will cause residues to carry over from one growing season to the next. Researchers found that foliar applications of dinotefuran to apple trees in the fall did not result in residues in the flowers the following spring (David Biddinger, pers. comm.).

Some neonicotinoids can persist for extended periods in soil (see Table 3.1) (Rouchaud et al. 1996; Maus et al. 2004a, b). Clothianidin, for example, has a soil half-life of between 148 and 1,155 days (5¼ and 38½ months), depending upon soil types (EPA 2003a). Soil residues may be present for several years following an application (Jones et al. 2014), and untreated plants may take up residues of neonicotinoids still present in the soil from previous applications (Bonmatin et al. 2003, 2005b). There is also evidence to suggest that repeated applications to the soil or repeated plantings of coated seeds can cause soil residues to increase steadily over time (DAR 2006; EFSA 2008; Goulson 2013).

Neonicotinoids are water soluble at varying levels depending on the product (Gervais et al. 2010), and, accordingly, have the potential to move into surface water or leach into groundwater under some uses. Untreated plants can, therefore, be unintentionally contaminated; thiamethoxam, for example, was detected in potato crops irrigated with contaminated groundwater (Huseth and Groves 2014). Some neonicotinoids, such as imidacloprid, may be persistent in water (Tisler et al. 2009).

In California, 89% of water samples from rivers, creeks, and agricultural drains contained imidacloprid (Starner and Goh 2012). In the Midwest, Hladik et al. (2014) detected neonicotinoids in all nine streams from which they took water samples, with multiple neonicotinoids common. Of the 79 samples, clothianidin was detected 75% of the time, thiamethoxam 47%, and imidacloprid 23%; dinotefuran and acetamiprid were each only detected once. Imidacloprid, thiamethoxam, and clothianidin

Table 3.1: Half-life in Soil of Neonicotinoids

Neonicotinoid	Half-life in Soil*
Acetamiprid	1–8 days[1]
Clothianidin†	148–1,155 days[2]
Dinotefuran	138 days[3]
Imidacloprid	40–997 days[4]
Thiacloprid	1–27 days[5]
Thiamethoxam†	25–100 days[6]

Notes:

* Aerobic soil metabolism

† Clothianidin is a primary metabolite of thiamethoxam

Sources:

1. EPA 2002
2. EPA 2003a
3. EPA 2004
4. Gervais et al. 2010
5. EPA 2003b
6. Syngenta Group 2005

were detected in groundwater in Wisconsin repeatedly over a five-year sampling period (Huseth and Groves 2014). In a study in Saskatchewan, clothianidin and thiamethoxam were found in the majority of water samples taken from wetlands (Main et al. 2014). A study of rainwater puddles in the corn fields of southwest Quebec detected one or more neonicotinoids in each of the 59 samples (Samson-Robert et al. 2014).

Although large knowledge gaps remain, persistence of neonicotinoids in plants, soil, and waterways are likely impacting a broad range of wildlife (Goulson 2013; Pisa et al. 2014; Chagnon et al. 2015; Morrissey et al. 2015).

3.2 Prophylactic Use of Neonicotinoids: A Shift Away from Integrated Pest Management

Given the important role of pollinators, as well as other beneficial insects, in our natural areas and farms, pest management decisions should balance the need to control pests with the importance of maintaining healthy beneficial insect populations.

Integrated pest management (IPM), which involves the use of various methods to reduce crop damage, provides an important framework to lessen the effects of insecticides on nontarget species. In particular, IPM encourages preventive measures to reduce initial pest build up. These include cultural practices such as planting pest-resistant crop varieties or rootstocks, removing crop residue, using trap crops, and creating on-farm habitat for predator and parasitoid insects that suppress pest species. IPM also requires monitoring of pest and beneficial insect populations so that growers know when to take action before pests reach economically damaging levels. When a pest outbreak does occur, IPM encourages the use of nontoxic options when available (e.g., pheromone mating disruption for moth pests) as an initial strategy, before resorting to pesticide use. Insecticides are employed as a last resort, ideally only treating areas where pests are documented above an economic threshold and then using the most selective product to kill the primary pest without disrupting biological control of other secondary pests. As insecticide uses are weighed, sometimes less selective (i.e., broad spectrum) pesticides are used in a selective manner by adjusting the timing of the application (see the Case Study: Incorporating Pollinator Health into Pennsylvania Apple IPM, pages 10–11).

Routine (calendar-based) applications and preemptive treatments without documented pest problems are contrary to the philosophy of IPM. The increasing prophylactic use of neonicotinoids, such as seed coatings, applied before pest damage has occurred (Sur and Stork 2003) represents a shift away from IPM. A prophylactic approach negates the principles of IPM because insecticides are used before their need is demonstrated, and it hinders the use of biological control agents. For example, in a recent field study researchers found that the use of thiamethoxam-coated seeds depressed the activity and density of the predatory ground beetle *Chlaenius tricolor*, thereby partially eliminating predation of crop-damaging slugs, resulting in a 5% reduction in soybean yield (Douglas et al. 2015).

This shift away from IPM strategies is in part due to a lack of applied research that is independent of pesticide company trials and a reduction in the use of cooperative extension technical support to train growers and private consultants in the adoption of new IPM practices. If growers are to implement pest management plans that incorporate the various IPM components, greater support for applied research by university and other independent scientists is needed.

Table 3.2: Examples of Neonicotinoid Products Used in the United States

Neonicotinoid	Registered Use in the United States[1]	Example Product Trademark Names[2]
Acetamiprid	Foliar spray for leafy and fruiting vegetables, cole crops, citrus fruits, pome fruits, small fruits, stone fruits, grapes, and cotton Foliar spray for flowers, trees, and shrubs	Assail Tristar Ortho Flower, Fruit and Vegetable Insect Killer* Ortho Rose and Flower Insect Killer*
Clothianidin	Seed treatment, foliar spray, or soil drench for a variety of field and tree crops Granules, soil drench, or foliar spray for turf, flowers, trees, and shrubs	Arena Poncho Sepresto Aloft Green Light Grub Control with Arena
Dinotefuran	Soil drench or foliar spray to a wide range of leafy and fruiting vegetables; also apples Granules, soil drench, or foliar spray for turf, flowers, trees, and shrubs Bait or granules for cockroach control	Venom Scorpion Green Light Tree & Shrub Insect Control with Safari Safari
Imidacloprid	Seed treatment, soil drench, granules, trunk injection (for trees), or spray to a wide range of field and tree crops, and pome fruits Soil drench, granules, trunk injection, or foliar spray for turf, flowers, trees, and shrubs Topical application on pets for flea control and to buildings for termite control	Admire Gaucho Imicide Provado Malice Sepresto Wrangler Bandit Bayer Advanced 3-in-1 Insect, Disease, & Mite Control Bayer Advanced 12 Month Tree & Shrub Insect Control Bayer Advanced 12 Month Tree & Shrub Protect & Feed Bayer Advanced Fruit, Citrus & Vegetable Insect Control Bayer Advanced All-in-One Rose & Flower Care concentrate Marathon Merit Monterey Once a Year Insect Control II Ortho Bug B Gon Year-Long Tree & Shrub Insect Control†
Thiamethoxam	Seed treatment, soil drench, injection, granules, or foliar spray to a wide range of field crops and pome fruits Soil drench, trunk injection, granules, or foliar spray for turf, flowers, trees, and shrubs	Actara Adage Cruiser Centric Flagship Meridian

Notes

1. Registered uses: agricultural (), ornamental (), and residential ()
2. In April 2016, Ortho announced the planned phase out of all neonicotinoid active ingredients in its lawn and garden products by 2021(*), with the most toxic neonicotinoid ingredients to be phased out by 2017(†).

Incorporating Pollinator Health into Pennsylvania Apple IPM

Integrated pest management (IPM) is a long-standing, science-based decision-making process with ecological roots. IPM uses multiple biological, cultural, physical, and chemical tactics to protect crops in a way that minimizes economic, human health, and environmental risks. IPM can adapt to address any pest complex (insect, disease, weed, vertebrate, etc.) and can be adjusted to incorporate ecosystem services such as the protection of pollinators or predators of crop pests. The IPM paradigm, already understood by growers, offers a valuable opportunity to help growers adopt pollinator protection practices (Biddinger and Rajotte 2015).

Over half of Pennsylvania's apple growers rely on native pollinators—like the ground-nesting cellophane bee (*Colletes* spp.), above, and mining (*Andrena* spp.) bees, below—found naturally living in and around orchards.

Pollination by bees, both wild and managed, is critical for apple production. A 2011 survey showed that over half of Pennsylvania's apple growers, including many large-acreage operations, do not rent honey bees for apple pollination. Honey bee hives are increasingly expensive to rent (costs have risen by more than 3-fold since 2006), and researchers have found that most growers in this region receive significant pollination from wild bees living in and around orchards. Therefore, many farmers are looking to sustain and increase pollination for their crops by increasing the number of wild bees on their farms. In what is being termed "integrated pest and pollinator management" (IPPM), IPM strategies and tactics are being designed to minimize pesticide use and guide the use of selective pesticides or broad spectrum insecticides in selective application methods or timings to help protect pollinators and ensure sustainable fruit pollination in the eastern U.S. (Biddinger and Rajotte 2015).

Given the pest pressure in apple orchards—more than two dozen major insect and mite pests, in addition to fungal and bacterial infections, attack the fruit and trees—IPM in apple production is quite complex. Conventional IPM tactics in Pennsylvania tree fruit include host plant resistance, biological control, sophisticated pest monitoring, pest predictions based on mathematical models, and highly specific pesticide applications. These techniques are developed through university research programs, and growers are taught how to implement the practices through comprehensive extension education programs.

Complicating pest control in Pennsylvania apple production, however, is the rosy apple aphid (*Dysaphis plantaginea*), a major pest which stunts and deforms fruit and can also reduce tree vigor. The rosy apple aphid is resistant to organophosphate and pyrethroid insecticides, and currently no clear commercially viable alternative control methods exist. However, the rosy apple aphid can be controlled by neonicotinoids, and applications must occur early in the growing season before bloom so that

the developing fruit will not be deformed. Post-bloom applications will control aphid colonies, but are too late to prevent fruit stunting.

While neonicotinoids are currently under scrutiny for their role in pollinator decline, two neonicotinoids, acetamiprid and thiacloprid, are significantly less toxic to bees than the others (imidacloprid, thiamethoxam, dinotefuran, and clothianidin) (see Table 5.1 on page 20 of this report). (Note: The use of thiacloprid has been voluntarily cancelled in the U.S. but it is still in use in other countries.) Recognizing the need for both effective control for the rosy apple aphid and protection of bees visiting apple bloom, it is critical to obtain a greater understanding of pollinator exposure to acetamiprid and thiacloprid. Direct contact with these two neonicotinoids is very unlikely to be a main route of exposure for bees in Pennsylvania apple orchards, since the chemicals are not typically applied during apple bloom and bees would not contact them during foraging. The main route of exposure of bees to acetamiprid or thiacloprid is ingestion of contaminated pollen and nectar from applications that were applied pre-bloom. Therefore, knowing the levels of pesticide in the nectar and pollen under field conditions is a critical step in determining the exposure level of bees subjected to low doses in multiple flower visits over time.

When the decision is made to use an insecticide, the chemical's selectivity toward beneficial insects is often overlooked in favor of a cheaper product that is advertised as having a longer residual or a broader spectrum. Although both clothianidin and thiamethoxam are registered for pre-bloom use and will effectively control the rosy apple aphid in apple orchards, researchers from Pennsylvania State University recommend the less-toxic, less-systemic neonicotinoid compounds acetamiprid and thiacloprid for this pest, even though they may be more expensive than the alternatives. Residues in the nectar and pollen from apple blossoms treated with thiacloprid and acetamiprid are much lower from the same pre-bloom application timing than the other neonicotinoids because they are less systemic and have shorter residual activity. Efficacy for all products on the aphid pest was equivalent. To further minimize exposure levels, the researchers confirmed pest control is still effective when acetamiprid and thiacloprid are applied 10–12 days earlier than the traditional timing (when buds are pink) during the earlier leaf budding, known as the ½-inch green stage. This shift gave the same level of management of the aphid pest, while residues in nectar and pollen fell below the 2 ppb detection level. (D. Biddinger, unpublished data). This IPPM research from Pennsylvania State University demonstrates that some neonicotinoids can be used within the context of IPM to control a problematic pest while minimizing risk to pollinators.

...

This overview was adapted from a case study written by David J. Biddinger[1,2], Neelendra K. Joshi[1,2,3], Sarah Shugrue[1,2], and Edwin G. Rajotte[2]:

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4

Routes of Neonicotinoid Exposure to Bees

Bees may be exposed to neonicotinoids in numerous ways, including direct contact with spray residue on plants or through ingestion of contaminated pollen or nectar. Other flower-visiting insects, including butterflies, flies, and beetles—many of which are pollinators—can also be affected in this way. However, the presence of systemic insecticides in plants poses a particular risk to bees, because they feed on nectar as adults and collect nectar and pollen to feed their offspring. This range of exposure routes was not considered during the registration process for neonicotinoids or ongoing regulation of insecticides by EPA, which registers and monitors pesticides in the United States (EPA 1996). In July 2014, the EPA published new risk assessment guidelines that, in part, emerged from a Society of Environmental Toxicology and Chemistry Pellston Conference on Pesticide Risk Assessment for Pollinators (Fischer and Moriarty 2014; EPA 2014a). The guidelines present valuable recommendations to assist researchers in designing studies to better evaluate the risks pesticides pose to bees, but they still fail to address the range of exposure routes that native pollinators face. As such, assessments could underestimate risk to pollinators.

One factor affecting bee exposure is the relationship between foraging distance and species size. Bumble bees, honey bees, alkali bees, and other large species can easily forage a mile or more from their nest, whereas small bees—sweat bees, blue orchard bees, leafcutter bees, and many others—may only fly a few hundred meters (Greenleaf et al. 2007). Their shorter flight distance may result in a disproportionate risk to small bees that nest near treated crops, because their limited foraging range results in ongoing exposure to neonicotinoids while they gather food or nest materials. In contrast, honey bee and bumble bee colonies will likely forage from a more diverse set of plants, over a much larger area, complicating their potential exposure levels.

The sections that follow describe nine routes by which bees can be exposed to neonicotinoids. It should be noted that the presence of a route of exposure is neither evidence of contamination nor of a hazard to bees, but simply one way in which bees may encounter neonicotinoids. Also, exposure does not equate to harm for bees. Contact with neonicotinoids may result in lethal, sublethal, or no effects.

4.1 Direct Contact

Direct contact with foliar spray may be the most obvious exposure route for bees. This can occur when applications are made while bees are actively foraging on flowers or nesting in the ground within the application area, or when pesticides drift onto adjacent habitat.

The body sizes of bees may influence how they react to contact neonicotinoid exposure. Many solitary bees are significantly smaller than honey bees and therefore receive a relatively higher contact dose because of the higher surface area to volume ratio. Tests of pesticide toxicity to worker bumble bees of the same species have confirmed that toxicity may correlate with body size: smaller bumble bees tend to have a lower LD_{50} and larger bees a higher LD_{50} (Thompson and Hunt 1999; Malone et al. 2000). (LD_{50}

is the lethal dose that kills 50% of study organisms; a small LD_{50} indicates a more toxic substance.)

4.2 Contaminated Pollen and Nectar

Pollen and nectar may be contaminated by neonicotinoids irrespective of how the chemicals are applied. Neonicotinoids have been found in pollen loads brought to hives by honey bees (Chauzat et al. 2006; Pettis et al. 2013), in pollen stored within honey bee hives (Bernal et al. 2010; Mullin et al. 2010; Krupke et al. 2012), in honey stored within hives (Chauzat et al. 2009), and in bee bread (Pohorecka et al. 2012; Giroud et al. 2013). The presence of these products in nectar and pollen delivers the active ingredient directly to bees and other pollinators. Some systemic insecticides, when applied in a manner designed to be taken up by the plant, can be very persistent staying in plant tissues for many months or even years, and may build up after repeated applications (Doering et al. 2004a; Oliver et al. 2010).

Exposure risks from contaminated pollen and nectar are likely greater for native bee larvae relative to European honey bee larvae. Honey bee larvae are primarily fed brood food, a substance secreted by adult workers and in which pesticide residues may have been reduced by the adult nurse bees, and consume only small amounts of diluted honey and pollen (Winston 1987). In fact, direct feeding on pollen comprises only about 5% of the total protein consumed during larval development (Babendreier et al. 2004). Larvae of bees native to North America typically feed directly on raw pollen, undiluted nectar, or both (Michener 2007), each of which may contain neonicotinoid residues.

4.3 Residue Contact

Exposure to neonicotinoid residues occurs when bees visit flowers or walk on leaves that have been directly treated with foliar spray. This is especially problematic when a neonicotinoid has a long persistence in the field. For example, clothianidin residues applied to foliage will remain toxic to honey bees for 5–21 days (EPA 2010).

Study Finds that Bees Prefer Neonicotinoid Contaminated Food

Some scientists have questioned whether bees can choose to avoid neonicotinoid contaminated nectar and pollen, thus avoiding or diluting their exposure. A study by Kessler et al. (2015) casts doubt on that theory. The researchers evaluated the ability of European honey bees (*Apis mellifera*) and buff-tailed bumble bees (*Bombus terrestris*) to taste or be repelled by the neonicotinoids imidacloprid, clothianidin, or thiamethoxam.

The study found that the bees were not repelled by any of the chemicals nor could they taste them. Furthermore, the researchers were surprised to find that both the honey bees and bumble bees preferred to eat either the imidacloprid- or thiamethoxam-laced sucrose. Kessler et al. (2015) concluded that bees cannot control their exposure to neonicotinoids, and that flowering crops treated with imidacloprid and thiamethoxam are a hazard to foraging bees.

. . .

The Link Between Application Rate and Risk

Risk is generally defined as hazard (i.e., toxicity) plus exposure. That equation serves as a reminder that it isn't enough to simply know the toxicity of a chemical. Additionally, people must take into account the potential exposure amount. In determining risk, it is important to keep in mind that there is significant variability in application rates between chemicals and even between products that contain the same chemical. Yet, the application rate has a direct link to the residue levels detected and the amount of a chemical to which a bee would be exposed.

In Section 5, we present research on the amount of each neonicotinoid that causes either lethality or other harm that could negatively affect pollinators. These numbers are only part of the story to determine risk. For example, we cannot assume that a "less toxic" pesticide is always the better choice because the application rate that is effective at controlling pests might be high enough to cause significant risk. It also is not accurate to state that a pesticide applied at very low amounts is somehow better than those that have high application rates because if the pesticide is harmful at very low amounts that low application rate could still lead to significant risk.

4.4 Particles Released During the Planting of Coated Seeds

Abraded seed coatings have drifted onto flowers adjacent to cropland during planting of neonicotinoid-coated seeds resulting in bee kills (Greatti et al. 2006; Tapparo et al. 2012; but see Schnier et al. 2003). Beekeepers in the Baden-Württemberg region of Germany suffered spring colony losses after the fixative agent that glues clothianidin to seed coats was not applied to rapeseed (canola) and sweet corn, and clothianidin dust released via seed abrasions during sowing drifted onto nearby blooming vegetation (de la Rúa et al. 2009; Pistorius et al. 2009). Even when fixative agents are used on coated seeds, the planting process may expose bees to lethal levels of neonicotinoids in aerial insecticidal powders (Tremolada et al. 2010; Girolami et al. 2012, 2013; Krupke et al. 2012; Tapparo et al. 2012).

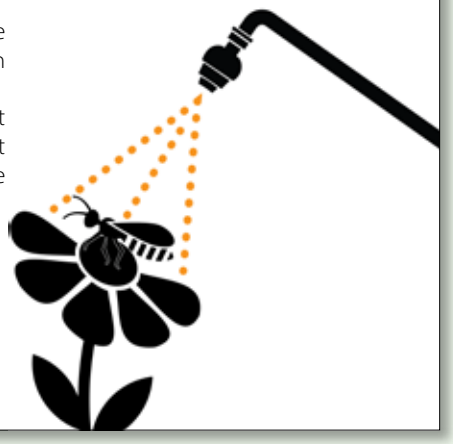
In the United States, where neonicotinoid-coated seed is used for many annual crops, talc is often added to the seed boxes of planters to aid the flow of the sticky coated seeds during planting (Krupke et al. 2012). Excess talc is exhausted during planting, either onto the soil or into the air behind the planter. Levels of clothianidin and thiamethoxam that far exceed levels known to be lethal to honey bees have been found in the talc exhausted from plantings of seed-treated corn (Krupke et al. 2012). Talc is highly mobile and can contaminate flowers within or near treated fields (Krupke et al. 2012), even beyond 100 meters into the field margin (CDRC 2014; Christian Krupke, pers. comm.). Alternative seed lubricants have since been proposed in an effort to reduce the release of active ingredient and dust with planting. Tested by University of Guelph researchers in 2013, a novel seed lubricant developed by Bayer CropScience had mixed results. The lubricant did reduce overall dust and active ingredient emissions; however, the concentration of neonicotinoids (clothianidin and thiamethoxam) in the dust that was exhausted was three times higher than known residues in exhausted talc (CDRC 2014).

The full extent to which bees are exposed to neonicotinoids during planting is unknown, but millions of acres of coated seed are planted each year (Krupke et al. 2012; Douglas and Tooker 2015). In 2012, over 40 beekeepers in Ontario reported significant losses of honey bees. The Health Canada Pest Management Regulatory Agency determined that the deaths were likely due to insecticide-contaminated dust, based on the detection of clothianidin residue in dead bees and the timing of deaths coinciding with planting of corn (Health Canada 2013).

Figure 4.1: Pesticide Exposure Pathways to Bees

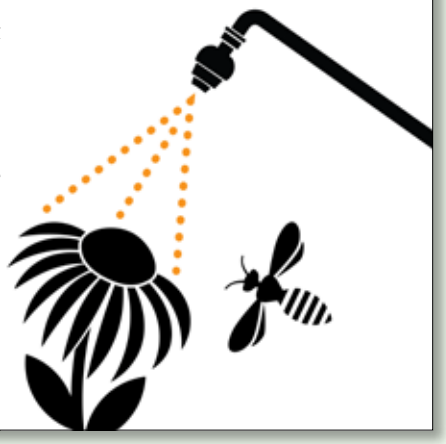
Direct Contact
occurs when pesticide sprays directly land on bees.

The risk of direct contact is highest when chemicals are sprayed on or near blooming crops, weeds, cover crops, or habitat areas.



Residue Contact
occurs when bees visit flowers or walk on leaves that have been previously treated with pesticides.

The risk of residue contact is especially problematic with chemicals with a long half-life.



4.5 Contaminated Nesting Areas

When neonicotinoids are applied to or drift onto areas of bare ground, even within fields, they may contaminate potential nest sites for ground-nesting bees. Nearly 70% of native bee species in North America nest in the ground, even within orchards and field crops. Squash bees (genus *Peponapis*), for example, frequently construct underground nests at the base of squash plants (Shuler et al. 2005), where they may come in contact with residues from soil drenches, chemigation, or seed coatings. (For information about how long residues remain in the soil, see Table 3.1.)

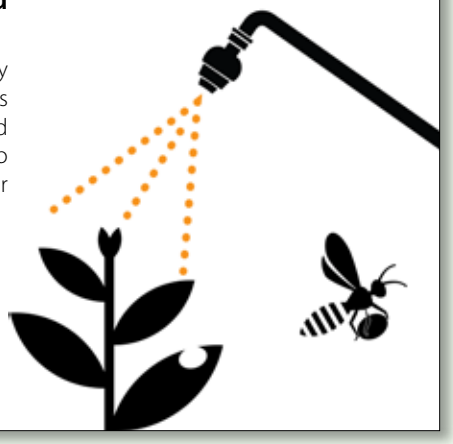
Similarly, application to shrubs in ornamental landscapes or spray drift into shrubby areas next to farm fields may contaminate nest sites for tunnel-nesting bees. Also, drift into overgrown habitat or forest edges may contaminate potential bumble bee nesting sites.

4.6 Contaminated Nesting Material

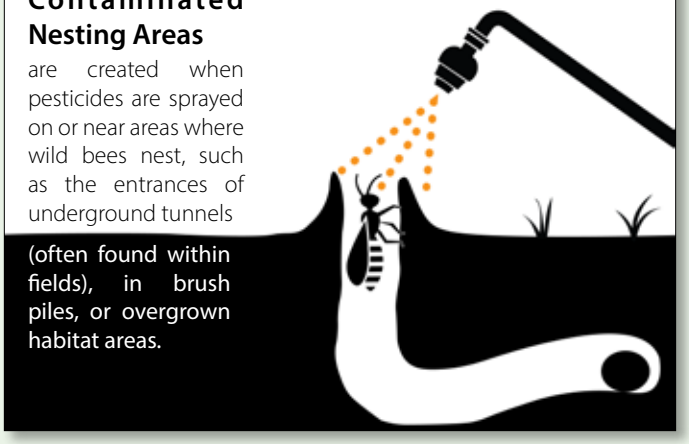
Many solitary bees may be exposed to neonicotinoids when the materials they use to construct their nests are contaminated. About 30% of native bee species use existing cavities—often those made by beetles in dead trees—or excavate their own small cavities in pithy plant stems. Many of these bees gather mud or plant materials to construct the brood cells, and in doing so may be exposed to neonicotinoid residues. For example, leafcutter bees (genus *Megachile*) use pieces of leaves to wrap their brood cells, and mason bees (genus *Osmia*) separate their brood cells with walls of mud. Both the leaf pieces and mud may be contaminated.

Although honey bees do not collect outside resources to construct their combs (wax is a glandular secretion), wax comb has been found to contain neonicotinoids (Mullin et al. 2010; Wu et al. 2011). Honey bee eggs and larvae exposed through residues in the brood comb may suffer effects that may later influence colony health. Exposure to sublethal levels of multiple pesticides in wax brood combs resulted in delayed development of honey bee larvae and reduced survivorship of adults (Wu et al. 2011).

Contaminated Nesting Material can include previously sprayed leaf sections or soil collected by solitary bees to construct or line their nests.



Contaminated Nesting Areas are created when pesticides are sprayed on or near areas where wild bees nest, such as the entrances of underground tunnels (often found within fields), in brush piles, or overgrown habitat areas.



4.7 Contaminated Water

Honey bees may be exposed to neonicotinoids when they gather water to cool their hives on warm days or to dilute their honey to feed to their offspring. Other bees may also be exposed to contaminated water; some ground-nesting bees will use water to moisten hard packed soil prior to excavating their nest or may collect wet soil to divide brood cells within their nest. Water sources may be contaminated by chemigation leaks, overspray, drift, or field runoff. A survey of water sources within half a mile of honey bee hives found that some had sublethal levels of imidacloprid (Johnson and Pettis 2014). A study of water puddles in cornfields found a wide range of agricultural pesticides including clothianidin, imidacloprid, and thiamethoxam at levels high enough to cause sublethal effects (Samson-Robert et al. 2014). Honey bees will also drink from leaking chemigation equipment (David J. Biddinger, unpub. data).

Neonicotinoids are water soluble (Gervais et al. 2010) and, accordingly, have the potential to move into surface water or leach into ground water under some uses. Some, such as imidacloprid, may be persistent in water (Tisler et al. 2009). In California, 89% of water samples from rivers, creeks, and agricultural drains contained imidacloprid (Starner and Goh 2012). In the Midwest, Hladik et al. (2014) detected neonicotinoids in all nine streams from which they took water samples, with multiple neonicotinoids common. Of the 79 samples, clothianidin was detected in 75% of them, thiamethoxam in 47%, and imidacloprid in 23%; dinotefuran and acetamiprid were each only detected once (Hladik et al. 2014). Clothianidin and thiamethoxam were found in the majority of water samples taken from wetlands in Saskatchewan, though detection fluctuated with the season between 16% and 91% of samples (Main et al. 2014).

4.8 Guttation Fluid

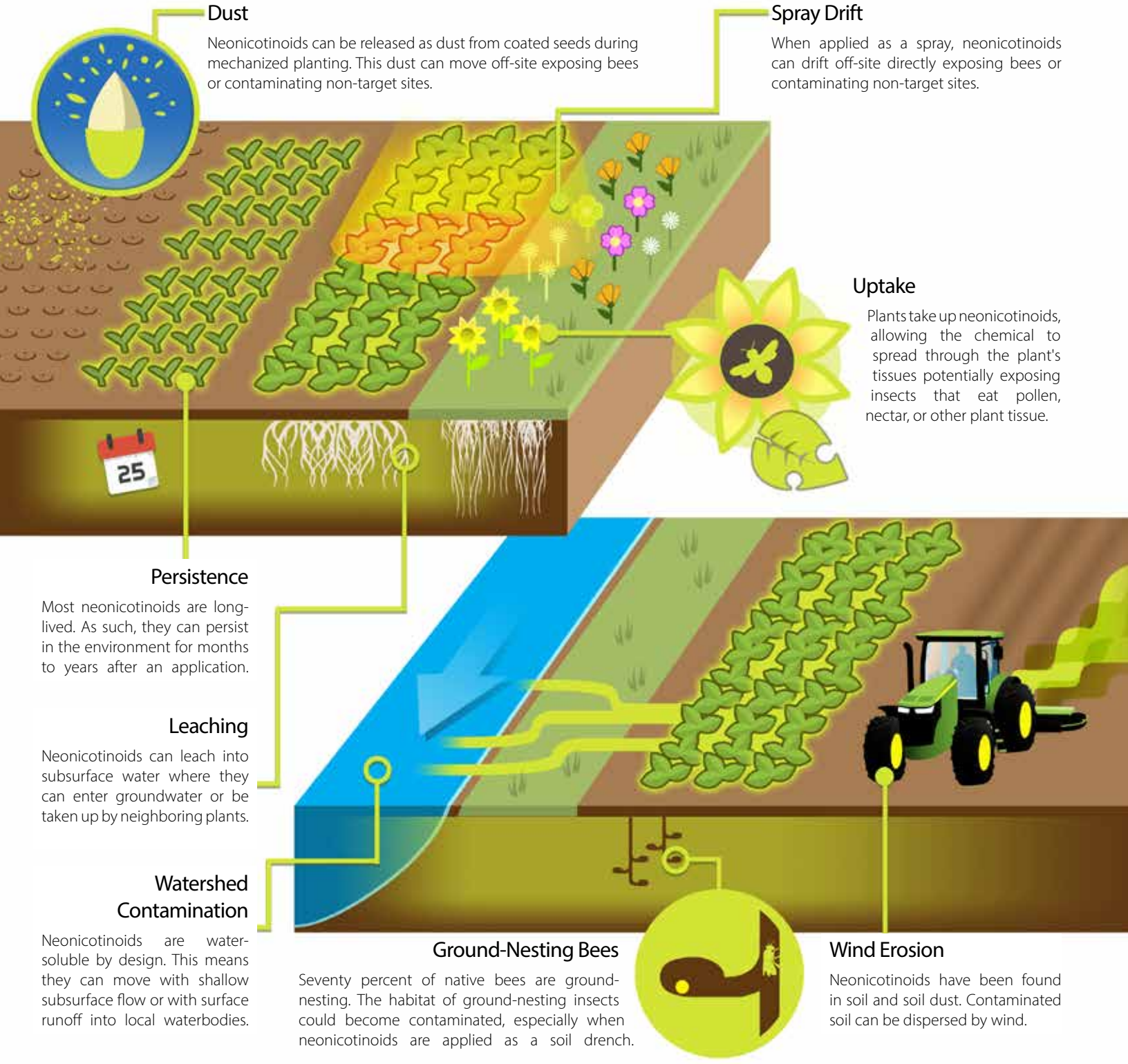
Guttation fluid is the water secreted by plants when soil moisture is high. It is typically seen in the morning, as droplets exuded at the tip of the plant or around leaf edges. Honey bees and other pollinators may collect these droplets from plants treated with systemic insecticides. Girolami et al. (2009) determined that guttations of seed-treated corn plants can contain high concentrations of imidacloprid, clothianidin, and thiamethoxam, and that these droplets are highly toxic to honey bees. Toxic levels of imidacloprid have also been reported in melon guttations (Hoffman and Castle 2012). The level of risk this contamination poses is unclear since the frequency at which honey bees actually consume guttations in a field setting is unknown.

4.9 Extrafloral Nectaries

Extrafloral nectaries are nectar-producing glands located outside the flower, often on leaves or stems. Extrafloral nectaries are not directly involved in pollination, though bees (and other beneficial insects) are attracted to them. Common plants with extrafloral nectaries include crops such as peaches, cotton, sunflower, and pumpkins, and plants found in yards such as morning glory, black locust, and willows. Given that neonicotinoids can be found in floral nectar, it is highly likely that neonicotinoid concentrations will also occur in extrafloral nectaries, though to date no studies have investigated their potential for contamination.

Figure 4.2: Neonicotinoid Movement in the Environment

Neonicotinoids are being found through the landscape in areas where they were not applied. This figure illustrates some of the main pathways for neonicotinoid movement in the environment and also shows how this movement could expose beneficial insects.



5

Effects of Neonicotinoid Exposure on Bees

5.1 Research Study Bias

Research investigating the effects of neonicotinoids on pollinators is primarily restricted to bees, largely the honey bee. Honey bees are the primary tested species because they are economically important, readily available in large numbers, and have existing test protocols. Nearly 60 studies have investigated effects of neonicotinoids on honey bees. As bumble bee colonies have become more available through commercial rearing, increased testing on bumble bees has occurred. To date more than 30 peer-reviewed studies involving neonicotinoids and bumble bees have been published.

To our knowledge, only seven studies of effects of neonicotinoids on solitary bees have been published. These have involved species that are managed to varying degrees for commercial pollination, including the blue orchard bee (*Osmia lignaria*), the alkali bee (*Nomia melanderi*), the alfalfa leafcutter bee (*Megachile rotundata*), the Japanese orchard bee (*O. cornifrons*), and the red mason bee (*O. bicornis*). The lack of studies relating to effects of neonicotinoids on wild, unmanaged species is of great concern because these bees comprise most of the bee species in North America and also contribute significantly to crop pollination (Winfree et al. 2007; Garibaldi et al. 2013).

Given that the life history traits of bees differentially affect their susceptibility to insecticides (Brittain and Potts 2011), and the evidence from a vast body of research that one insect species will not respond to insecticides like any other (Robertson et al. 2007), native bees and honey bees no doubt respond differently to neonicotinoid exposure (Biddinger et al. 2013).

Of the neonicotinoids that have been investigated, imidacloprid is by far the most studied (approximately ¾ of neonicotinoid studies), followed by clothianidin, thiamethoxam, acetamiprid, and thiacloprid. Dinotefuran is the least studied, despite the fact that it is currently allowed for use on bee-visited ornamental plants as well as several flowering vegetable crops, and may be used under an emergency registration for late season control of the brown marmorated stink bug on bee-pollinated fruit trees in some mid-Atlantic states.

Honey bees are the subject of the majority of scientific studies on bees. Information about solitary bees—the majority of species—is lacking.

5.2 Honey Bees and Neonicotinoids

Honey bees (*Apis mellifera*) live in large perennial colonies consisting of a single queen, tens of thousands of worker bees, and several hundred male drones (Winston 1987). There is a division of labor within the colony, with worker bees of different ages taking on different roles (for example, as nurse bees, nest entrance guards, or food-gathering foragers), while the queen is responsible for egg-laying. The growth of a colony is closely tied to its food stores. Honey bees are generalist foragers that gather nectar and pollen from a wide range of flowers through the seasons. The remarkable communication between foragers and their ability to navigate to food sources is important to colony survival. The colony social structure and generalist foraging contributes to the convenience of managing honey bees for agricultural pollination: thousands of bees nest in an easy-to-move hive box that can be transported from crop to crop. Honey bee colonies are trucked across the country to pollinate almonds in California, citrus in Florida, blueberries in Maine, and various crops in other states. Honey bees are responsible for at least $15 billion worth of crop pollination each year in the United States (Morse and Calderone 2000). However, the prevalence of honey bees in flowering crops frequently exposes them to a wide range of agrochemicals, including neonicotinoids.

5.2.1 Lethal Toxicity of Neonicotinoids to Honey Bees

Based on laboratory estimates of oral and/or contact LD_{50}, clothianidin, dinotefuran, imidacloprid, and thiamethoxam, are considered highly toxic to honey bees, and acetamiprid and thiacloprid are considered moderately toxic (Table 5.1). Acetamiprid and thiacloprid have structural differences that make them less toxic to honey bees than the other four (Iwasa et al. 2004; Jones et al. 2006). As a result, the contact LD_{50} for thiacloprid in honey bees is 816 times larger than that of imidacloprid (Iwasa et al. 2004) and 323 times higher for acetamiprid than imidacloprid (Biddinger et al. 2013). Neonicotinoid spray can be toxic to honey bees, either through direct contact with the spray or via residue on a recently sprayed leaf (Costa et al. 2014).

Although not a route of exposure that was considered in earlier risk assessments, bee contact with neonicotinoid-laced dust or powder became a concern after bee deaths were reported in Europe (de la Rúa et al. 2009; Pistorius et al. 2009; Tremolada et al. 2010) and the United States (Krupke et al. 2012). In the U.S., concentrations in exhausted talc used to keep coated seeds flowing inside planters were as high as 15,030 ppm (clothianidin) or 13,240 ppm (thiamethoxam), levels that are lethal on contact to honey bees (Krupke et al. 2012). As there are many field crops that utilize neonicotinoid seed coatings (e.g., corn, soybean, sunflower, wheat, and canola) that are planted on millions of acres of land in the U.S., it is clear that this route of exposure presents a risk that needs remediation.

Most neonicotinoids demonstrate greater toxicity to honey bees by oral consumption than by contact (Suchail et al. 2000). Many of the metabolites (breakdown products) of neonicotinoids are also toxic to bees. Whereas some are less toxic than the parent compound, others are just as toxic or more so. For example, olefin-imidacloprid is approximately two times more toxic than imidacloprid (Suchail et al. 2001) and thiamethoxam actually breaks down into another highly toxic neonicotinoid, clothianidin (Nauen et al. 2003).

A Note About Numbers

Studies involving neonicotinoids use different units to measure residue levels. These include parts per billion (ppb), micrograms per liter (µg/L), micrograms per kilogram (µg/kg), and milligrams per kilogram (mg/kg). To help with comparing figures between studies, throughout this report a conversion into ppb is given in parentheses after each figure, where needed. See the conversion table on page 67 for more details.

The oral lethal concentration of imidacloprid needed to kill 50% of a test population (the LC_{50}) of honey bees from a short term acute exposure is 185 ppb (CA DPR 2008; Schmuck et al. 2001). While imidacloprid residues of 185 ppb in pollen and nectar are unlikely to result from applications of label rates of coated seed, such residue levels could occur in plants under certain circumstances. For example, as discussed in Section 6.3, soil drenches (Doering et al. 2004b, 2005a, 2005b) and trunk injections (Maus et al. 2004b) of imidacloprid at label rates approved for home and garden use resulted in residue levels in blossoms well above the LC_{50} for honey bees. It is also possible that residue levels above 185 ppb could occur in other circumstances which have not yet been tested, such as residues in annual or perennial crops that result from repeated applications that may be additive over time because residues accumulate in soil and plants.

Although it appears unlikely that acute lethal doses of residues in pollen and nectar are typically found in agricultural settings, there are some unknown factors that still need to be resolved, such as how rate and timing of application influence residue levels. (See Chapter 6 for a detailed discussion of neonicotinoid levels found in crops.) However, available research indicates that neonicotinoid levels pose a much bigger risk in the pollen or nectar of home and garden trees and shrubs, primarily because of higher label application rates. (The case study on pages 50–53 gives a comparison of agricultural and garden application rates for apple trees.)

In contrast with acute lethal exposure, chronic exposure in doses much lower than LD_{50} levels is far more likely to occur in agricultural settings, given that honey bees repeatedly visit crops while foraging. However, laboratory studies investigating rates of mortality after chronic sublethal exposure are complicated by many types of measures and sometimes present conflicting results and conclusions. One study found no significant differences in the mortality of untreated bees and bees exposed to doses of imidacloprid between 2 and 20 µg/kg (2–20 ppb) for 39 days (Schmuck et al. 2001). In contrast, another study observed high rates of mortality after 30 days with small doses of 4 and 8 µg/L (4 and 8 ppb) (Moncharmont et al. 2003).

Table 5.1: Lethal Toxicity of Neonicotinoids to Honey Bees

Neonicotinoid	⚠	Known Toxicity to Honey Bees†	
		Contact LD_{50}	Oral LD_{50}
Acetamiprid	M	7.1 µg[2]–8.09 µg[3]	8.85–14.52 µg[3]
Clothianidin	H	0.022 µg[2]–0.044 µg[4]	0.00379 µg[5]
Dinotefuran	H	0.024 µg[2]–0.061 µg[6]	0.0076–0.023 µg[6]
Imidacloprid	H	0.0179 µg[4]– 0.243 µg [7]	0.0037 µg[7]– 0.081 µg[8]
Thiacloprid	M	14.6 µg[2]–38.83 µg[9]	8.51–17.3 µg[9]
Thiamethoxam	H	0.024 µg[10]– 0.029 µg[2]	0.005 µg[10]

Notes

⚠ Toxicity Classification:
Highly toxic (H) = LD_{50} <2 µg;
Moderately toxic (M) = LD_{50} 2–10.99 µg;
Slightly toxic (S) = LD_{50} 11–100 µg;
Practically nontoxic (N) = LD_{50} >100 µg

† Per bee

Sources:

1. WSDA 2010
2. Iwasa et al. 2004
3. EC 2004b
4. EPA 2003a
5. EC 2005
6. EPA 2004
7. Schmuck et al. 2001
8. Nauen et al. 2001
9. EC 2004a
10. Syngenta Group 2005

Suchail et al. (2001) found that doses of 0.1, 1, and 10 μg/L (0.1, 1, and 10 ppb) of imidacloprid or its six metabolites induced high rates of mortality in bees from a single colony after only eight days of exposure. If these results are applicable in the real world, they have important implications because the very low doses tested are similar to those that bees might encounter regularly in a field setting (e.g., residues from coated seed) (Rortais et al. 2005). However, in a subsequent study, Schmuck (2004) used similar procedures as Suchail et al. (2001) to feed two imidacloprid metabolites to bees from four different colonies. Neither metabolite caused mortality at the rates reported by Suchail et al. (2001), although mortality rates varied quite a bit between colonies. It is important to note that the metabolites found to be the most toxic by Suchail et al. (2001) were not tested by Schmuck (2004).

There are several potential reasons why these study results vary. Schmuck (2004) suggests that the age of the bees may influence results, specifically that older worker bees are more susceptible to chemicals and, if used in studies, may influence mortality rates. Even within a species, responses to pesticides vary (Robertson et al. 2007). The ability to tolerate and break down insecticides can vary between colonies, and may be a reflection of the health and physiological condition of the bees (Smirle and Winston 1987). Sensitivity to imidacloprid appears to vary between colonies (Schmuck et al. 2001; Schmuck 2004), as well as between subspecies (or race) of honey bees (Suchail et al. 2000; Sandrock 2014b).

5.2.2 Delayed Toxicity and Sublethal Effects of Neonicotinoids on Honey Bees

Death is not the only negative outcome from pesticide exposure. An amount of pesticide smaller than the level determined to cause mortality, but that still causes an adverse effect, is referred to as a sublethal dose. At sublethal levels, chemicals may disrupt cognitive abilities, communication, various behaviors, and physiology. The ability for a honey bee colony to collect and store food depends on coordination and communication between workers. Exposure to chemicals that compromise the ability of worker bees to forage and communicate with others may negatively affect colony health (Desneux et al. 2007). Studies of honey bee response to sublethal doses of neonicotinoids have included measurements of foraging and feeding behaviors, learning, predator avoidance, navigation, and mobility.

Studying a system as complex as the interactions between an insecticide and a honey bee hive and understanding what would happen in a farm setting is difficult, and there is no single approach that allows control of all necessary variables. Studies have been done in laboratories, in semi-field conditions (typically done within large, outdoor enclosures), and as field trials. Each of these approaches has design limitations but each also provides information that contributes towards a better overall picture of the effects occurring at both individual and colony levels as bees interact with the insecticides. Laboratory experiments where honey bees were given a single contact or oral sublethal dose of 20 ppb or greater of a neonicotinoid indicate that imidacloprid alters learning (Guez et al. 2001; Lambin et al. 2001), motor activity (Lambin et al. 2001; Medrzycki et al. 2003), and memory (Decourtye et al. 2004a); clothianidin impairs foraging behaviors (Schneider et al. 2012); and acetamiprid impairs activity, memory, and sucrose sensitivity (El Hassani et al. 2008). Laboratory experiments in which honey bees were given multiple sublethal doses of 20 ppb or greater of neonicotinoids found that chronic exposure to imidacloprid impairs learning and foraging (Decourtye et al. 2003; Han et al. 2010) and to thiamethoxam decreases sucrose sensitivity and memory (Aliouane et al. 2009). Chronic exposure to a dose of 4.8 ppb imidacloprid (2.1 ppb in sugar and 2.7 ppb in a pollen mixture) decreased the development of the hypopharyngeal glands (which produce royal jelly) and altered respiration in newly emerged adult bees, effects that have consequences for individual bees as well as overall colony health (Hatjina et al. 2013).

Honey bee larvae can also be exposed to neonicotinoids when contaminated pollen and nectar

The complex workings of a honey bee hive make it challenging to determine pesticide exposure levels. Different castes of bees eat different types of honey products that could have different contamination levels. Honey bees also share food through trophallaxis, a mouth-to-mouth transfer of nectar.

are brought into the hive. Yang et al. (2012) exposed honey bee larvae to imidacloprid added to the brood cell and found that survival and pupation declined at chronic doses (4 days) of 24 ng/bee (150 µg/L or ca. 150 ppb) and above. Worker bees treated as larvae with sublethal chronic (4 days) doses as low as 0.25 µg/L (ca. 0.25 ppb) (highest dose tested was ca. 2.5 ppb) had reduced olfactory response as adults, an impairment that can impact the health of the colony if other workers are likewise exposed (Yang et al. 2012). To gauge how the developing metabolism of larvae is affected by neonicotinoid exposure, Derecka et al. (2013) provided colonies of free-flying honey bees with nectar containing imidacloprid at a concentration of 2 µg/L (ca. 2 ppb) for 15 days. Their analyses of the larvae molecular profiles obtained from RNA gene sequencing showed that imidacloprid altered the physiology of the larvae, affecting energy metabolism pathways.

All semi-field experiments reviewed here involved entire colonies exposed to contaminated pollen or syrup inside tunnels or flight cages. Chronic exposure to a syrup contaminated with 24 ppb of imidacloprid reduced brood production, foraging activity, and food stores (Decourtye et al. 2004b). Similarly, a chronic exposure to 48 ppb of imidacloprid in a sugar syrup also showed impaired foraging activity (Ramirez-Romero et al. 2005). At imidacloprid doses of 500 ppb and up, doses that exceed the lowest estimate of the LC_{50}, some bees failed to return to the hive (Bortolotti et al. 2003; Yang et al. 2008). In contrast, at 0.5 or 5 µg/L (0.5 or 5 ppb) in syrup, imidacloprid did not cause significant differences in population, capped brood area, or adult activity, suggesting that at very low doses imidacloprid may not be measurably harmful to colonies (Faucon et al. 2005). In a statistical analysis of results from 13 laboratory and semi-field studies that investigated sublethal effects of imidacloprid on honey bees, Cresswell (2011) found that imidacloprid residues (estimated as 0.7–10 µg/L [0.7–10 ppb]) in nectar of sunflower and canola planted with coated seed reduced adult honey bee performance between 6–20%.

Recently, in order to better simulate real-world field conditions, several experiments have exposed individual foragers from free-flying colonies to neonicotinoids in field settings. By monitoring radio-tagged honey bees displaced from their hive, Henry et al. (2012) learned that foraging honey bees exposed to a sublethal dose of thiamethoxam had reduced homing ability and survival. Though the study tested a dose (1.34 ng/bee [67 ppb]) above what might be expected in pollen or nectar of seed-treated plants, the doses were realistic for treated ornamental plants or some crops treated via soil drench or foliar spray (see Section 6). Fischer et al. (2014) found that 25 ppb clothianidin, 75 ppb imidacloprid, or 12,500 ppb thiacloprid also elicited a reduction in homing ability and navigation. Tan et al. (2014) trained bees to visit specific feeders in order to evaluate sublethal effects from field-relevant exposure to imidacloprid. In one study they evaluated predator avoidance decision-making ability. The researchers found that when foraging on nectar containing 34 ppb (40 µg/L) imidacloprid, the Asian honey bee (*Apis cerana*) showed no aversion to a feeder with a hornet predator, whereas control bees exhibited significant predator avoidance. The study also evaluated foraging ability, finding that *A. cerana* foraging was also inhibited by field-relevant, sublethal exposure amounts (17.2 ppb [20 µg/L] and 34 ppb [40 µg/L]) of imidacloprid (Tan et al. 2014).

A common approach to field studies has been to expose free-flying colonies to typical residue

levels in agricultural settings (although application area is typically less than two acres, quite unlike most real world agricultural settings) and monitor whole colony health. Researchers placed hives near fields planted with imidacloprid-treated corn or sunflower seeds (Stadler et al. 2003; Nguyen et al. 2009), clothianidin-treated canola seeds (Cutler and Scott-Dupree 2007; Pohorecka et al. 2012), or thiamethoxam-treated canola seeds (Pohorecka et al. 2012; Pilling et al. 2013) and monitored colonies over time in comparison to hives near control plots of untreated plants. Measurements of residues in bees, wax, honey, or pollen were collected and the bees themselves were monitored for colony mortality (Nguyen et al. 2009); bee mortality in front of the hive, colony weight, brood present, and worker longevity (Cutler and Scott-Dupree 2007); honey production, brood, and colony weight (Stadler et al. 2003); brood area, worker biomass, colony health, and food storage (Pohorecka et al. 2012); and mortality in front of hive, foraging behavior, colony strength, colony weight, brood development and food storage (Pilling et al. 2013). No significant negative effects on honey bee colonies from seed coated with imidacloprid, clothianidin, or thiamethoxam were observed in any of these studies (Stadler et al. 2003; Cutler and Scott-Dupree 2007; Nguyen et al. 2009; Pohoreka et al. 2012), including one that observed colonies for four years (Pilling et al. 2013).

One important limitation of these early field studies is that the application area is just a tiny fraction of the total honey bee colony foraging range (e.g., Cutler and Scott-Dupree 2007). Honey bees typically forage two miles or more from the hive (Winston 1987; Beekman and Ratnieks 2000), and colonies use relatively few patches within their range and change them regularly (Visscher and Seely 1982). A two-mile radius encompasses over 8,000 acres (3,240 ha); a two-acre (0.8 ha) experimental field covers just 0.025% of that foraging range. Although a honey bee colony may be located near a treated field, given their flight range capabilities and the recruitment of foragers to nectar-rich locations, there is no guarantee that the bees will forage primarily in the treated field. In a study conducted in corn fields, less than 15% of the corn available to the honey bees was in treated fields (Nguyen et al. 2009); corn is a crop that bees generally avoid when other sources of pollen are available. In another study, with field sizes of 1 ha (2.5 ac), treated and untreated canola fields were within 300 m (330 yd) of each other (Cutler and Scott-Dupree 2007), a distance well within a honey bee's flight range. Honey bees may also visit wildflowers in addition to crops; nearly 50% of pollen collected from hives in Spain came from non-crop species (Bernal et al. 2010). If bees have other forage options besides the treatment plots, it is hard to assess effects of treatment, or to determine the impact of hundreds (or millions) of hectares of treated crop, compared to a single hectare.

Responding to some of the above issues, two large-scale field studies reviewed the effects on honey bees from foraging on canola plants grown from seeds coated with the insecticide clothianidin (Cutler et al. 2014; Rundlof et al. 2015). Neither study found significant effects to the honey bees. Cutler et al. (2014) reported that the honey bees foraged almost exclusively on the canola during the 14-day experiment and pollen residues were between 0.5 ppb and 1.9 ppb. These studies provide valuable insights for risk assessments of honey bees, although, as with all studies, there are limitations. Since many commercial bee colonies are moved from crop to crop throughout the spring and summer the exposure duration to a single crop likely does not represent actual exposure potential throughout their foraging season.

Sandrock et al. (2014b) chronically exposed

While corn is not a preferred foraging source for honey bees, they will still collect corn pollen. An estimated 79–100% of conventional corn seed is treated with neonicotinoids, and bees are exposed to these chemicals when foraging in and around fields planted with coated seed.

The Risks of Neonicotinoid Replacements

Pollinators better served by implementing IPM and reducing use of insecticides

The increase in scientific knowledge about the risks of neonicotinoid insecticides clearly warrants reassessment of their current uses. Still, if pollinator protection efforts are to be effective they should look to IPM methods, and avoid simply replacing harmful pesticides with other chemicals of concern (Biddinger and Rajotte 2015).

The risks of replacing one pesticide by another are well documented. This is illustrated by the fact that the EPA registered many neonicotinoid products as either "reduced risk" or "replacements" in order to provide pesticide options less toxic than organophosphates and other older pesticide classes (EPA 2015a), and now, as the concerns about currently legal uses increase, the EPA is registering new products and working to provide replacement options to farmers and other pest management professionals.

A quick look at some of the new replacement chemicals being registered shows that we may again be providing replacement products of equal or greater concern for pollinators and other beneficial insects. For example, in 2013 the insecticide sulfoxaflor was registered for use in the U.S. Some scientists define sulfoxaflor as a neonicotinoid (Cutler et al. 2013). It has similar mode of action to neonicotinoids (mode of action is the term used to describe how an insecticide kills or otherwise controls insects), but, for regulatory purposes, sulfoxaflor is not a neonicotinoid.

Like neonicotinoid insecticides, sulfoxaflor is non-selective/broad-spectrum, and thus kills a wide range of insects, including many non-target beneficial insects, if they are exposed. It is designated as highly toxic to honey bees and is highly toxic to bumble bees (EPA 2012b). It is also systemic which provides pollinators with a direct route of exposure through contaminated pollen and nectar depending on the timing and method of application.

Concerns for the use of sulfoxaflor recently diminished when a federal court concluded that EPA violated federal law when it approved sulfoxaflor without reliable studies regarding the impact that the insecticide would have on honey bee colonies. As a result, sulfoxaflor may not be used in the U.S. unless, and until, EPA obtains the necessary information regarding impacts to honey bees and re-approves the insecticide in accordance with law. Still, EPA has begun approving emergency uses of sulfoxaflor.

Another replacement insecticide registered by EPA in 2015, flupyradifurone, also has characteristics that show it too may be problematic. Flupyradifurone is very similar to the neonicotinoids. Although it is considered practically nontoxic to adult honey bees through a short-term contact exposure, when ingested flupyradifurone is highly toxic to adult honey bees (EPA 2015b)—and as a systemic insecticide, ingestion is a likely exposure route for bees and other pollinators.

The farmers and others that must respond to pest pressures need information on sustainable pest management. State and federal agencies must prioritize independent university-based applied commodity research for grower adoption of IPM. Sustainable and effective IPM includes: preventive measures, monitoring of pest populations, use of nontoxic management such as conservation biological control, and preferentially selecting targeted least-toxic insecticides options when other methods are ineffective.

There is no silver bullet for managing pests. But, IPM can provide a valuable framework to design practices that reduce dependence on chemical pest control while still providing long-term, effective, and ecologically sound pest management (Biddinger and Rajotte, 2015).

Toxicity Classification: A pesticide's classification is determined by EPA. As there are generally multiple LD_{50}s for each pesticide, EPA uses a predetermined criteria to select one LD_{50} for contact and one for oral exposure to classify the pesticide's toxicity:

- Highly toxic = LD_{50} <2 µg;
- Moderately toxic = LD_{50} 2–10.99 µg;
- Slightly toxic = LD_{50} 11–100 µg;
- Practically nontoxic = LD_{50} >100 µg

(Sources: WSDA 2010; EPA 2015b; EPA 2012b.)

honey bee colonies for a period of 46 days during late spring and early summer to field-realistic levels of both thiamethoxam and clothianidin (5.31 ppb [µg/kg] and 2.05 ppb [µg/kg] respectively). After the 46-day period, researchers documented a 28% decline in adult bees, 13% decline in brood, 29% decline in honey production, and 19% decline in pollen collection of the bees exposed to the neonicotinoids in comparison to the unexposed controls. Although the colonies recovered by the late fall and overwintered successfully, the following spring the exposed colonies exhibited significantly lower numbers of adult bees and overall deceleration of colony growth compared to controls. Furthermore, because the researchers evaluated two distinct strains of bees, they were able to demonstrate that genetic background influences both short- and long-term effects of neonicotinoids on colony performance (Sandrock et al. 2014b).

The above mentioned studies evaluated contamination levels associated with plants grown from coated seeds. Residue levels from coated seeds are much lower (less than 20 ppb) than residues from plants treated by methods such as soil drench (see Section 6). Therefore, evaluating exposure level to residues from coated seed could underestimate exposures that result from other application methods commonly used in the U.S.

A study by Dively et al. (2015) evaluated responses of entire honey bee colonies to exposure to neonicotinoids at levels representative of both plants grown from coated seeds as well as other treatment methods. The three-year study found negative impacts to honey bee colony health and reduced overwintering success from imidacloprid exposure at the two higher levels of 20 µg/kg (20 ppb) and 100 µg/kg (100 ppb). Negligible effects were noted at the lower level of 5 µg/kg (5 ppb). As the imidacloprid dose increased, so did the infestations of the parasitic *Varroa* mite. Significantly higher levels of the mites were found in the colonies exposed to the highest dose. In contrast to other studies, this study did not find statistically significant impact to foraging from any treatment level.

Without sufficient replication of treated fields and adequate numbers of hives placed adjacent to each field, field studies investigating effects of neonicotinoids on honey bee colonies are likely to produce inconsistent results. Appropriate replication is also needed in order to overcome the high variability in vigor among honey bee colonies (Cresswell 2011). A colony's capacity for detoxification may vary due to genetics, age, and diet (Wahl and Ulm 1983; Smirle and Winston 1987; Meled et al. 1998). Duration of field studies is another important consideration, because colonies and their food stores within the hive persist for multiple years. Without at least a complete year of hive monitoring, it is difficult to know the full effects of neonicotinoids on colony health.

Lastly, it can be very challenging to find suitable study sites with sufficient replication for field trials, especially if the compounds tested, or products with similar compounds, have been on the market for quite some time. The prevalence of other treated bee food sources increases the likelihood of confounding the study. Field studies should be conducted before products and related compounds are approved by the EPA and become widely available for consumers.

5.2.3 Potential for Additive, Multiplicative, and Synergistic Effects Between Neonicotinoids and Other Agrochemicals

The effects of each insecticide on nontarget organisms is typically considered in isolation, though insecticides are often used in combination with other chemicals in the field. Various fungicides, for example, are often sprayed in combination with insecticides. A synergistic interaction between pyrethroid insecticides and the demethylase inhibitor (DMI) fungicides (e.g., triflumizole) has been demonstrated in honey bees. For example, triflumizole increases the toxicity of pyrethroid insecticides by delaying metabolism and detoxification (Pilling and Jepson 1993; Pilling et al. 1995). Some neonicotinoids are thought to interact similarly with this group of fungicides.

In laboratory bioassays using formulated product in water, Biddinger et al. (2013) found that

when honey bees were topically exposed (i.e., applied directly to the body of the bees) to a mixture of acetamiprid and the fungicide fenbuconazole, the acute lethal toxicity (LC_{50}) of the mixture was almost five times greater than exposure to acetamiprid alone (fenbucaonazole is minimally toxic to honey bees). Toxicity of imidacloprid to honey bees was only additive when mixed with fenbuconazole (Biddinger et al. 2013). In laboratory tests using the pesticide chemical in its pure form (the technical grade active ingredient) dissolved in ethanol, Iwasa et al. (2004) found that DMI fungicides increased the toxicity of acetamiprid and thiacloprid as much as 244-fold, but not imidacloprid. However, when honey bees were exposed to foliage treated with acetamiprid and triflumizole products under semi-field conditions, no differences in mortality rates were seen (Iwasa et al. 2004). The difference in toxicity could be due to the use of a pesticide product (a mixture of the pure pesticide with other ingredients to create a product ready for sale) in the semi-field test, and the use of the technical grade active ingredient using a penetrant such as alcohol or acetone in the laboratory test, but this has not been explicitly tested.

Schmuck et al. (2003) also found that DMI fungicides increased toxicity of thiacloprid to honey bees significantly in the laboratory, but no adverse effects were seen in bees exposed to sprayed vegetation in a semi-field setting. More research on the field effects of the synergistic interaction between fungicides and neonicotinoids is needed (Blacquiere et al. 2012).

In addition to fungicides, other pesticides, including those used in the hives for mite control, may also act synergistically with neonicotinoids. The combined effects of sublethal imidacloprid and coumaphos (an organophosphate used in hives to control *Varroa* mites) appear to be additive, inhibiting neural pathways in the brain of honey bees which results in reduced cognitive function (Palmer et al. 2013) and impaired honey bee olfactory learning and memory (Williamson and Wright 2013).

Future insecticide screening should include potential additive and synergistic effects of pesticide mixtures, with an emphasis on mixtures most likely to be encountered in approved uses on crops or within hives.

5.2.4 Neonicotinoids, Colony Collapse Disorder, and High Annual Honey Bee Colony Losses

Colony collapse disorder (CCD) is the large-scale loss of European honey bees that was first observed in the United States during the winter of 2006–2007. The addition of CCD to the typical winter maladies of colony starvation, *Varroa* loss, and disease, led beekeepers to see an increase in winter losses from around 15% to upwards of 30%. In colonies that succumbed to CCD, beekeepers were mystified to discover that a majority of worker bees left hives and did not return, despite the presence of a queen, brood, and food stores. The cause of CCD remains unexplained, although it appears that no single factor alone is responsible. Research suggests that CCD is a syndrome caused by multiple factors, including pesticides, pathogens, parasites, and poor habitat that work individually but probably also in combination (USDA 2010).

The failure of foraging bees to return to their hives has led many people to suggest that a link exists between CCD and the behavioral disruptions observed with sublethal exposure to neonicotinoid insecticides. As of yet, no single insecticide or combination of insecticides have been linked to CCD or the abnormally high annual colony losses beekeepers are experiencing, though many chemicals have been found in hives (e.g., Mullin et al. 2010). Researchers who compared gene expression in honey bees from healthy colonies and from collapsed colonies found no link between expression of genes that code for proteins associated with the detoxification of insecticides and collapsed colonies (Johnson et al. 2009). This suggests that insecticide exposure, whether to neonicotinoids or another class, is not a primary factor in CCD.

However, pesticide exposure may interact with other factors such as viruses, pathogens, or parasites to weaken colony health and increase susceptibility to CCD or to colony failure (USDA 2010).

For example, dietary exposure to the fungicides chlorothalonil and pyraclostrobin or to miticides used to control in-hive parasites (amitraz and fluvalinate) increases *Nosema* infection (Pettis et al. 2013). Several studies have also demonstrated the combined effects of infection by honey bee gut parasites (*Nosema apis* and *N. ceranae*) and sublethal levels of neonicotinoids. Pettis et al. (2012) found that sublethal imidacloprid exposure in brood food fed to honey bee larvae led to increased susceptibility to *Nosema* in adult bees. Alaux et al. (2010) found that when they occurred together, imidacloprid, *N. apis*, and *N. ceranae* increased mortality more than neonicotinoid exposure or *Nosema* infection alone and reduced the ability to sterilize food. A reduced ability to sterilize stored food could make colonies more susceptible to other pathogens. Similarly, Vidau et al. (2011) found a synergistic interaction between infection with *N. ceranae* and exposure to sublethal levels of thiacloprid that increased honey bee mortality. Sublethal levels (0.1–10 ppb) of clothianidin or imidacloprid have also been linked to higher viral loads of deformed wing virus (Di Prisco et al. 2013), which causes debilitating deformities in adult bees and is transmitted by parasitic *Varroa* mites. Interestingly, bees exposed to an organophosphate insecticide, chlorpyrifos, showed no signs of increased viral loads (Di Prisco et al. 2013).

By studying gene expression in clothianidin-exposed bees, Di Prisco et al. (2013) found a mechanism by which neonicotinoids can compromise the immune system of honey bees: exposure to clothianidin inhibits a protein involved in activating the immune system response, allowing viruses such as deformed wing virus to proliferate.

Neonicotinoids and other agrochemicals currently do not appear to be a direct cause of CCD or abnormal colony losses. However, the evidence suggests they are a contributing factor to the decline of colonies already stressed by poor diet, pathogens, or parasites. Although beekeepers have reported fewer incidents of CCD in recent years, they continue to report over-winter losses that are greater than the acceptable winter morality rate, including the second highest annual loss rate of 42.1% of colonies in 2014–2015. It is increasingly important that future studies focus on interactions of multiple factors suspected of contributing to CCD and over-winter losses.

These empty hive bodies, from a single commercial beekeeper, once housed hundreds of honey bee colonies. Beekeepers in the United States continute to have greater than acceptable winter and summer losses of colonies, despite fewer reported incidents of CCD in recent years. Neonicotinoids are considered to be one of several stressors linked with honey bee declines.

5.2.5 Documented Concentrations of Neonicotinoids with Lethal and Sublethal Effects

The information presented in the Table 5.2 (opposite) is compiled from studies that investigated the effects of oral doses of neonicotinoids on bees. For the ease of comparison here, we have converted doses from µg/bee or concentrations of mg/kg to ppb. It should be noted that although the concentrations can provide a helpful frame of reference for residue levels in pollen or nectar that are likely to be harmful to bees, it is difficult to know the actual dose that is ingested by bees without further information on the various amounts of nectar or pollen collected and consumed by bees within a given time frame. Known harmful levels of concentration allow us to extrapolate exposure levels to bees and the resulting impact of that exposure.

5.3 Bumble Bees and Neonicotinoids

There are 46 species of bumble bees (genus *Bombus*) in North America. They are active from spring to fall—all year in warmer regions—and are important pollinators of both crops and wildflowers. On a bee-per-bee basis, bumble bees pollinate crops such as cranberries, blueberries, and tomatoes more effectively than honey bees because they fly in cooler weather and in lower light levels, and because they buzz pollinate (sonicate) the flowers to release pollen through pores in the anthers.

Bumble bees are social bees, with small colonies (typically no more than a few hundred individuals and frequently much smaller) that last a single season. Colonies are founded in the spring by a queen, who establishes a nest under a clump of grass or in an old rodent burrow. She then secretes wax to form brood cells and honey pots for temporary storage of nectar. The queen rears the first generation of bees and once they are active, she remains inside the nest to lay eggs. The daughter-workers cooperate to raise additional offspring and find food. As larvae, bumble bees consume a mixture of pollen and nectar, which in contrast to honey bees, is not stored for lengthy periods within the nest before consumption. In this way, the colony increases in numbers throughout the growing season. New queens are reared in the late summer, mate, and then overwinter. Other members of the colony die when winter arrives.

As generalist foragers that visit a wide range of flowers and habitats, bumble bees have the potential to be exposed to neonicotinoids in agricultural settings as well as in parks and yards. Bumble bees are highly important crop and native plant pollinators, so it is crucial to better understand their response to neonicotinoid exposure.

Information on toxicity of neonicotinoids to bumble bees is limited, but studies have increased with the commercial availability of colonies. Most of these studies examined effects of imidacloprid, but a few investigated the impacts of clothianidin, thiamethoxam, and thiacloprid.

5.3.1 Lethal Toxicity of Neonicotinoids to Bumble Bees

Laboratory studies of acute toxicity—a single incident in which contact is made—demonstrate that imidacloprid and clothianidin are very toxic to bumble bees. Acute contact exposure to imidacloprid or clothianidin is very harmful (Marletto et al. 2003; Scott-Dupree et al. 2009; Gradish et al. 2010), and an acute oral dose of imidacloprid is very toxic (Marletto et al. 2003). Mommaerts et al. (2010) determined an LC_{50} of 120 ppb (33 ng/bee) for thiamethoxam. Clothianidin is apparently slightly more toxic to bumble bees through contact exposure than imidacloprid (Scott-Dupree et al. 2009). The amount of acetamiprid, dinotefuran, thiacloprid, and thiamethoxam that can kill bumble bees (the acute lethal dose) has not been evaluated.

Table 5.2: Concentrations of Neonicotinoid Insecticides Known to Cause Harm When Ingested by Bees

A.I.	Effect	Exposure	Honey Bees	Bumble Bees	Solitary Bees
Acetamiprid		Acute*	≥442,500 ppb[1]	——	——
		Chronic	——	——	——
		Acute	5,000 ppb[2]	——	——
		Chronic	5,000 ppb[3]	——	——
Clothianidin		Acute	≥190 ppb[4]	——	——
		Chronic	——	——	——
		Acute	25 ppb[5]	2.1 ppb[14]	——
		Chronic	2.05 ppb[6†]	17 ppb[15‡]	0.45 ppb[19]
Dinotefuran		Acute	≥380 ppb[7]	——	——
		Chronic	——	——	——
		Acute	——	——	——
		Chronic	——	——	——
Imidacloprid		Acute	≥185 ppb[8]	——	——
		Chronic	0.10 ppb[9¤]⟹≥20 ppb[10]	59 ppb[16]	——
		Acute	75 ppb[5]		
		Chronic	0.25 ppb[11]	0.3 ppb[17]	30 ppb[20]
Thiacloprid		Acute	≥425,500 ppb[12]	——	——
		Chronic	——	18,000 ppb[16]	——
		Acute	12,500 ppb[5]	——	——
		Chronic	——	12,000 ppb[16]	——
Thiamethoxam		Acute	≥250 ppb[13]	——	——
		Chronic	——	120 ppb[16]	——
		Acute	——	——	——
		Chronic	5.31 ppb[6†]	2 ppb[18]	2.87 ppb[19]

Notes

* Acute lethal exposure concentrations are based upon reported LD_{50} levels (Table 5.1) and were converted to concentrations by dividing the LD_{50} by 20 mg, the standard consumption rate of sucrose consumed by a bee during an LD_{50} test (Schmuck et al. 2001; CA DPR 2008).

† This study exposure combined 5.31 ppb thiamethoxam with 2.05 ppb clothianidin.

‡ Scholer and Krischik 2014 noted effects to bumble bees as low as 9 ppb of clothianidin but their conclusions stated that "negative impacts" occurred at 17 ppb.

¤ The results of this study[9] have been called into question, so we have included the results from another similar study[10]

Sources:

1. EC 2004b
2. El Hassani et al. 2008
3. Aliouane et al. 2009
4. EC 2005
5. Fischer et al. 2014
6. Sandrock et al. 2014b
7. EPA 2004
8. Schmuck et al. 2001
9. Suchail et al. 2001
10. Schmuck 2004
11. Yang et al. 2012
12. EC 2004b
13. Syngenta Group 2005
14. Moffat et al. 2015
15. Scholer and Krischik 2014
16. Mommaerts et al. 2010
17. Laycock and Cresswell 2013
18. Elston et al. 2013
19. Sandrock et al. 2014a
20. Abbott et al. 2008

Key: Lowest reported lethal concentrations* | Lowest reported sublethal effects level | —— No data available

The effect of chronic neonicotinoid exposure—that is, repeated exposure over a period of time—on bumble bee mortality is less known. Mommaerts et al. (2010) demonstrated that following a chronic oral exposure to imidacloprid, all the bees exposed to doses of 2,000 ppb died within several weeks. No significant mortality was observed at a dose of 10 ppb. In experiments requiring bees to leave the nest to retrieve food, mortality at the above doses was much swifter, though again no significant mortality was observed at 10 ppb. These authors also found that of the three neonicotinoids tested (imidacloprid, thiacloprid, and thiamethoxam) thiamethoxam caused the highest mortality and thiacloprid the least. In a separate study, bumble bees were exposed in a flight cage to blooming cucumbers treated with a foliar spray of imidacloprid applied at field dose (50 ml/hl, Confidor 200 SL) (Incerti et al. 2003); a third of the bumble bees died within 48 hours of exposure.

5.3.2 Delayed Toxicity and Sublethal Effects of Neonicotinoids on Bumble Bees

Several laboratory studies have found that bumble bees exhibit sublethal effects after chronic oral exposure to imidacloprid. Bumble bees appear to be affected by dietary concentrations of imidacloprid at levels lower than honey bees, perhaps because, unlike honey bees, bumble bees do not metabolically degrade imidacloprid effectively while continuing to ingest it (Cresswell et al. 2014). Although no negative effects of colony health or foraging ability were seen in bees fed imidacloprid-contaminated pollen at a low dose (7 ng/g [7 ppb]), reduced foraging ability and trembling was seen in bees fed a higher dose (30 ng/g [30 ppb]) (Morandin and Winston 2003). In addition, reduced drone production and longer foraging times were seen in bees fed lower doses (10 ppb) of imidacloprid (Mommaerts et al. 2010). Bumble bees fed both imidacloprid-contaminated nectar and pollen (16 µg/kg [16 ppb]) had lower worker survival rates and reduced brood production (Tasei et al. 2000). In another study (Laycock et al. 2012), 13-day exposure to imidacloprid in syrup to the workers of queenless bumble bee micro-colonies was detrimental to worker bee fecundity (workers can lay male brood when rendered queenless). Effects were seen at doses of 1.27–63.5 ppb. At the very low, field-realistic dose of 1.27 ppb, imidacloprid reduced brood production by 42%. A 14-day exposure to levels between 0.3 and 10 ppb in syrup could reduce brood production by between 18% and 84%, and reduced pollen consumption was observed at doses of 0.2 ppb and 4.4 ppb (Laycock and Cresswell 2013). When given an additional 14 days without exposure, colonies recuperated somewhat and brood production was only reduced by 2% to 19%, though these results may not be environmentally relevant when the bloom period of a treated crop extends beyond 14 days or if bumble bees consume contaminated pollen as well as nectar (Laycock and Cresswell 2013). Colonies fed either 16 ppb imidacloprid or 17 ppb clothianidin for 11 weeks experienced queen mortality, and reduced colony consumption and colony weight (Scholer and Krischik 2014). These results may not directly translate to a field setting, where bumble bees would likely have access to untreated food sources during the course of an 11-week period.

Mommaerts et al. (2010) reported significantly reduced brood production (yielding only 14% of the unexposed colonies) from micro-colonies fed thiamethoxam in sugar water at 100 ppb. However, colonies receiving sugar with 10 ppb thiamethoxam showed no significant reduction of brood (Mommaerts et al. 2010). Another study found that thiamethoxam reduced production of drones in micro-colonies fed 10 µg/kg (ppb) thiamethoxam in a honey water solution and 10 µg/kg (10 ppb) in a pollen paste (Elston et al. 2013). Feeding rates and wax cell production were also significantly reduced; these effects were also seen in micro-colonies fed a lower dose of 1 µg/kg (1 ppb) in honey water and 1 µg/kg (1 ppb) in pollen paste (Elston et al. 2013). Reduced feeding rates were also found by Laycock et al. (2014) but were only seen at thiamethoxam doses of 98 and 39 µg/kg (98 and 39 ppb) in syrup. Worker mortality was also reduced at doses of 98 µg/kg and brood production was reduced at doses of 39 µg/kg and 98 µg/kg. Doses between 1 and 11 µg/kg (1 and 11 ppb) of thiamethoxam had no

The effects of neonicotinoids cannot be assessed in isolation. Bees can be exposed to multiple pesticides. Pollen and nectar can contain mixtures of fungicides and neonicotinoids. These mixtures can increase the toxicity of neonicotinoids.

significant effect on brood production or food consumption, suggesting that imidacloprid may have a greater impact on bumble bee colonies than does thiamethoxam (Laycock et al. 2014).

Bumble bees exposed to thiamethoxam in nectar (4 ppb) and clothianidin in pollen (1.5 ppb) during the growth stage of the colony had reduced worker production and longevity, and overall colony reproductive success was also reduced (Fauser-Misslin et al. 2014). In a study in which bumble bees were fed clothianidin-contaminated pollen at doses of 6 or 36 ppb, bees did not exhibit significant sublethal effects on pollen consumption, newly emerged worker weights, amount of brood, or the number of workers, males, and queens (Franklin et al. 2004). Bumble bees exposed to thiacloprid in sugar water in large doses (12,000 ppb) reduced nest reproduction by 36%, but colonies exposed at lower doses (1,200 ppb and lower) had no significant loss of reproduction (Mommaerts et al. 2010). Because acetamiprid and dinotefuran are also used on plants visited by bumble bees, it would be worthwhile investigating the sublethal impacts of these neonicotinoids, since bumble bees do not necessarily respond similarly to all neonicotinoids.

Studies conducted in semi-field conditions, exposing bumble bee colonies to neonicotinoid-contaminated nectar or foliage within glasshouses or flight cages, have found results similar to laboratory studies. Bumble bee colonies within flight cages exposed to clothianidin-treated (at label rates for grub control) weedy turf with flowering white clover (with average concentration of 171 ppb in nectar) had reduced foraging activity and increased worker mortality during the six day exposure period (Larson et al. 2013). In the six weeks following initial exposure, colonies had significantly fewer adults and honey pots, had reduced colony weight, and failed to produce any new queens (Larson et al. 2013). Mommaerts et al. (2010) provided bumble bee colonies with sugar water containing 2–20 ppb of imidacloprid and placed pollen 3 meters from the hives. After two weeks, colonies fed doses of 10 and 20 ppb were not producing offspring; only colonies fed 2 ppb exhibited no sublethal effects. Tasei et al. (2001) observed unaltered bumble bee activity on imidacloprid seed-treated sunflowers (treated at a rate of 0.7 mg a.i./seed). In contrast, Al-Jabr (1999) found that foraging activity of bumble bees was significantly reduced on tomatoes treated with soil drenches of imidacloprid (at a rate of 130 mg a.i./pot). Additionally, less food was stored and fewer adults survived in colonies after exposure to soil-treated tomatoes (Al-Jabr 1999). Rates of neonicotinoid application to plants (as soil drenches, trunk injections, or foliar sprays) are often much higher than the rates applied to seeds, and the risks to bees also increase correspondingly.

Gill et al. (2012) exposed bumble bee colonies to a concentration of 10 ppb of imidacloprid in nectar and allowed workers to forage freely in the field, while monitoring worker activity through radio frequency tagging. Exposed bumble bees experienced impaired foraging efficiency; 50% more workers from exposed colonies did not return to their colonies than did workers from control colonies, and returning workers were significantly less efficient at collecting pollen (Gill et al. 2012). Exposed colonies allocated more workers to collect pollen as colony demand for pollen grew, which may have impaired brood development. Brood production was reduced by 22% in imidacloprid-exposed colonies (Gill et al. 2012). Additionally, combined exposure to both 10 ppb of imidacloprid and label rates of the pyrethroid λ-cyhalothrin caused significantly higher worker mortality and forager losses (Gill et al. 2012).

After exposing bumble bee colonies to 6 ppb of imidacloprid in pollen and 0.7 ppb nectar for 14

Neonicotinoids Might Be Additional Stressor for Already Compromised Monarchs

Monarch butterfly (*Danaus plexippus*) populations in North America have dwindled precipitously over the last two decades. It is estimated that Eastern monarch populations that overwinter in Mexico have dropped by over 80%. Western monarchs that overwinter on the California coast have suffered a 74% drop. These declines are so severe that the U.S. Fish and Wildlife Service is considering listing the North American monarch as threatened under the Endangered Species Act.

Key factors of monarch decline include the loss of milkweed (*Asclepias* spp.) breeding habitat due to increased use of herbicides on genetically modified, herbicide-resistant corn and soybeans, lands being converted to agriculture, deforestation of overwintering sites, and climate change. The contribution of other stressors to the monarch decline are just beginning to be evaluated. If monarch recovery efforts are to be successful, a greater understanding of the broader set of risks is needed.

Two new studies evaluated the risks of neonicotinoids to monarchs (Krischik et al. 2015; Pecenka and Lundgren 2015). Both concluded that these long-lived, systemic insecticides harm larval monarchs when their milkweed host plants are contaminated.

Estimates by Douglas and Tooker (2015) show that approximately 90% of all conventional corn seed is treated with neonicotinoids prior to planting. A significant portion of monarch breeding habitat is next to field crops, including corn. To better understand the potential risk, Pecenka and Lundgren (2015) investigated the lethal and sublethal levels of the neonicotinoid clothianidin for monarch larvae. Using an exposure time of 36 hours, which is likely shorter than exposure time monarchs would experience in the field, the researchers observed sublethal effects (reduced larval size) due to clothianidin at 1 ppb, but not at 0.5 ppb.

The lethal concentration for 10% of the population was 7.72 ppb. Milkweed plants growing near corn fields were also tested for clothianidin. The average detection level was 1.14 ppb clothianidin, with a maximum detection of 4 ppb on a single plant. These contamination levels may be higher than what would be commonly expected as the wet spring season pushed back corn planting and reduced the field dissipation time. By comparing contamination levels in the milkweed growing adjacent to corn fields with the levels that caused visible impact to monarch larvae, the authors concluded that field-realistic levels could contribute to monarch population declines.

Another study (Krischik et al. 2015) evaluated the impact to monarchs and painted lady (*Vanessa cardui*) butterflies when their host plants were treated with legal ornamental application rates of imidacloprid. Larval survival of both species of butterflies was significantly reduced when they fed on vegetation of host plants treated with a single application of imidacloprid at 300mg a.i./3 gallon pot. That same application rate of imidacloprid led to contamination levels of 6,030 ppb in the flowers of tropical milkweed (*A. curassavica*). The exposure analysis

days, Whitehorn et al. (2012) then allowed the colonies to develop in the field. After 8 weeks, the bumble bee colonies had an 85% reduction in the production of new queens, as well as significantly reduced colony growth rates (Whitehorn et al. 2012). Feltham et al. (2014) suggest that significantly reduced pollen foraging, the result of exposure to field-realistic doses of imidacloprid, is a potential causal mechanism for reduced queen production. After feeding for fourteen days in the lab on a dose of 0.7 ppb imidacloprid in sugar water and 6 ppb in pollen, bumble bees were released in the field and their foraging behavior was monitored using radio frequency identification technology (Feltham et al. 2014). Exposed bumble bees brought back significantly less forage and 31% less pollen per hour than unexposed bees; the resulting pollen limitation likely affects queens that require abundant food during development (Feltham et al. 2014).

The sublethal impacts on reproduction observed by Whitehorn et al. (2012) and Gill et al. (2012) may have disproportionate consequences. For example, decreased queen production results in fewer bumble bee colonies in future years and may lead to substantially reduced bumble bee populations. That trace amounts of neonicotinoids may impact bumble bee reproduction, thereby impacting populations is cause for concern (Gill et al. 2012; Laycock et al. 2012; Whitehorn et al. 2012; Fauser-Misslin et al. 2014).

Another study using radio frequency identification technology to track the movements of

individual bumble bees gave the bumble bees an imidacloprid-dosed sucrose solution for the four-week duration of the research (Gill and Raine 2014). The bees were able to forage freely and no pollen was provided to them. The imidacloprid amount selected was a field relevant dose (10 ppb). Gill and Raine (2014) found that while the control bees experienced improved foraging performance over time, the imidacloprid-exposed bees actually had a reduction in foraging performance. Foraging performance was measured by the average amount of pollen brought back from foraging bouts. Bumble bees exposed to imidacloprid on average brought back less pollen from foraging bouts in week four than in the previous three weeks. In comparison, bumble bees from the control colonies brought back larger pollen loads per bout as they got older (Gill and Raine 2014).

Moffat et al. (2015) assessed the accumulation of field realistic levels (0.21–2.1 ppb) of clothianidin and imidacloprid in bumble bee brains. They found that the bees rapidly accumulated levels of clothianidin capable of disturbing normal nervous system function after an acute (minutes-long) exposure, but it took three days of exposure to imidacloprid before levels that affected the nervous system were detected in the brain. In a follow-up field study, the researchers found that colonies exposed to equivalent field-relevant levels of imidacloprid showed a 55% reduction in the number of live bees compared to unexposed controls. Furthermore, the nest condition of exposed colonies was severely compromised by fungal contamination and some weakened colonies were overrun by wasps.

There have been several field experiments in which bumble bee colonies were placed close to imidacloprid-treated plants and then monitored for changes over time in numbers of adults, offspring, or other colony health parameters. A study in France placed colonies within a 16 ha (39.5 acre) field of seed-treated sunflowers, as well as control hives in an 18 ha (44.5 acre) untreated field 20 km (12.5 miles) away (Tasei et al. 2001). Bees were marked and recaptured to assess their ability to find their way back to their nest after exposure to treated fields. Although sunflower pollen was only roughly 25% of the pollen collected, loss of workers and growth of colonies did not appear to be significantly affected by field treatment (Tasei et al. 2001).

In a similar study, by Cutler and Scott-Dupree (2014), commercial colonies of bumble bees were placed near corn fields and monitored for changes. Half the colonies were placed next to organic sites and the other half were placed near conventional sites where clothianidin-coated seed was planted. Pollen tested from the conventional sites had clothianidin residue levels between 0.1 and 0.8 ppb. Because other forage was available to the bees, corn pollen made up only 0–2.6% of the pollen collected. The only endpoint measured that showed an effect was that significantly fewer worker bees were recovered from

found that survival of adult butterflies fed imidacloprid was not significantly reduced. The authors hypothesize that adult butterflies may secrete the insecticide rather than metabolize it.

These studies provide a valuable initial look into potential risks to monarchs from neonicotinoids. Concerns were found from both the movement of neonicotinoids from agricultural crops to adjacent monarch habitat and the ornamental use of neonicotinoids on butterfly host plants. The risks shown through these studies raise new questions that warrant further research. Furthermore, since monarch populations have seen such a dramatic decline, these potential stressors should be considered in the creation and restoration of monarch habitat.

Monarch larvae can be exposed to pesticides while feeding on contaminated milkweed plants. Research indicates that field-realistic levels of neonicotinoids could harm already at-risk monarch populations.

colonies placed next to corn treated with neonicotinoids, although the researchers thought the lower number of workers was not biologically significant.

A study by Rundlof et al. (2015) compared impacts on bumble bees from canola treated with clothianidin, cyfluthrin (a non-systemic insecticide), and the fungicide thiram to those from canola treated only with the fungicide. The study found that the insecticide seed coating was negatively related to colony growth and reproduction. More specifically, the weight of the colonies that foraged on canola treated with insecticides were considerably smaller than the weight of the colonies foraging on canola treated only with the fungicide (Rundlof et al. 2015).

Field studies can be confounded by pre-existing contamination from the compounds being tested. This is most relevant when the compounds tested, or products with similar chemicals, have been on the market for some time. A recent study of the impacts of neonicotinoids on bumble bees, performed by United Kingdom's Food and Environment Research Agency exemplifies this issue. Although the study found no clear or consistent relationship between colony health measurements and exposure to imidacloprid, thiamethoxam, or clothianidin, some of the control bumble bees (those that were supposed to provide data on the health of bumble bees without neonicotinoid exposure) were accidentally exposed in the field (Thompson et al 2013). A re-analysis of the data, that compensated for the accidental exposures, found that queen production was lower in colonies exposed to higher levels, thus suggesting that colonies in or near farmland will be negatively impacted by neonicotinoid exposure (Goulson 2015). This example demonstrates the value of conducting field studies before products and related compounds are approved and become widely available for consumers.

Two field studies conducted by Bayer CropScience AG placed bumble bee colonies near 18 or 30 ornamental shrubs treated with soil drenches of imidacloprid. Visitation of bees to blossoms of treated shrubs and other nearby untreated potted flowers was monitored, as was adult mortality in front of the hives (Maus et al. 2006, 2007). Visitation to treated shrubs was lower than to untreated (and plenty of alternate forage was also available), and dead bees were seen in treated plots but not untreated plots (Maus et al. 2006, 2007). The 2007 study also included residue analyses of bees found dead from the colonies that survived (<1.7 μg/kg [<1.7 ppb]), though the researchers inexplicably did not measure residues in the bees from colonies that died midway through the experiment (Maus et al. 2007).

Additional studies tracking behavior and colony health after exposure to treated ornamental plants would be especially valuable since approved application rates for neonicotinoids in home and garden settings are typically significantly higher than those allowed on crops.

5.3.3 Parasites, Neonicotinoids, and Bumble Bees

Bumble bees across North America are in decline, with several previously common species now absent from much of their former range (Cameron et al. 2011a). The causes of these declines are not fully understood, but loss or fragmentation of habitat, pesticide use, overgrazing, climate change, low genetic diversity, pathogens, and parasites are all likely playing a role (Hatfield et al. 2012). Several parasites have been identified as of concern to wild North American bumble bees, including *Crithidia bombi*, a protozoan gut parasite (Cameron et al. 2011b), which can impact the health of individual bumble bees as well as of the colony (Schmid-Hempel 2001). A recent study demonstrated that, when combined with a sublethal infection of *C. bombi*, chronic exposure to low level, realistic doses of thiamethoxam (4 ppb) and clothianidin (1.5 ppb) significantly reduced queen survival (Fauser-Misslin et al. 2014). Since the fate of the colony is directly linked to queen bumble bees, queen loss can decrease the number of queens available to establish the subsequent year's colonies, thus reducing the population size over time.

5.4 Solitary Bees and Neonicotinoids

The United States and Canada have more than 3,500 species of native bees, which are greatly varied in their social behavior, habitat requirements, and floral preferences. While some native species such as bumble bees form colonies, the majority lead solitary lives. Each female establishes and provisions her own nest; some species are gregarious and will nest in large aggregations. Insecticide exposure can significantly impact solitary bee populations, because if a female solitary bee dies due to insecticide contact while foraging, her nest remains incomplete. In contrast, a dead worker honey bee or bumble bee can be replaced because the egg-laying female (the queen) is protected within the hive.

Most native bees nest in the ground, digging narrow tunnels. Others tunnel into pithy plant stems or nest in preexisting cavities, such as tunnels left behind by borer beetles in trees. Native bees may use mud, resin, leaves, petals, or plant fibers in their nest construction. Their wide range of lifestyles deserves more attention in neonicotinoid studies, as these bees may come into closer contact with residues in contaminated soil or leaves through their nest construction than do honey bees or bumble bees. For example, squash bees (*Peponapis* spp., *Xenoglossa* spp.) have recently been demonstrated to be the primary pollinators of squash and pumpkin plants across much of the United States (Jim Cane, pers. comm.). They also nest in the ground between the crop plants and may easily come into contact with soil-applied systemic insecticides.

5.4.1 Lethal Toxicity and Sublethal Effects of Neonicotinoids on Solitary Bees

There are only seven published studies of the impacts of neonicotinoids on solitary bees, and four of these investigated imidacloprid. Five managed bee species were tested: blue orchard bees (*Osmia lignaria*), alkali bees (*Nomia melanderi*), alfalfa leafcutter bees (*Megachile rotundata*), Japanese orchard bees (*O. cornifrons*), and red mason bees (*O. bicornis*). Blue orchard and alkali bees are native to North America. The alfalfa leafcutter bee and Japanese orchard bee have both been introduced to North America from Europe and Asia, respectively. The red mason bee is native to Europe, where studies were done. Three of the seven studies investigated the effects on mortality in a laboratory setting, while the other four investigated sublethal effects in both laboratory and semi-field settings.

Although LD_{50} levels have not been determined across the five species tested, laboratory studies demonstrated that acute contact with imidacloprid is highly toxic to alkali bees (Stark et al. 1995; Mayer and Lunden 1997), alfalfa leafcutter bees (Stark et al. 1995; Mayer and Lunden 1997; Scott-Dupree et al. 2009), blue orchard bees (Scott-Dupree et al. 2009), and Japanese orchard bees (Biddinger et al. 2013). Clothianidin on contact was toxic to blue orchard bees and alfalfa leafcutter bees (Scott-Dupree et al. 2009), and acetamiprid on contact was moderately toxic to Japanese orchard bees (Biddinger et al. 2013). The effects of acute oral or chronic oral exposure of imidacloprid or clothianidin on solitary bees are unknown. Effects of other neonicotinoids such as dinotefuran or thiacloprid have not been studied.

Several studies demonstrated that toxicity of neonicotinoids can vary significantly among species

Studies demonstrate that larvae of blue orchard bees take longer to develop in the nest when their food supplies are tainted by neonicotinoid residues.

Studies demonstrate that that not only are honey bees are not accurate at predicting neonicotinoid toxicity to solitary bees, neither are individual species of solitary bees. While imidacloprid is toxic to many bee species on acute contact, it is not equally toxic to members of the family Megachilidae. Studies have shown that imidacloprid is more toxic to blue orchard bees (above) than clothianidin, whereas clothianidin is more toxic to alfalfa leafcutter bees (below).

The effects of neonicotinoids on most native ground-nesting bee species—many of which remain undescribed—have not been studied.

of bees (Arena and Sgolastra 2014). Scott-Dupree et al. (2009) showed that imidacloprid was more toxic to blue orchard bees than clothianidin, but clothianidin was more toxic to alfalfa leafcutter bees than imidacloprid. Biddinger et al. (2013), using formulated products in water, found that acute contact exposure of imidacloprid was 12 times less toxic to Japanese orchard bees than to honey bees; in contrast, acetamiprid was 26 times more toxic to Japanese orchard bees than to honey bees. Toxicity to honey bees is not a suitable predictor of toxicity to all bee species, and honey bees should not be a surrogate for thousands of species of native bees, nor for tens of thousands of species of other nontarget insects.

In a field study designed to understand effects on larval development and adult emergence, Abbott et al. (2008) injected imidacloprid into the pollen provisions in blue orchard bee nests. Several doses were used, one that would be commonly encountered in the field (3 ppb), a medium dose (30 ppb), and a high dose thought to be unlikely to be found in a field setting (300 ppb). In a second trial, the researchers fed pollen contaminated with the same doses to blue orchard bee larvae in a laboratory setting. In both trials, they monitored larval development, emergence time, weight, and mortality. Lethal effects were expected, but not observed at higher doses in both field and lab; researchers speculated larvae may have selectively eaten around the treated portion of the pollen stores. A sublethal effect of lengthened larval development time was seen at doses of 30 ppb and 300 ppb (Abbott et al. 2008). It is difficult to know if this longer development time was the result of slower food intake or effects of the chemical. Future studies should address consumption rates of provisions.

Abbott et al. (2008) also performed a field experiment using alfalfa leafcutter bees and clothianidin at low (6 ppb), medium (30 ppb), and high (300 ppb) doses injected into pollen stores. Again, no lethal effects were observed, even at the higher dose. Minor sublethal effects on development were observed but overall alfalfa leafcutter bees, at least in the larval stage, appear resistant to the effects of clothianidin at the doses tested.

Sandrock et al. (2014a) experimentally tested the effects of chronic, field-realistic, sublethal levels of either thiamethoxam or clothianidin on the reproductive fitness of red mason bees. Bees in flight

cages were able to freely reproduce and forage on nectar contaminated with either 2.87 ppb thiamethoxam or 0.45 ppb clothianidin. While neonicotinoid exposure did not impact adult mortality or longevity, it did reduce reproductive capacity. Females exposed to neonicotinoids completed fewer nests and constructed fewer brood cells per nest, demonstrating a 50% reduction in offspring compared to non-exposed females (Sandrock et al. 2014a). Additionally, neonicotinoid exposure increased the ratio of male offspring to females, which further contributes to a loss of reproductive potential (males do not contribute to nest building or provisioning activities). A reduction in offspring and reduced female numbers due to neonicotinoid exposure could have major negative impacts on populations of solitary bees.

Further research into areas including the sublethal effects of neonicotinoids on foraging and behavior of adult solitary bees is needed. Additionally, since nearly 70% of wild bees in North America nest in the ground, effects of neonicotinoids in the soil on ground-nesting bees would be helpful in understanding risk. Research has already demonstrated that soil-applied imidacloprid has been found to impair parasitoid wasps seeking underground prey (e.g., Rogers and Potter 2003), yet effects on soil-nesting bees are generally unknown.

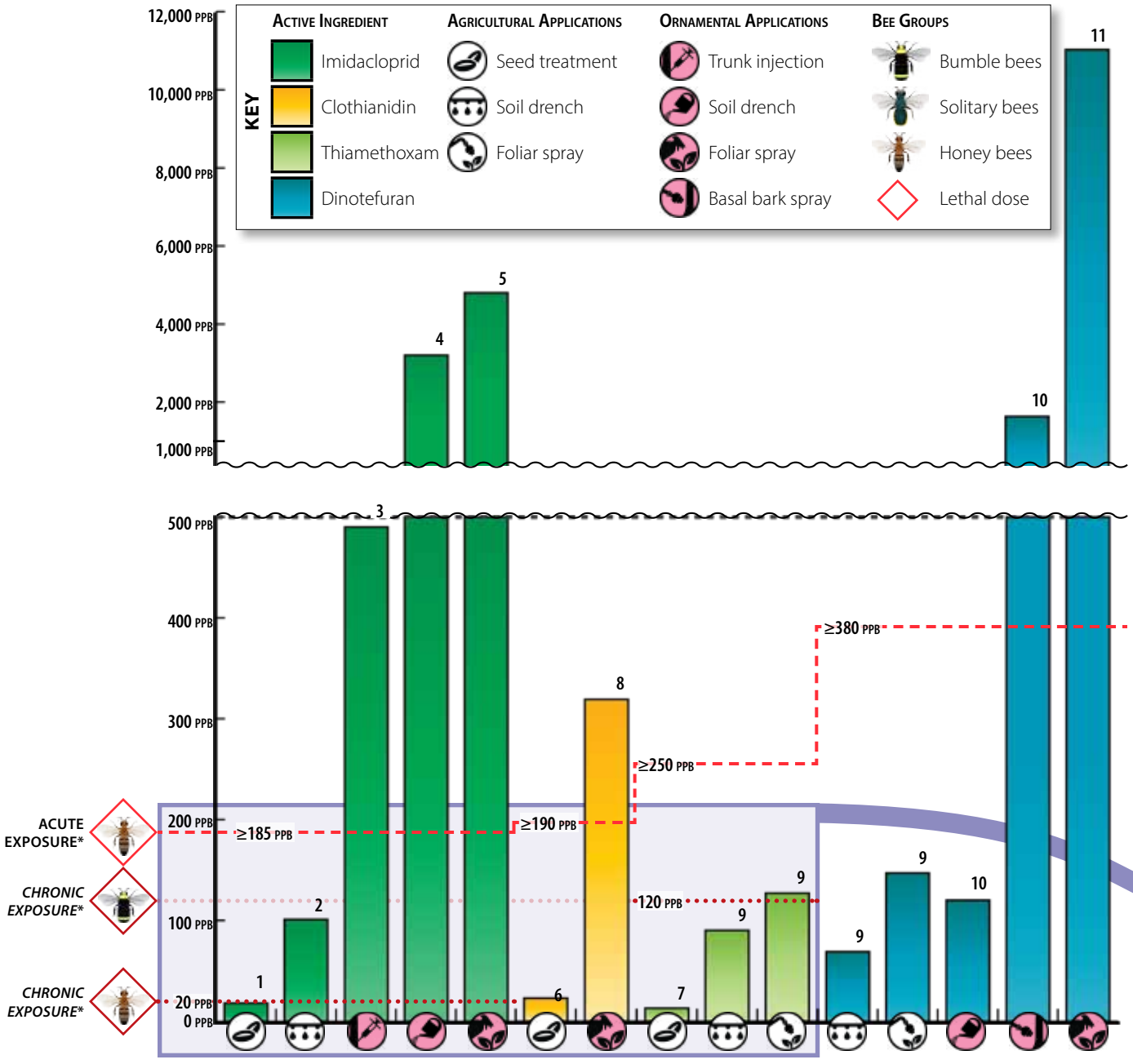
The alkali bee is native species that has become an important managed pollinator of alfalfa (bottom) across the western states, but little is known about how this ground-nesting species (at top, the bee is beside its nest entrance) responds to different neonicotinoids.

A large-scale field study by Rundlof et al. (2015) evaluated some of these sublethal effects to soil nesting bees. More specifically, the study evaluated the impact to wild bees from insecticide-coated seeds. They found reduced density of wild bees foraging in fields where seeds had been treated with the insecticides clothianidin and cyfluthrin and the fungicide thiram, compared to the fields planted with seeds coated with only the fungicide. Furthermore, the study found that insecticide seed coatings correlated with reduced nesting of the wild red mason bee. Red mason bees built brood cells in six of the eight fields planted with only the fungicide but in none of the fields treated with the insecticides (Rundlof et al. 2015).

Consideration of solitary bees is not currently required in pesticide risk assessments and regulations (e.g., Blacquiere et al. 2012). However, using cavity-nesting bees like the blue orchard bee in field tests might be more informative than toxicological studies examining honey bees given the more limited foraging range and higher crop fidelity of most solitary species. As the acreage of neonicotinoid-treated crops increases there are proportionately fewer areas of untreated plants. Smaller native bees that have shorter flight ranges may increasingly need to forage in treated areas.

Additionally, solitary bees might be a more straightforward study model compared with social bees because solitary bees have a direct link between an individual and reproductive success. Comparative studies on solitary bees with differing life-history traits (nesting materials, foraging specialization, number of generations per growing season) would provide a better understanding of the broader environmental impacts of systemic insecticides. Wild solitary bees are important for food security (Garibaldi et al. 2013) as well as for biodiversity, and should be considered in risk assessments.

FIGURE 6.1-1: Known Residues (in ppb) in Plants from Various Application Methods



Sources (Figs 6.1-1 and 6.1-2):

1. Bonmatin et al. 2005a
2. Dively and Kamel 2012
3. Maus et al. 2004b
4. Doering et al. 2005a
5. ODA 2014d
6. Rundloff et al. 2015
7. Pohorecka et al. 2012
8. Larson et al. 2013
9. Dively and Kamel 2012
10. ODA 2013a
11. ODA 2013d
12. Abbott et al. 2008
13. Decourtye et al. 2004b
14. Tan et al. 2014
15. Fetham et al. 2014
16. Whitehorn et al. 2012
17. Laycock and Creswell 2013
18. Yang et al. 2012
19. Fischer et al 2014
20. Larson et al. 2013
21. Mommaerts et al 2000
22. Sandrock et al. 2013
23. Fauser-Misslin et al. 2013
24. Henry et al 2012
25. Elston et al. 2013
26. Sandrock et al. 2013

** See Table 5.2 on page 29*

6

Neonicotinoid Residues and Persistence

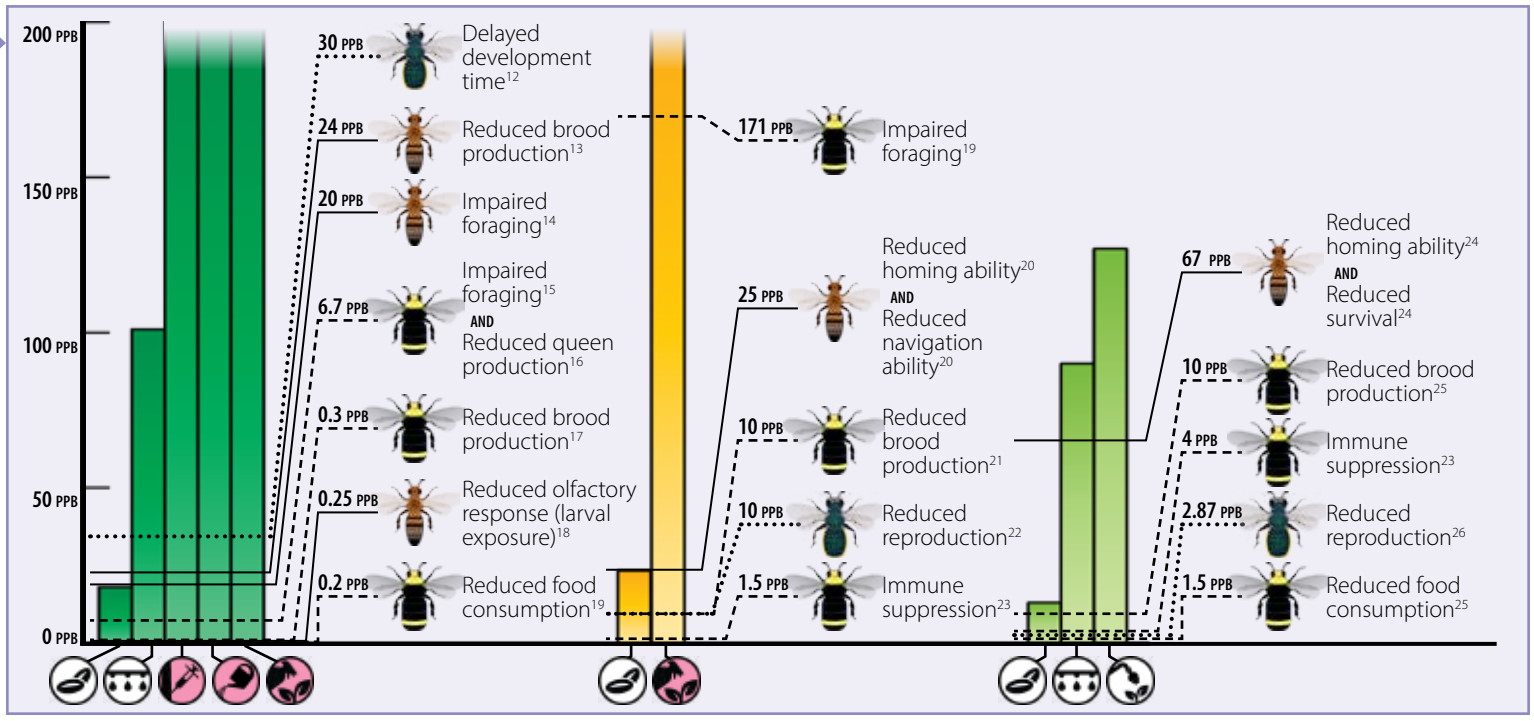
6.1 Neonicotinoid Persistence and Residue Levels in Plants

Farmers of bee-pollinated crops seeking to control pests do not want to harm the pollinators they need for production. Given that the levels of exposure that cause lethal or sublethal effects in bees vary, that different bee species respond differently to exposure, and that some negative effects to bees have been reported at very small amounts of residue (see Table 5.2, page 29), it is extremely important to understand the levels at which neonicotinoid residues occur in real-world settings.

The amount of neonicotinoid that reaches pollen and nectar is a very small part of the dose applied to the plant (Laurent and Rathahao 2003), and there are a number of factors that influence residue levels. For example, neonicotinoids have differing characteristics in the soil. Imidacloprid is more readily absorbed by and mobile in plants by soil application than is acetamiprid (Horowitz et al. 1998, as cited by Buchholz and Nauen 2001). In general, clothianidin, dinotefuran, and thiamethoxam have somewhat similar soil mobility characteristics as imidacloprid, and all four apparently have long half-lives in soil (see Table 3.1). In some types of soil, clothianidin has a half-life of up to 1,155 days (more than three years), but at minimum, remains in the soil for about a year (EPA 2003a).

Figure 6.1-2: Documented Sublethal Effects of Neonicotinoids in Bees

This figure compares plant residues, found after legal applications were made, with pesticide levels that have been shown to cause harm. It provides a blunt assessment of potential risk. This overlay is limited by the fact that residue levels do not necessarily equal exposure level.



A survey of soil residues on farms in the U.K. found clothianidin residues in fields that had previously been planted with thiamethoxam-coated seeds. (Clothianidin is a break-down product of thiamethoxam.) Two years after planting, clothianidin residues in soils in the center of fields were 1.2–10.3 µg/kg (ppb); three years following planting, soil residues were 0.32–4.5 µg/kg (ppb) (Jones et al. 2014). Imidacloprid also appears to remain in soil for a long time; one study found a half-life of over two years in sand and sand–dolomite soil material used for bedding plants (Baskaran et al. 1999). Fertilizers can slow neonicotinoid degradation in the soil: imidacloprid readily adsorbs to organic matter such as cow manure and will linger three times longer in soil with organic fertilizer (Rouchaud et al. 1996). In contrast to other neonicotinoids, acetamiprid and thiacloprid degrade quickly in soil (half-lives are estimated at 8 and 27 days respectively) (EPA 2002, 2003b).

With such persistence in soil, it would be expected that imidacloprid, clothianidin, thiamethoxam, and dinotefuran residues would accumulate in the soil from repeated applications over time, but data is limited. Two industry studies monitored residues in soils during six years of repeated applications of imidacloprid; both studies indicated some degree of accumulation. Soil residues increased steadily over time following soil spray applications of imidacloprid (Confidor 70 WG) in apple orchards, and appeared to plateau in the final years of the study with residues of 45–77 ppb (DAR 2006). Over a six year period of repeated plantings of barley seed coated with imidacloprid at a low application rate, residues climbed from 6–18.5 ppb in the first year to 34–49 ppb in year six (DAR 2006). At a higher application rate, year one residues were 16.6 –26.6 ppb and year six residues were 66.1–87.6 ppb) (DAR 2006)—and showed no signs of reaching a plateau (EFSA 2008). Monitoring of residues was not continued beyond the six years, so currently it is unknown how long residues remained at measureable levels in the soil following repeated applications. However, it has been demonstrated that plants can pick up residues in soil from prior applications (Bonmatin et al. 2005b). Untreated plants that follow repeated applications of neonicotinoids (e.g., a cover crop of red clover planted following a rotation of treated corn, soybean, and wheat) will likely take up residues from the soil, though the extent to which this may occur is still unknown and worthy of investigation.

Cloyd and Bethke (2011) suggest that residue concentrations in pollen or nectar may vary with plant and flower morphology; this should be investigated further. Residue concentrations in pollen and nectar also may fluctuate. There is evidence that residues—from seed coatings at label rates—increased in sunflower heads over time to an average of 8 ppb (Bonmatin et al. 2005b). In longer-lived plants (i.e., trees, shrubs, or perennial plants), this accumulation may be more significant. Samples of rhododendron blossoms taken 356 days after treatment at label rates had higher levels of imidacloprid (0.0518–0.1804 mg/kg [51.8–180.4 ppb]) than did samples taken from the same plants 17 days after treatment (<0.0015–0.0168 mg/kg [1.5–16.8 ppb]) (Doering et al. 2004c).

In the United States, five main neonicotinoid application methods—seed coatings, soil drenching (including chemigation), trunk injections, basal bark, and foliar sprays—are employed to varying degrees and deliver a range of doses. In this chapter, we review known measurements of neonicotinoid residues that result from these application methods.

Pesticides are applied to a wide variety of crops and ornamental plants in the United States. Below, a crop duster in sprays herbicide on fields in January in advance of planting. While herbicides generally do not cause direct harm to bees, they can reduce forage and quality of nesting habitat.

6.2 Residue Levels from Neonicotinoid Application to Agricultural Crops

6.2.1 Application by Seed Coating in Agricultural Crops

Residue levels in pollen or nectar that result from neonicotinoid-coated seed are more studied than those resulting from other applications. This may relate to the frequency of use of coated seed compared with other application methods, though this cannot be confirmed. Currently pesticide use, including the planting of coated seeds, is not tracked at a national level in the United States.

- Imidacloprid residues in pollen of seed-treated (at label rates) corn, a wind-pollinated crop from which bees collect pollen, were 2.1 ppb on average and up to 18 µg/kg (ppb) (Bonmatin et al. 2005a).
- Imidacloprid residues in sunflower nectar after seed coating contained 1.9 ppb (Schmuck et al. 2001). Studies of imidacloprid residues in sunflower pollen after seed coating at label rates found concentrations of an average of 3 ppb (Bonmatin et al. 2005a) or 3.9 ppb (Schmuck et al. 2001), and a maximum of 11 ppb (Bonmatin et al. 2005b).
- Bonmatin et al. (2003, 2005b) detected trace levels of imidacloprid at 1–2 µg/kg (ppb) in untreated sunflowers grown one year after a seed-treated crop had been planted in the same soil.
- Reported clothianidin residues in canola vary between studies. Maximum concentrations were 3 ppb in pollen and 3.7 ppb in nectar from canola seed treated with clothianidin at label rates (Cutler and Scott-Dupree 2007). In a subsequent study (Cutler et al. 2014), the maximum clothianidin residues were 1.9 ppb in pollen. Pohorecka et al. (2012) reported maximum levels of 3.7 ppb clothianidin in pollen and 10.1 ppb in nectar from seed-treated canola. Rundlof et al. (2015) reported maximum pollen residues of 23 ppb and nectar residues at 16 ppb.
- Krupke et al. (2012) reported residue levels of 3.9 ppb of clothianidin in pollen of corn grown from seed coated at label rates. The maximum corn pollen residues reported by Cutler and Scott-Dupree (2014) was 0.8 ppb.

Neonicotinoid use has increased rapidly due to the planting of coated seeds. This practice is generally used as 'insurance' against possible pest outbreaks. Yet, the EPA and agricultural researchers question the ability of coated soybean seed to manage common crop pests. Neonicotinoid use could drop considerably by halting these prophylactic uses.

- Thiamethoxam residues of 1.7 ppb were measured in corn pollen of plants grown from thiamethoxam-treated seed at label rates (Krupke et al. 2012).
- In a first year planting of treated corn seed, Pilling et al. (2013) report residues of up to 12 ppb thiamethoxam in corn pollen, as well as residues of up to 6 ppb of clothianidin, the primary metabolite of thiamethoxam. In the second year of planting treated corn seed, up to 7 ppb thiamethoxam was found in corn pollen, with clothianidin residues at or below 3 ppb (Pilling et al. 2013).

With over 3,500 species of native bees in the U.S. and Canada, many pesticide-treated crops depend on less-visible solitary bees for pollination services, like this tiny sweat bee pollinating canola blossoms.

- Thiamethoxam was detected in canola nectar and pollen at a maximum of 12.9 ppb in nectar and 9.9 ppb in pollen respectively (Pohorecka et al. 2012).
- Pilling et al. (2013) recorded thiamethoxam residues up to 4 ppb in canola nectar and up to 4 ppb in canola pollen, with the metabolite clothianidin at a maximum of 1 ppb in either nectar or pollen. Residues in seed-treated canola following a seed-treated barley crop were slightly higher, with around 4.5 ppb in nectar and up to 6 ppb in pollen, with the metabolite clothianidin at a maximum of 4 ppb in pollen (Pilling et al. 2013).

Concerns for pollinators and other beneficial insects from the exposure to residues that result in pollen and nectar from the use of coated seeds are surfacing (Douglas et al. 2015; Douglas and Tooker 2015; Pecenka and Lundgren 2015; Rundlof et al. 2015), although it is still an area under debate. It has been suggested by some that coated seed may be less harmful to pollinators than other application methods because concentration of the insecticide decreases over time as the biomass of the growing plant increases (Krischik et al. 2007). One estimate found that only 0.005% of the imidacloprid absorbed by a sunflower plant is translocated to the pollen following the use of coated seed (Laurent and Rathahao 2003). However, studies examining residues in pollen and nectar following repeated use of coated seed over time are needed. Soil residues climbed higher each year of successive planting of neonicotinoid-seed treated crops and did not plateau after six years of planting (EFSA 2008). Repeated annual plantings of seed-treated crops may lead to increased residue levels in pollen and nectar that may pose more of a risk to bees, since residues from previous seasons remain in the soil.

Residues from coated seeds are often reported in studies as benchmark levels of residues observed in the field (e.g., Franklin et al. 2004; Blacquiere et al. 2012) even though other methods of application are registered, are commonly used in the United States, and often deliver a higher residue level in pollen and nectar.

6.2.2 Application by Soil Drench in Agricultural Crops

Studies examining neonicotinoid residues in crop plants that result from soil applications are less common than studies of coated seed.

- Apple trees ('James Grieve') treated by soil application at label rates had imidacloprid residues at 12 ppb or lower in blossoms 197 days after treatment (Doering et al. 2004a).
- Imidacloprid applied to pumpkins at label rates in transplant water resulted in residues of 30.1–86.6 ppb in pollen and 3.8–11.9 ppb in nectar. Plants first treated with a half rate in transplant water with the remaining half applied by drip irrigation at flowering had much higher levels: 52.3–101 ppb in pollen and 9–13.7 ppb in nectar (Dively and Kamel 2012).
- Squash varieties treated with imidacloprid via either surface spray to the soil during seed planting or chemigation five days after transplanting had average concentrations of imidacloprid of 14 ppb (range: 6–28) in pollen and 10 ppb (range: 5–14 ppb) in nectar (Stoner and Eitzer 2012).

- Citrus trees treated 50 or 55 days before bloom by soil drench at the maximum label rate (0.5 lbs/ac) had imidacloprid residues between 2.9 and 39.4 µg/l (2.9 and 39.4 ppb) (Byrne et al. 2013). Imidacloprid was present in nectar with residues between 2 and 16 ng/ml (2 and 16 ppb) in citrus trees treated either 227 or 232 days before bloom (Byrne et al. 2013).
- Dinotefuran applied with half of the labeled application rate in water used on pumpkin transplants with the remaining half applied by drip irrigation three weeks later was found in pollen at concentrations of 44–69.2 ppb and in nectar at 7.1–10.6 ppb (Dively and Kamel 2012).
- Thiamethoxam applied to pumpkins with half of the labeled application rate in water used on transplants with the remaining half applied by drip irrigation three weeks later was found in pollen at 54.8–90.4 ppb and in nectar at 7.8–12.2 ppb (Dively and Kamel 2012). Although clothianidin was not applied to the pumpkins, Dively and Kamel (2012) found it to be present in pollen and nectar at about half the levels of thiamethoxam, because clothianidin is a metabolite of thiamethoxam (Nauen et al. 2003). Total residues in pollen ranged from 68.6 to 131.6 ppb and in nectar from 10.2 to 18.6 ppb (Dively and Kamel 2012).
- Squash varieties treated with thiamethoxam via either surface spray to the soil during seed planting or chemigation five days after transplanting had average concentrations of thiamethoxam of 12 ppb (range: 5–35) in pollen and 11 ppb (range: 5–20 ppb) in nectar (Stoner and Eitzer 2012).

Squash bees (*Peponapis* spp. and *Xenoglossa* spp.) are important pollinators of pumpkins, squash, and other plants in the genus *Cucurbita*, because they exclusively collect cucurbit pollen for their offspring. Above: two females collect nectar from a zucchini flower; below: males congregate inside a squash flower, waiting for females.

Soil drench residues in pollen and nectar are higher than levels reported from seed applications. Imidacloprid, at the levels reported by Stoner and Eitzer (2012) and Dively and Kamel (2012), caused sublethal effects on honey bees, bumble bees, and solitary bees (see Table 5.2). According to Dively and Kamel (2012), the highest amounts of imidacloprid in pollen and nectar resulted from split applications, one half of which came during flowering. Labels of neonicotinoid products registered for use on squash (and some other continuously blooming crops) allow application during bloom, so bees that forage on squash blossoms after treatment will be exposed to higher concentrations of neonicotinoids. The application of thiamethoxam resulted in the simultaneous presence of both thiamethoxam and clothianidin (a break-down product of thiamethoxam) residues in nectar and pollen (Dively and Kamel 2012), potentially leading to an additive effect of these two compounds on bees.

6.2.3 Application by Trunk Injection in Agricultural Crops

While blossom residues in several ornamental landscape plants have been measured (see Section 6.3.3), we are unaware of residue measurements taken for pollen or nectar in tree crops after trunk injections. However, research (e.g., Maus et al. 2004b) clearly indicates that residues from trunk injections are a significant risk if trees are insect-pollinated or visited by pollinators.

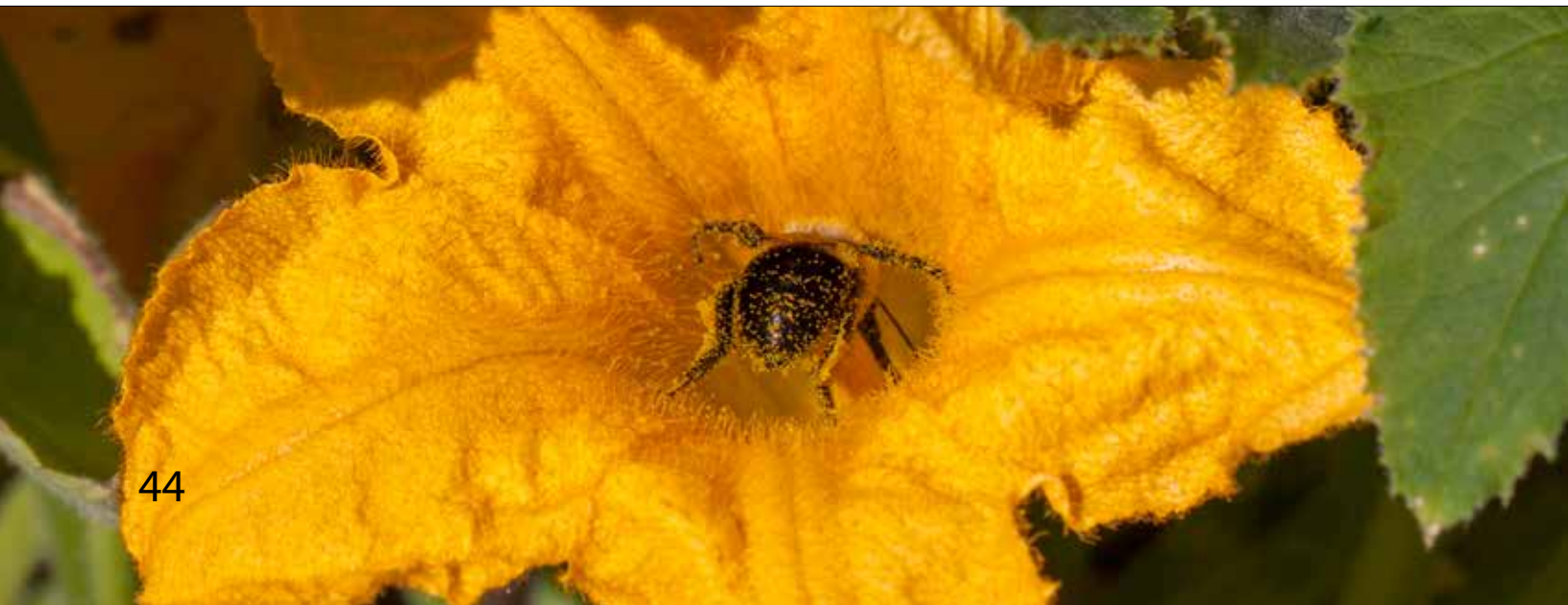
6.2.4 Application by Foliar Spray in Agricultural Crops

Studies examining neonicotinoid residues in crop plants that result from foliar spray applications are also less common than studies of coated seed. Having additional data on residues in pollen and nectar from foliar applications would be valuable because some labels allow multiple applications within a growing season.

- Dinotefuran applied as a foliar spray to pumpkins at label rates (two half-rates at 4 and 6 weeks after transplanting, bloom started approximately at 5 weeks) was found in pollen at concentrations of 36–147 ppb and in nectar at 5.3–10.8 ppb (Dively and Kamel 2012).
- Thiamethoxam applied as a foliar spray to pumpkins at label rates (two half-rates at 4 and 6 weeks after transplanting, bloom started approximately at 5 weeks) was found in pollen at concentrations of 60.7–127 ppb and in nectar at 6.7–9.1 ppb. Clothianidin (a break-down product of thiamethoxam) was also present in pollen and nectar but in lower concentrations; total residue levels ranged from 70.5-162.1 ppb in pollen and 7.4-12.4 in nectar (Dively and Kamel 2012).
- Acetamiprid applied to canola either one week or two weeks before bloom resulted in average residues of 2.4 ppb (maximum of 13.3 ppb) in nectar and 4.1 ppb (maximum of 26.1 ppb) in pollen, while thiacloprid applied to canola at the same time resulted in average residues of 6.5 ppb (maximum of 208.8 ppb) in nectar and 89.1 ppb (maximum of 102.2 ppb) in pollen (Pohorecka et al. 2012).

Current studies indicate that application at flowering results in higher residue levels (e.g., Dively and Kamel 2012) than applications made at planting (e.g., Stoner and Eitzer 2012). In the U.S., while there are some limits on foliar application during bloom, in general, neonicotinoids can be applied to crops such as cucurbits anytime within the crop cycle (aside from preharvest restrictions), including during flowering as long as certain requirements, such as notifying bee keepers, are met.

High concentrations of dinotefuran and thiamethoxam have been detected in the pollen and nectar of pumpkin flowers of plants that received foliar spray applications after transplant.



6.3 Residue Levels from Neonicotinoid Application in Ornamental Settings

6.3.1 Application by Seed Coating in Ornamental Settings

While residues in several species of crop plants have been measured (see Section 6.2.1), to our knowledge, no measurements are available of pollen or nectar residues of ornamental plants after coated seeds are planted.

6.3.2 Application by Soil Drench in Ornamental Settings

Soil drenches are a common way to treat ornamental plants, many of which are visited by bees and other pollinators. A series of studies conducted by Bayer CropScience AG showed that imidacloprid remained in shrubs and trees for months or years after application by soil drench. The findings from those studies are outlined below. Also listed below are the reported findings from bee kill incidents resulting from soil drench applications.

- Blossoms of soil-treated *Rhododendron* shrubs (at label rates) contained imidacloprid residues of 27–850 ppb 175 days (nearly six months) after treatment (Doering et al. 2004b).
- Imidacloprid residue levels of up to 19 ppb were still present in *Rhododendron* shrub blossoms 3–6 years after soil applications (Doering et al. 2004c).
- Five months after *Eucalyptus* trees were treated with the label rate of imidacloprid via soil injection, imidacloprid was found in floral nectar at a concentration of 286 ppb alone and 660 ppb including its metabolites (Paine et al. 2011).
- Soil applications of imidacloprid to horse chestnut trees (*Aesculus hippocastanum*) (at label rates) resulted in residue levels below the threshold of detection (5 ppb) at 412 days (more than 13 months) after treatment (Maus et al. 2004a). Measurements at earlier dates are unknown because they were not taken during this study.

Treating ornamental shrubs by soil drench can result in long-lasting residues in nectar and pollen. Imidacloprid was found in rhododendron blossoms more than three years after treatment.

The long half-life of imidacloprid allows it to persist in plants at high levels for months. *Eucalyptus* trees tested positive for imidacloprid and its metabolites at nearly equal levels five months after treatment.

- Shrubs in the genus *Amelanchier* (serviceberry, shadbush) had imidacloprid residue levels of 66–4,560 ppb in blossoms 540 days (18 months) after soil applications (at label rates), and leaves had residues from 56–3,200 ppb 186 days (six months) after treatment (Doering et al. 2005a).
- Imidacloprid residues in blossoms of Cornelian cherry (*Cornus mas*) ranged from 1,038 to 2,816 ppb at 505 days (nearly 17 months) after soil application (at label rates) (Doering et al. 2005b).
- Reported bumble bee deaths at a golf course appeared to result from exposure to imidacloprid in the pollen or nectar of littleleaf linden trees (*Tilia cordata*) that received soil treatment. Numerous dead bumble bees were observed directly under flowering trees treated by soil injections, and residues recovered in the dead bees included 146 ppb imidacloprid and 138 ppb of its toxic metabolite, olefin-imidacloprid (CA DPR 2009).
- Imidacloprid residues were detected at 210 ppb in the leaves of *Tilia* trees four months after a soil drench application. Residue testing was performed because bumble bees were found dead under the trees. The application rate was 0.13 fl oz/inch of tree diameter at breast height. In total, the applicators treated approximately 65 trees (only 10 of which were *Tilia*) in an area just over two acres in size. The application was within legal limits per tree but was in excess of the maximum application rate on a per acre basis (ODA 2013b).
- Imidacloprid residues were detected at levels between 72 ppb and 440 ppb in the leaves of *Tilia* trees seven weeks after a soil drench application performed in accordance with label instructions. Reported residues in flowers were between 20 ppb and 69 ppb. Residue testing was performed because bumble bees were found dead under the trees. The application rate was 0.1 fl oz/inch of tree diameter at breast height (ODA 2013c). One year after the incident, residue monitoring from the tree with the highest detection level found 31 ppb in the flowers and 160 ppb in the leaves (ODA 2014a).
- Imidacloprid residues were detected at 53 ppb in the flowers and 290 ppb in the leaves of *Tilia* seven months after a soil injection application performed in accordance with the label. Residue testing was performed because bumble bees were found dead under the trees. The application rate was 0.05 fl oz/inch of tree diameter at breast height (ODA 2014b).

Tilia trees are widespread ornamental trees with blossms that are highly attractive to pollinators, including bumble bees. Neonicotinoids are sometimes used to kill aphids that feed on *Tilia* sap and excrete honeydew (a sticky sugary liquid), and these aphid control efforts have led to multiple bee kills. Investigations into these incidents found that *Tilia* blossoms can continue to contain harmful levels of neonicotinoids during the year after an application.

- Nine littleleaf linden (*Tilia cordata*) trees were treated with dinotefuran by soil drench at the labeled rate of 0.25 fl oz/inch of diameter at breast height (40 other littleleaf lindens at the site received a foliar application that same day). Residue testing was performed as part of an investigation into the estimated 50,000 bumble bees found dead under the trees. Two weeks after the application, blossom and leaf samples were taken. Residue levels were 12 and 120 ppb in the blossoms and 390 and 970 ppb in the leaves of the trees treated by soil drench (ODA 2013a). One year after the incident, residue monitoring of the same two trees found 24 ppb and 76 ppb in the flowers and 650 ppb and 630 ppb in the leaves respectively. Dinotefuran had been applied to the trees in years prior to 2013 (ODA 2014a).

6.3.3 Application by Trunk Injection in Ornamental Settings

While there have been numerous studies looking at translocation of neonicotinoids into leaves and cambium of ornamental trees, few have looked at residues in pollen or nectar. We also include residue data from bee kill incidents resulting from trunk injection applications.

- Trunk injections to horse chestnut trees resulted in imidacloprid residues of 5–283 ppb in blossoms just seven days after treatment at label rates (Maus et al. 2004b).
- Compared with soil applications, trunk injections at label rates more quickly resulted in higher residues in leaves (Maus et al. 2004b).
- Imidacloprid residues were detected at 490 ppb in the flowers and 2,200 ppb in the leaves of *Tilia* four weeks after a trunk injection application performed in accordance with the label. Residue testing was performed because bumble bees were found dead under the trees. The application rate was 4 mL/inch of tree diameter at breast height (ODA 2014c).

6.3.4 Application by Foliar Spray in Ornamental Landscapes

Few studies have measured residues in pollen or nectar of ornamental plants after foliar sprays are applied. We also include residue data from bee kill incidents resulting from foliar spray applications.

- After turf was treated with clothianidin, nectar from white clover flowers in the turf had an average concentration of 171 ppb (range: 89–319 ppb) one week after application (Larson et al. 2013).
- Approximately 40 littleleaf linden (*Tilia cordata*) trees received a foliar treatment of dinotefuran at a mid-range label rate of 6 fl oz/100 gal of water (nine other littleleaf lindens at the site received a soil drench application that same day). Residue testing was performed as part of an investigation into the estimated 50,000 bumble bees found dead under the trees. Two weeks after the application, dinotefuran residue levels in blossoms were 7,400 ppb and 11,000 ppb. The leaves contained residues of 3,800 ppb and 5,400 ppb dinotefuran (ODA 2013a). Dinotefuran had been applied to the trees in previous years. One year after the incident, the leaves and blossoms of the same trees were tested, dinotefuran was not detected (ODA 2014a).
- Imidacloprid residues were detected at 4,800 ppb in the flowers and 1,900 ppb in the leaves of *Tilia* the day after a foliar application to the trees. Residue testing was performed as part of an investigation into the estimated 5,000 bumble bees found dead under the trees. The application rate of 1 fl oz/100 gal of water is a legal label rate. The application also included a small amount of an acephate product that had been left in the tank from an application the day prior (ODA 2014d).

Risks Associated with Neonicotinoid Applications to Ornamental *Tilia* Trees

On July 2, 2013, the Oregon Department of Agriculture (ODA) received the fourth report of a bee kill in three weeks. Bumble bees were found dead and dying under *Tilia* spp. trees (commonly called basswood or linden) in the parking lot of a golf club. The trees attract aphids when the flowers are in bloom. While the aphids don't harm the trees, they drip their excess honeydew, which falls onto the cars parked below. In order to avoid the nuisance honeydew, the golf course had the trees treated by soil drench, prior to bloom, with imidacloprid.

ODA initiated an investigation of the application, ultimately finding that the applicator followed label instructions. In fact, the application rate was the minimum rate of 0.1 fl oz/inch of the trunk diameter at breast height.

In total, since June of 2013 ODA has investigated and confirmed seven incidents of mass bee kills caused by the application of neonicotinoids to *Tilia*. The first case was in Wilsonville, Oregon, where an estimated 50,000 bumble bees (calculated to represent at least 160 distinct colonies) were found dead in a parking lot after the foliar application of dinotefuran to *Tilia* trees. That incident is the single largest native bee kill ever recorded in North America.

Of the seven bee kills, five incidents were caused by prophylactic applications made up to seven months prior to bloom. The treatment methods included tree injection, soil drench, soil injection, and basal bark applications. The other two incidents were caused by foliar applications.

Only one incident had a label violation: exceedance of the annual per-acre application rate, although the rate per tree was within legal limits. While no other label violations were found, some of the other incident investigations uncovered minor violations, such as record keeping mistakes.

In June of 2014, approximately one year after the 2013 incidents, ODA re-sampled the leaves and flowers of the trees originally sampled. Residues were found in all trees treated by non-foliar systemic methods. Dead bees were only seen at one site, in small numbers, and the cause of their death was not evaluated.

In response to the incidents, ODA established a rule prohibiting the application of the most-toxic (nitroguanidine) neonicotinoids—clothianidin, dinotefuran, imidacloprid, and thiamethoxam—regardless of application method, on *Tilia*.

A review of these incidents raises questions as to whether the regulatory changes made in response should be considered more broadly. Should Oregon's prohibition be imposed at the federal level? *Tilia* trees can produce mannose in its nectar that may be slightly toxic to bumble bees. That natural toxin might add to the impact from neonicotinoids, putting bumble bees at greater risk when *Tilia* are treated. A second question is whether the new regulation should extend to other pollinator attractive trees? *Tilia* are treated when they overhang areas, such as parking lots, where honeydew is unwanted. Bees killed over pavement are much easier to spot than bees that fall in the grass or landscaping beds. All the reported incidents occurred over pavement in areas frequented by people. Have other similar incidents, involving other tree species, gone unnoticed?

These incidents also raise questions as to short- and long-term sublethal effects from neonicotinoid applications to trees. If legal application rates caused acute bee kills, are other subtle yet harmful effects arising from applications to trees? In addition, based on the data showing residues one year post application, does the risk increase over time if applications occur annually?

Further information about application rates and detected residues for these incidents can be found in Section 6.3 Residue Levels From Neonicotinoid Application in Ornamental Settings on page 45.

Sources:

1. ODA 2013a.
2. ODA 2013b
3. ODA 2013c.
4. ODA 2013d.
5. ODA 2014a.
6. ODA 2014b.
7. ODA 2014c
8. ODA 2014d
9. ODA 2014e.
10. ODA 2015.

6.3.5 Application by Basal Bark Spray in Ornamental Settings

There is no known study that has measured residues in pollen or nectar of ornamental plants following basal bark applications. We include residue data from bee kill incidents resulting from basal bark applications.

- Thirteen weeks after a basal bark application of dinotefuran at label rates to *Tilia* trees residues were detected at levels of 630 and 24,000 ppb on leaves, and 27 ppb and 1,600 ppb in the flowers. The tree with residues at 24,000 ppb on leaves and 1,600 ppb in the flower had dead bumble bees underneath. That tree also reportedly appeared stressed and had more blossoms than other *Tilia* trees in the area. One year after the incident, residue monitoring of the same two trees had a "no-detect" and 100 ppb in the flowers, and 190 ppb and 2,100 ppb in the leaves. Dinotefuran had been applied to the trees in years prior to the incident. (ODA 2013d, 2014a).

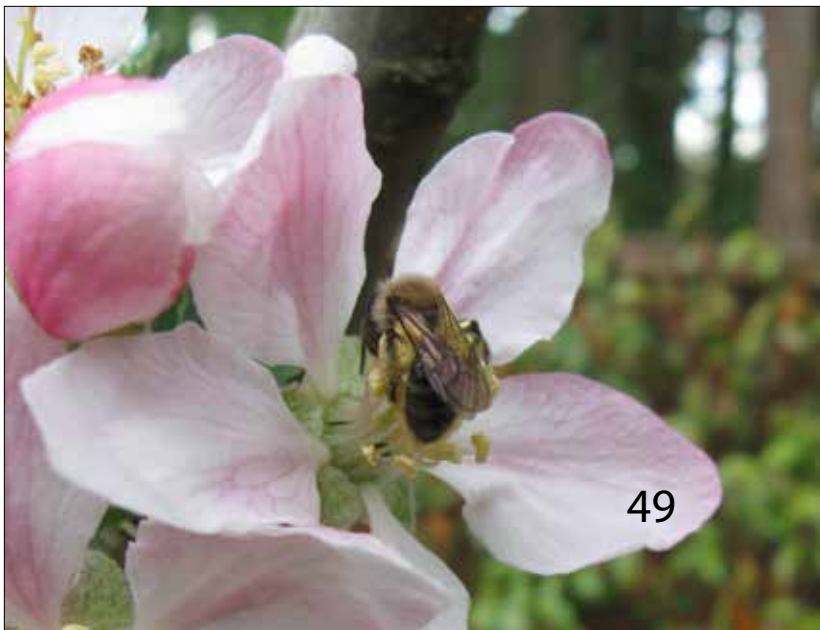
6.4 Rates of Application in Ornamental vs. Agricultural Settings

Research to date indicates that neonicotinoid residues in agricultural crops and ornamental plants may pose a risk to bees. Residues in pollen or nectar are not expected to reach acute lethal levels in agricultural field settings under label rates of applications, but chronic exposure may put bees at risk (e.g., see Section 5.2) and bees may experience detrimental sublethal effects at the levels recorded under some applications (e.g., see Sections 5.2, 5.3, and 5.4). In contrast, residue levels in some ornamental plants far exceed the estimated level of lethal concentration (LC_{50}) for honey bees (see Section 5.2.1 for more information about LC_{50}), which suggests that ornamental use of neonicotinoids poses high risks to bees.

In a comparison between a home product approved for garden use and a professional product approved for agricultural use, a homeowner treating trees in their garden can apply 12 to 16 times the amount of imidacloprid allowed in an agricultural setting, and in certain circumstances it could be twice as much again—or more. (See Case Study: Comparison Between Agricultural and Backyard Products, pages 50–53.)

There is much we do not understand about the movement of neonicotinoids in plants. To ensure adequate protection of pollinators and other plant-visiting beneficial insects, further research is needed to help us understand how several factors (i.e., application method, rate, timing, etc.) contribute to variation in pollen and nectar residue levels, and whether we can manage the use of these products to eliminate their damage to pollinators.

The amount of imidacloprid that is allowed to be applied to apple trees in backyards is many times higher than is allowed in commercial orchards.



Comparison Between Agricultural and Backyard Products

Product	Agricultural Use		Ornamental Use	
Trade Name	Admire Pro Systemic Protectant™	Provado 1.6 Flowable Insecticide™	Bayer Advanced 12 Month Tree & Shrub Insect Control II™ concentrate	
Active ingredient (A.I.)	Imidacloprid	Imidacloprid	Imidacloprid	
A.I. content (%)	42.8%	17.4%	2.94%	
A.I. per gallon	4.6 lbs A.I./gal	1.6 lbs A.I./gal	0.26 lbs A.I./gal	
Application rate*	10.5 fl oz/ac	40 fl oz/ac†	0.5 fl oz/inch of tree trunk circumference	
Application distribution	200 trees/ac‡	200 trees/ac‡	30" circumference	60" circumference
TOTAL A.I. per tree	**0.03 oz A.I./tree**	**0.04 oz A.I./tree**	**0.4875 oz. A.I./tree**	**0.975 oz A.I./tree**

Notes:

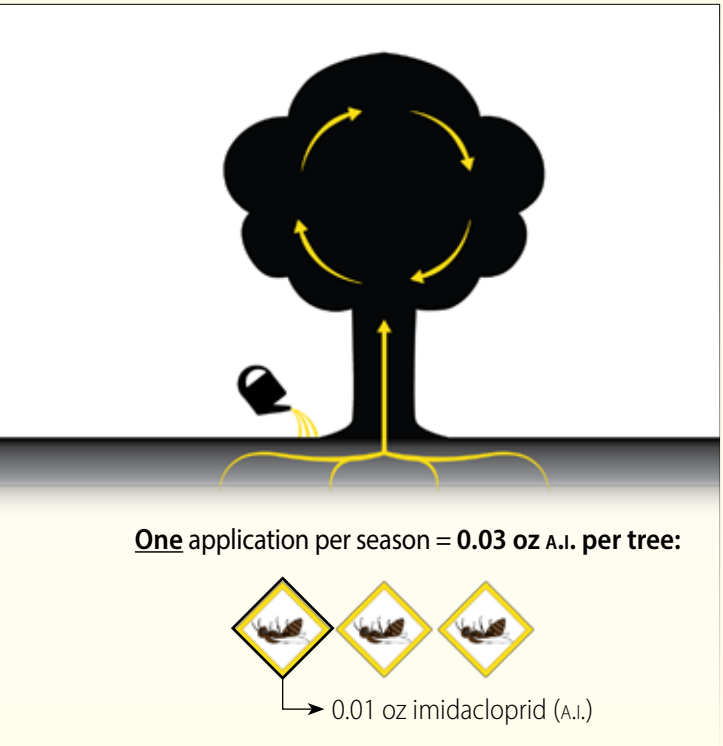
* The legal maximum application rate in one season. For some products this may occur over multiple smaller applications.

† Note that for many apple crop pests (e.g., aphids), applications of Provado are well below 40 fl oz/ac. For explanation of abbreviations and symbols, see Conversions chart on page 65.

‡ Assuming that there are 200 trees per acre in the agricultural setting—a conservative estimate based upon the *Pennsylvania Tree Fruit Production Guide* (available at: http://agsci.psu.edu/tfpg)—that average 60" in circumference (19" diameter) at the base in Pennsylvania orchards.

Agricultural Soil Drench Insecticide: Admire Pro Systemic Protectant™ (EPA Registration # 264-827)

FIGURE: The total A.I. (imidacloprid) applied per tree per season using Admire Pro Systemic Protectant™ soil drench insecticide in an agricultural setting.



The Admire Pro Systemic Protectant™ label (accessed January 7, 2010) shows that the product is comprised of 42.8% A.I. and 57.2% inert ingredients. The label also reports that this percentage equates to 4.6 lbs. of A.I. per gallon.

4.6 lbs A.I./gal	←	Admire Pro Systemic Protectant™ *(per label)*
÷ 128 fl oz/gal	←	(128 fluid ounces in one gallon)
= 0.0359 lbs A.I./fl oz	⇒	Total imidicloprid per fluid ounce
0.0359 lbs A.I./fl oz	↵	
× 10.5 fl oz/ac	←	The maximum legal application rate
= 0.377 lb A.I./ac	⇒	Pounds of imidacloprid per acre
0.377 lb A.I./ac	↵	
÷ 16 oz/ lb	←	(16 ounces in one pound-mass)
= 6.032 oz A.I./ac	⇒	Total imidacloprid per acre
6.032 oz A.I./ac	↵	
÷ 200 trees/ac	←	200 trees per acre (see Table 6.1‡)
= 0.03 oz A.I./tree	⇒	Total imidacloprid applied per season

Apple trees bloom early in the season and provide important nectar and pollen resources for early-emerging species like mason bees (left), bumble bees (middle), and mining bees (right).

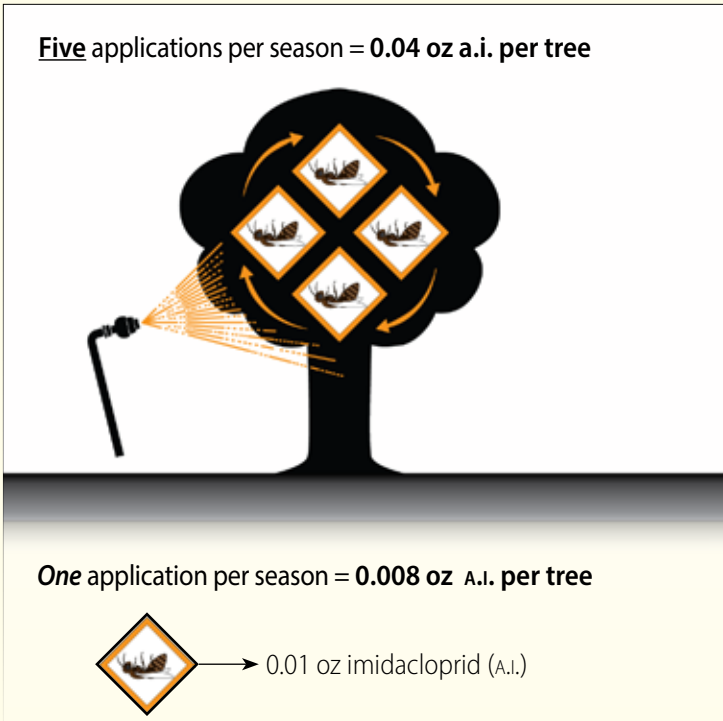
Agricultural Foliar Insecticide: Provado 1.6 Flowable Insecticide™ (EPA Registration # 264-763)

The Provado 1.6 Flowable Insecticide™ label (accessed June 2, 2014) shows that the product is comprised of 17.4% A.I. and 82.6% inert ingredients. The label also reports that this percentage equates to 1.6 lb. of A.I. per gallon.

1.6 lbs A.I./gal	←	Provado 1.6 Flowable Insecticide *(per label)*
÷ 128 fl oz/gal		
= 0.0125 lbs A.I./fl oz	⇒	Total imidacloprid per fluid ounce
0.0125 lbs A.I./fl oz	↵	
× 40 fl oz/ac	←	The maximum legal application rate*
= 0.5 lb A.I./ac	⇒	Pounds of imidacloprid per acre
0.5 lb A.I./ac	↵	
÷ 16 oz/lb		
= 8 oz A.I./ac	⇒	Total imidacloprid per acre
8 oz A.I./ac	↵	
÷ 200 trees/ac	←	200 trees per acre‡
= 0.04 oz A.I./tree	⇒	Total imidacloprid applied per season

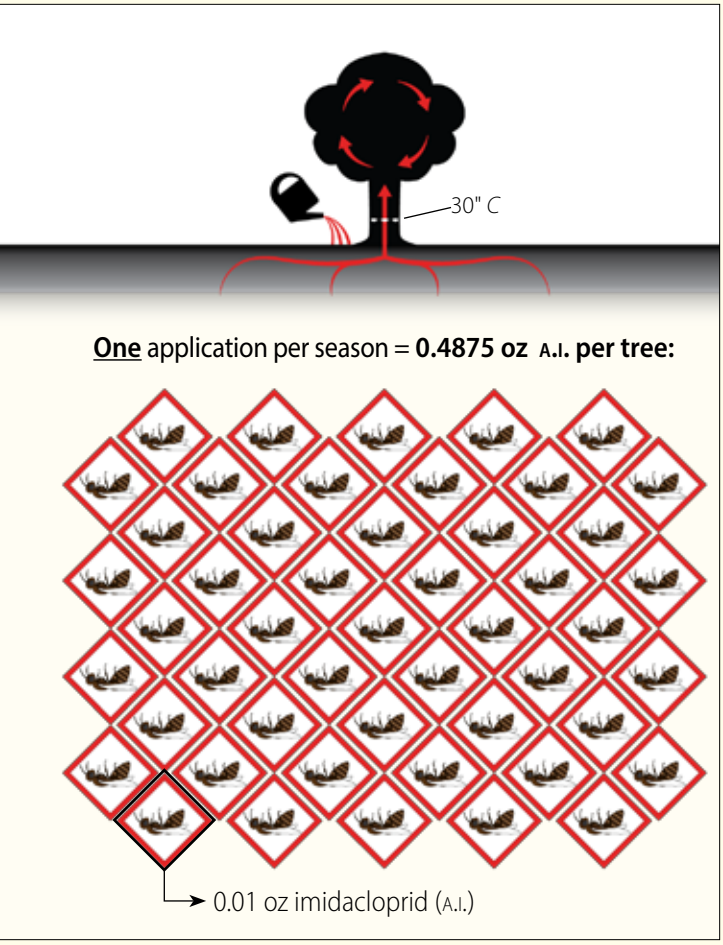
**The maximum legal agricultural application rate of Provado 1.6 Flowable Insecticide™ is 8 fl oz/ac × 5 applications per season = 40 fl oz/ac*

FIGURE: The total A.I. (imidacloprid) applied per tree per season using Provado 1.6 Flowable Insecticide™ foliar spray insecticide in an agricultural setting.



Ornamental or Backyard Soil Drench Insecticide: Bayer Advanced 12 Month Tree & Shrub Insect Control II™ (EPA Registration # 72155-56)

FIGURE: The total A.I. (imidacloprid) applied to a 30" circumference tree using Bayer Advanced 12 Month Tree & Shrub Insect Control II™ soil drench insecticide in an ornamental or backyard setting.



The Bayer Advanced 12 Month Tree & Shrub Insect Control II™ concentrate label (accessed February 10, 2012) shows that the product is comprised of 2.94% A.I. and 97.06% inert ingredients. We contacted the U.S. EPA to get the weight of A.I./gal of concentrate, 0.26 lbs A.I./ gal.

0.26 lbs A.I./gal	← Bayer Advanced 12 Month Tree & Shrub Insect Control II™ *(per EPA)*
÷ 128 fl oz/gal	
= 0.00203125 lbs A.I./ fl oz	⇒ Pounds of imidicloprid per fluid ounce

0.00203125 lb A.I./fl oz	↵
÷ 16 oz/lb	
= 0.0325 oz A.I./fl oz	⇒ Total imidicloprid per fluid ounce

Assuming that a mature apple tree in a backyard has a circumference of 30" (9.5" in diameter), the amount of imidacloprid applied to the tree is:

0.0325 oz A.I./fl oz	↵ Total imidicloprid per fluid ounce
× 30"	← Mature tree with a 30" trunk circumference
× 0.5 fl oz/inch	← Recommended application rate
= 0.4875 oz A.I./tree	⇒ Total imidacloprid applied to 30" tree

This would make the relative application rate of imidacloprid in Bayer Advanced 12 Month Tree & Shrub Insect Control II™

12× greater than Admire[1] and

16× greater than Provado[2].

Additional Notes:

1. The yearly application rate of Admire Pro Systemic Protectant™ in an agricultural setting—see p. 50.
2. The maximum legal agricultural application rate of Provado 1.6 Flowable Insecticide™ applied per tree per season—see p. 51.
3. Based on a small ornamental apple tree with a 30" trunk circumference at the base.
4. The average mature apple tree trunk in Pennsylvania orchards is 60" in circumference (19" diameter) at the base.

LEFT: In addition to bees, apple blossoms are highly attractive to other floral visitors—such as butterfles.

Furthermore, based on data from Pennsylvania apple orchards, the average tree base is 19" in diameter (60" in circumference)—twice that assumed for the backyard calculation. If Bayer Tree and Shrub Insect Control II™ were applied to a garden tree of that diameter, the allowed application would be.

0.0325 oz A.I./fl oz	⤶	Total imidicloprid per fluid ounce
× 60"	←	Mature tree with a 60" trunk circumference
× 0.5 fl oz/inch	←	Recommended application rate
= 0.975 oz A.I./tree	⇒	Total imidacloprid applied to 60" tree

This would make the relative application rate of imidacloprid in Bayer Advanced 12 Month Tree & Shrub Insect Control II™

24× greater than Admire[1] and

32× greater than Provado[2].

In addition, for many apple crop pests such as aphids, applications of Provado are well below the maximum allowed for a season (40 fl oz/ac). Sometimes 3–8 fl oz of A.I./ac provides enough aphid control for the whole season. If only 8 fl oz of Provado is applied in a season, the amount of imidacloprid per tree is:

0.0125 lbs A.I./fl oz	⤶	Total imidicloprid per fluid ounce (see p. 51)
× 8 fl oz/ac	←	A single application at legal application rate
= 0.1 lb A.I./ac	⇒	Pounds of imidacloprid per acre

0.1 lb A.I./ac	⤶	
÷ 16 oz/ lb		
= 1.6 oz A.I./ac	⇒	Total imidacloprid per acre

1.6 oz A.I./ac	⤶	
÷ 200 trees/ac		
= 0.008 oz A.I./tree	⇒	Total imidacloprid applied per season

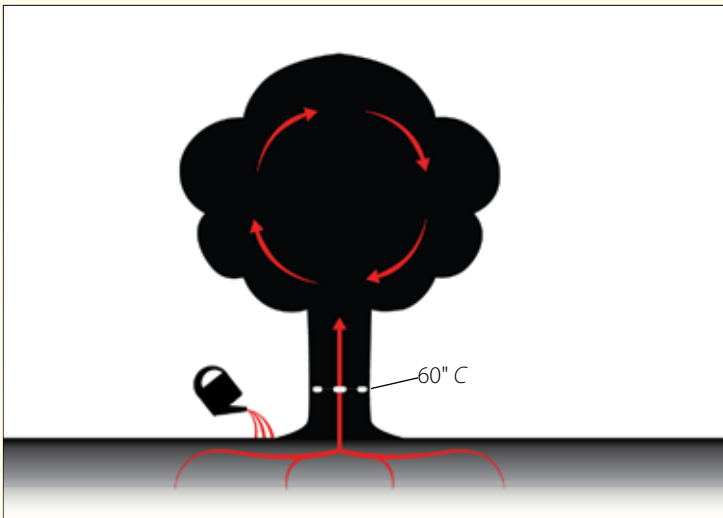
This would make the relative ornamental application rate of imidacloprid in Bayer Advanced *[...]* Insect Control II™

60× greater for a small tree[3] and

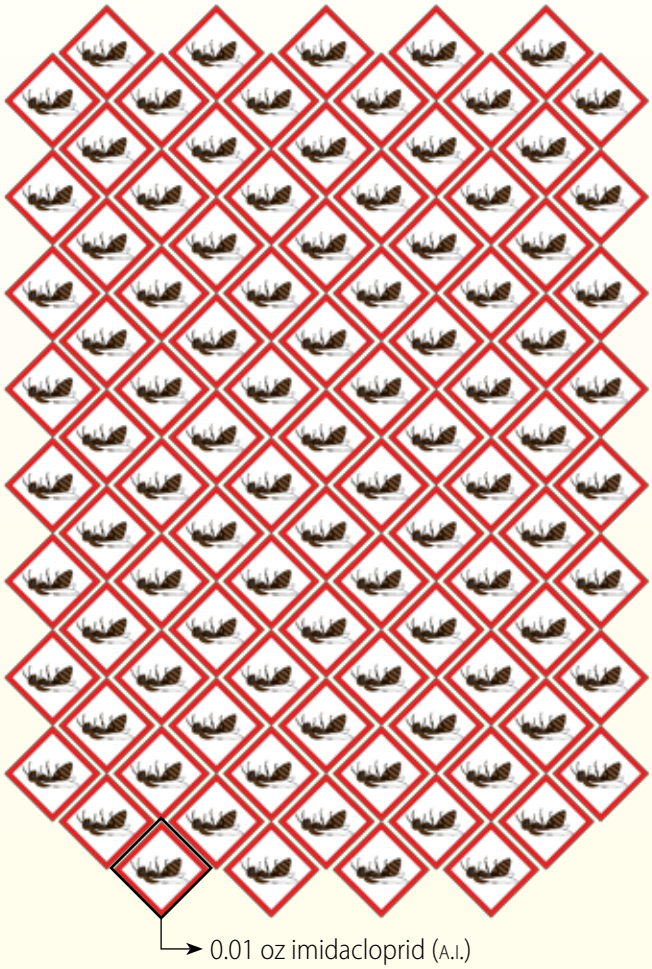
120× greater for an average tree[4]

than a single application of Provado[2] at the agricultural rate.

Figure: The total A.I. (imidacloprid) applied to a 60" circumference backyard tree using Bayer Advanced 12 Month Tree & Shrub Insect Control II™ soil drench insecticide in an ornamental or backyard setting.



One application per season = **0.975 oz A.I. per tree:**



Mining bee (*Andrena* sp.) and sweat bee (*Lasioglossum* sp.) pollinating raspberry.

7

Conclusions

Neonicotinoids have been widely adopted for pest control in agricultural, commercial, and residential landscapes. Consequently, bees are exposed to residues from many sources including in soil, on plants, in water, and in nectar or pollen. An ever-growing body of science points to the fact that this large scale use of neonicotinoids is harming bees.

Research to date indicates that neonicotinoid residues in both agricultural crops and ornamental plants may pose a risk to bees. In agricultural field settings, when neonicotinoids are applied at currently approved rates, residues in pollen or nectar from a single season of applications are not expected to reach levels high enough to cause sudden mortality of bees. Still, research is finding that chronic exposure (i.e., at low concentrations over a long period of time) may put bees at risk. Research findings indicate that bees experience detrimental sublethal effects, such as changes in foraging behavior, reduced predator avoidance, delayed development, or reduced reproduction, at very low residue levels that could realistically be expected under currently approved application rates. Furthermore, the residue levels in some ornamental plants far exceed the level of lethal concentration for honey bees and bumble bees, which suggests that non-agricultural use of neonicotinoids potentially poses more blatant risks to bees than agricultural use.

Along with their risks to pollinators, the use of systemic insecticides can pose risks to other benign and beneficial insects that prey upon crop pests, recycle organic matter, support soil health, or feed other wildlife such as songbirds (e.g., Hopwood et al. 2013). While their systemic activity is considered to offer some degree of protection to beneficial predators and parasites from contact, the harm to beneficial insects caused by neonicotinoids can conflict with the integrated pest management strategy of supporting natural predators.

Also in conflict with the principles of integrated pest management, is the current prophylactic use of neonicotinoids such as the planting of coated seeds. Treatments, performed without documented need, fail to employ a fundamental component of integrated pest management, that of monitoring for pests and employing control techniques only when pest thresholds have been reached.

Furthermore, the long-term persistence of most neonicotinoids and potential for accumulation from repeated applications negates many mitigation

Solitary bees such as this mining bee foraging on a cherry blossom are essential to biodiversity. The pollination services they provide support humans, lifestock, and wildlife.

strategies typically employed to reduce harm to bees. For example, nighttime spraying, not spraying during bloom, and relocating honey bee hives become irrelevant to pollinator protection wherever long-residual systemic insecticides are used.

Limited independent applied research shows promise that some neonicotinoids, specifically the less-toxic, shorter-lived acetamiprid and thiacloprid, can be used in a targeted manner within an integrated plan, without causing significant harm to pollinators and other beneficial insects.

With pollinators and global biodiversity in decline, and with worldwide neonicotinoid use expanding, more robust risk assessments and risk management strategies are critically needed to both elucidate and diminish the role neonicotinoids are playing in this decline. Existing research demonstrates that many of the current uses of neonicotinoids can cause lethal and sublethal effects on pollinators and other beneficial insects. Applications of neonicotinoids should be limited until we have expanded data on if and how neonicotinoid use on a specific plant may be managed to provide pest protection without exposing beneficial insects to sublethal or lethal levels. Without clear evidence that they are not causing long-term harm to nontarget species such as pollinators, the use of neonicotinoids should be restricted to applications that will not affect these vital insects.

For More Information

Ongoing review of research related to neonicotinoids and pollinators has helped the Xerces Society formulate recommendations regarding federal regulation, pesticide risk assessment, areas to advance research, and best management practices to protect pollinators. For guidance specific to pesticides and pollinator habitat go to: www.xerces.org/protect-pollinators-from-pesticides. Furthermore, to access Xerces annotated bibliography of new research released since this report was completed go to: www.xerces.org/neonicotinoids-and-bees.

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Conversions

Quantity	Measurement		Equivalent Value	
	Symbol	Unit	Metric	U.S. Customary
Mass	g	gram	1 g	0.0353 oz
	kg	kilogram	1000 g	2.205 lb
	lb	pound	0.454 kg	16 oz
	mg	milligram	10^{-3} g	3.5274e-5 oz
	ng	nanogram	10^{-9} g	3.5274e-11 oz
	oz	ounce	28.35 g	1/16 lb
	µg	microgram	10^{-6} g	3.5274e-8 oz
Volume	fl oz	fluid ounce	29.57 mL	1/128 gal
	gal	gallon	3.785 L	128 fl oz
	L	liter	1000 cm^3	0.2641 gal
	mL	milliliter	10^{-3} L	0.0338 fl oz
	µL	microliter	10^{-6} L	3.3814e-5 fl oz
Fraction	ppm	parts per million	1 µg/g	n/a
			1 mg/kg	
			1 µg/mL*	
			1 mg/L*	
			1000 ppb	
	ppb	parts per billion	1 µg/kg	n/a
			1 ng/g	
			1 ng/mL*	
			1 µg/L*	
			0.001 ppm	

Notes:

* Contaminants in solution are expressed as mass per volume of water (mg/L). Because 1 mg/L is equal to 1 mg/kg in water, and 1 mg/kg equals 1 ppm, contaminated solutions can also be expressed as parts per million (ppm). It is a slightly less accurate expression, because if the solution is not entirely water (e.g., sugar solution fed to bees), the calculations do not convert precisely.

Mining bee (*Andrena* sp.) pollinating plum blossoms.

Glossary

A.I.: Active ingredient (e.g., imidacloprid).

ACUTE: Single exposure, or short term exposure (less than 24 hours).

CENTRAL NERVOUS SYSTEM: Part of the nervous system, specifically the brain and nerve cord.

CHRONIC: Repeated exposures over a long period of time (days, months).

CONCENTRATION: Amount of pesticide or other chemical in a quantity of liquid or solid (e.g., expressed as mL/L, µg/kg).

DOSE: Amount of a compound that is ingested by or applied to an organism. It may be expressed in mg of chemical per kilogram of body weight (mg/kg) or the weight of chemical per individual (ng/bee).

FECUNDITY: Number of offspring produced.

FORAGING: Searching behavior of animals (e.g., for food).

GENE EXPRESSION: Process by which genetic information stored in DNA is interpreted to synthesize proteins.

HALF-LIFE: Time required for half of the chemical residue (whether it be in soil, water, plant, or animal) to break down.

INSECTICIDE: Type of pesticide used to kill insects.

IPM: Integrated Pest Management. An approach to pest management that utilizes knowledge of the pest's life cycle, established action thresholds, and a combination of pest control measures to manage pests in the most environmentally sensitive way possible.

LC_{50}: Lethal Concentration. The concentration of toxicant that induces mortality in 50% of the study organisms, usually expressed as parts per million (ppm) or parts per billion (ppb) for dietary studies, or as mg/L for toxicants dissolved in water. The lower the LC_{50}, the more toxic the substance.

LD_{50}: Lethal Dose. The dose that induces mortality in 50% of study organisms, usually expressed as the weight of the substance per individual (ng/bee) or unit of body weight (mg/kg). The LD can be applied topically (contact LD_{50}) or fed to the study organism (oral LD_{50}). The lower the LD_{50}, the more toxic the substance.

METABOLITE: Compound that results from an organism's metabolic processes. (For example, when we consume starch, it is broken down by our bodies into glucose, which is then further metabolized into a unit of energy.)

NOEL: No Observable Effects Level. The greatest concentration of pesticide that causes no detectable behavioral, physiological, or biochemical change in the animal under study.

PARASITE: Organism that completes its development by feeding on another organism (internally or externally), weakening but often not directly killing its host. (For example, mosquitoes feeding on humans.)

PARASITOID: Insect that completes its development by feeding on another arthropod (internally or externally), eventually killing its host. (For example, a braconid wasp that lays its eggs inside the body of tobacco hornworm caterpillars.)

PER: Proboscis Extension Reflex. This term is used to refer to a test that uses the extension of the proboscis in response to an olfactory stimulus such as nectar as a measurable reaction.

SEMI-FIELD STUDY: A study done in an enclosed space such as a greenhouse, large cage inside or outside, or flight tunnel, where bees have enough room to fly and forage more naturally. These studies often involve entire colonies of social species.

SUBLETHAL DOSE: Dose or a concentration that does not induce significant mortality but may induce other detrimental effects.

SUBLETHAL EFFECT: Effect (behavioral, physiological) on individuals that survive the exposure to a pesticide.

SYSTEMIC INSECTICIDE: Insecticide which can be absorbed by plants and can poison insects that feed on the plant's various tissues. Systemic insecticides can also enter the bloodstream of some animals (e.g., household pets) and will poison insects that feed on those animals (e.g., fleas).

TOXICITY: Ability of a compound to cause damage to an organism.

TRANSLOCATION: Movement of a substance throughout a plant's various tissues from the site of absorption. (For example, foliar spray applied to leaves is translocated through shoots, leaves, roots, and flowers).

Additional Acknowledgements

Photographs

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Elizabeth Sellers [flickr.com/esellers/]: alfalfa leafcutter bee on prairie coneflower (page 36).

Amber Vinchesi, Washington State University: alkali bees on alfalfa flower (2) and exiting ground nest (p. 37).

Kevin Wood [flickr.com/kdwood2]: crop duster spraying herbicide (p. 40).

The Xerces Society/Nancy Lee Adamson: bumble bee on peach blossom (page ix); mining bees pollinating blueberries (p. 2); mining bee pollinating apples (p. 10); honey bee collecting corn pollen (p. 23); bumble bees on sunflower (p. 31); male squash bees in a squash flower (p. 43); bumble bee and mining bee pollinating apples (p. 51); swallowtail butterfly on apple blossom (p. 52); bumble bees collecting corn pollen (back cover).

The Xerces Society/Thelma Heidel-Baker: monarch caterpillar on milkweed (p. 33).

The Xerces Society/Sarah Foltz Jordan: mining bee and small sweat bee pollinating raspberry (p.54); mining bees pollinating cherry (p. 55) and plum (p. 68).

The Xerces Society/Matthew Shepherd: commercially available garden pesticides (p. 1), orange-rumped bumble bee on *Rhododendron* sp. blossom (p. 2), backyard apple tree in bloom and visiting mining bee (p. 49).

The Xerces Society/Mace Vaughan: female blue orchard bee capping tunnel nest (p. 35), sweat bee pollinating canola flower (p. 42).

Artwork

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U.S. Geological Survey [www.usgs.gov]**:** Figures 3.1.1–3 (p. 4–6)

Woodcutter Manero [woodcutter.es]: "Agricultural use" and "Residential use" icons for Table 3.2 (p. 9).

The Xerces Society/Justin Wheeler: Figure 4.2 (p. 17).

The Xerces Society/Sara Morris: Figure 4.1 (p. 14–15); Table 5.2 (p. 28); Figures 6.1-1 & 6.1–2 (p. 38–39); Case Study (p. 50–53).

Bumble bees collecting corn pollen.

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