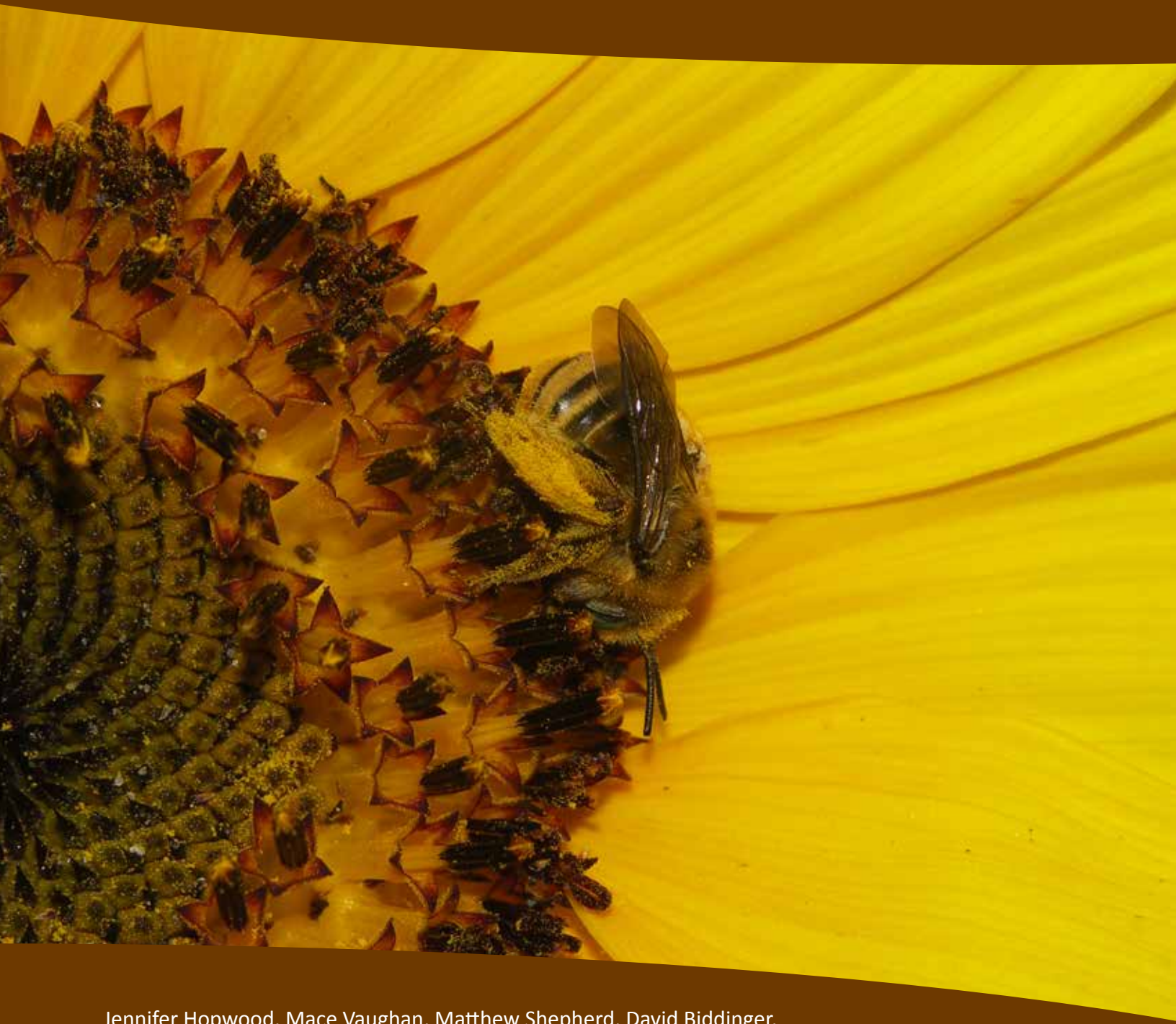
# ARE NEONICOTINOIDS KILLING BEES?

## A Review of Research into the Effects of Neonicotinoid Insecticides on Bees, with Recommendations for Action



Jennifer Hopwood, Mace Vaughan, Matthew Shepherd, David Biddinger,
Eric Mader, Scott Hoffman Black, and Celeste Mazzacano

The Xerces Society for Invertebrate Conservation

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The Xerces Society for Invertebrate Conservation

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THE XERCES SOCIETY
FOR INVERTEBRATE CONSERVATION

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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**BRING BACK THE POLLINATORS**
A Xerces Society Conservation Campaign



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**Front Cover Photograph**
Long-horned bee (*Melissodes* sp.) foraging on sunflower. Photograph by Mace Vaughan/The Xerces Society.

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# EXECUTIVE SUMMARY

Neonicotinoid pesticides were first registered for use in the mid 1990s. Since then, these chemicals have become widely adopted for use on farm crops, ornamental landscape plants, and trees. Of the six neonicotinoids commonly used on plants, the most widely used is imidacloprid. Neonicotinoids are systemic chemicals; they are absorbed by the plant and are transferred through the vascular system, making the plant itself toxic to insects.

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 absorbed into the plant, neonicotinoids can be present in pollen and nectar, making these floral resources toxic to pollinators that feed on them. The 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 outside of the bloom period. In addition, neonicotinoids persist in the soil and in plants for very long periods of time.

Across Europe and the United States, a possible link to honey bee die-offs has made neonicotinoids controversial. Several European countries have reexamined the use of neonicotinoids in crops such as corn, canola, and sunflower. In the United States and elsewhere, a number of opinion articles, documentary films, and campaigns have called for the ban of neonicotinoids.

This report reviews research on the impact of these pesticides on bees. We also identify knowledge gaps, highlight research needs, assess current regulations, and make recommendations for protecting bees.

## Findings

The following findings are divided into three sections. In the first section, we present clearly documented information about neonicotinoid impacts on bee, i.e., facts that are supported by an extensive body of research. 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 products.

**Neonicotinoid insecticides have been applied to hundreds of thousands of acres of farmland. Impacts on bees have been demonstrated, but there are still many things that are not known about the effects neonicotinoids have on these and other pollinators. (Photograph: USDA-ARS/Brian Prechtel.)**

### Clearly Documented Facts

#### *Exposure of bees to neonicotinoids*

- Neonicotinoid residues found in pollen and nectar are consumed by flower-visiting insects such as bees. Concentrations of residues can reach lethal levels in some situations.
- Neonicotinoids can persist in soil for months or years after a single application. Measurable amounts of residues were found in woody plants up to six years after application.
- Untreated plants may absorb chemical residues in the soil from the previous year.
- Products approved for home and garden use may be applied to ornamental and landscape plants, as well as turf, at significantly higher rates (potentially 120

times higher) than those approved for agricultural crops.

- Direct contact with foliar neonicotinoid sprays is hazardous to pollinators, and foliar residues on plant surfaces remain toxic to bees for several days.
- Neonicotinoids applied to crops can contaminate adjacent weeds and wildflowers.

*Effects on honey bees*

- Imidacloprid, clothianidin, dinotefuran, and thiamethoxam are highly toxic to honey bees.
- Thiacloprid and acetamiprid are mildly toxic.
- After plants absorb neonicotinoids, they slowly metabolize the compounds. Some of the resulting breakdown products are equally toxic or even more toxic to honey bees than the original compound.
- Honey bees exposed to sublethal levels of neonicotinoids can experience problems with flying and navigation, reduced taste sensitivity, and slower learning of new tasks, which all impact foraging ability.

*Effects on bumble bees*

- Laboratory studies demonstrate that imidacloprid and clothianidin are highly toxic to bumble bees.
- Bumble bees exposed to sublethal amounts of neonicotinoids exhibit reduced food consumption, reproduction, worker survival rates, and foraging activity.

**Bumble bees and solitary bees respond differently to neonicotinoids than do honey bees. Current regulatory testing doesn't address these differences. (Photograph: Mace Vaughan/The Xerces Society.)**

*Effects on solitary bees*

- Clothianidin or imidacloprid spray is toxic to blue orchard and alfalfa leafcutter bees.
- Residue of imidacloprid on alfalfa foliage increases rates of mortality of alfalfa leafcutter and alkali bees.
- Blue orchard bee larvae required more time to mature after consuming sublethal levels of imidacloprid in pollen.

**Inferences from Research Results**

*Exposure of bees to neonicotinoids*

- Application methods other than seed coatings (foliar sprays, soil drenches, and trunk injections) apply a higher dosage per plant and may result in much higher—even toxic—levels of neonicotinoid residues in pollen and nectar.
- Application of neonicotinoids shortly before and during bloom may lead to higher residue levels in pollen and nectar.
- Application by soil drench or trunk injection may result in high residue levels in blossoms of woody ornamental species more than a year after treatment.

*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 neonicotinoids may make honey bees more susceptible to parasites and pathogens, 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 significantly increased the toxicity of neonicotinoids to honey bees in laboratory tests, but the full effects of this interaction in field settings are unclear.
- Bumble bees and solitary bees respond differently to neonicotinoids than do honey bees.
- Pesticide residues from seed treatment have been found in hives. Neonicotinoid-treated corn seed is planted on millions of acres annually in the United States. Although we do not know the full scope of the impact of this exposure on bees, we do know that bees close to corn fields can come into contact with lethal levels of abraded seed coatings and dust, bees may collect contaminated pollen, and that plants (e.g., weeds) growing around seed-treated fields can become contaminated with systemic insecticides.

**Knowledge Gaps**

*Exposure of bees to neonicotinoids*

- How do residue levels in pollen and nectar increase in concentration over time with repeated application? Given that residues can persist for long periods, repeated applications to perennial plants may cause concentrations to accumulate to sublethal or lethal levels. These data are critical for managing impacts to pollinators.
- How do residues from repeated applications and/or repeated planting of seed treated annual crops accumulate in the soil over time, resulting in higher residue levels in the pollen and nectar of annual crops, as well as in crop weeds?
- What is the degree of risk posed by neonicotinoid contamination of non-target plants growing near treated plants?
- How soon after product application do neonicotinoid residues appear in pollen and nectar, and does its appearance vary with application method?
- Is the combined presence of neonicotinoids and their break-down products in pollen or nectar more toxic to bees than the individual chemicals? As a neonicotinoid breaks down inside a plant, bees may be exposed to residues of both the parent compound and its metabolites.
- Does the movement of neonicotinoids vary with the type of plant (e.g., herbaceous vs. woody), by functional group (e.g., forbs vs. legumes vs. grasses), or by the size of plant?
- 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*

- Do honey bees experience delayed effects of neonicotinoids during adverse weather conditions (e.g., winter or drought) when stored foods are consumed? Because honey bees store food for times of dearth, chemical exposure is likely delayed beyond field study timelines.
- What are the acute and chronic toxicities of neonicotinoids to bees other than honey bees? Given the contribution of bumble bees and solitary bees to agricultural and native plant pollination in temperate landscapes, it is vital that we better understand the effects of these chemicals on all bees.
- What is the full extent of the sublethal effects of neonicotinoids on foraging, reproduction, and other behaviors of adult bees?
- What is the full extent of the sublethal effects of neonicotinoids on larval bees?
- What effects do soil residues have on ground-nesting bees—the majority of bee species—exposed to neonicotinoids through soil applications (drenches, chemigation, granules)?
- What are the effects of neonicotinoid residues on bees that construct nests from contaminated plant tissues? About 30% of bee species construct nests by using leaf pieces, plant resins, or holes in stems and tree trunks.
- How do neonicotinoids affect other pollinators such as butterflies, moths, beetles, flies, and wasps? Although these insects make minor contributions to crop pollination, they serve important roles within crop systems and other ecosystems.

# Recommendations

Bees provide essential services in agriculture, in natural ecosystems, and in the support of overall biodiversity. A large—and growing—body of research demonstrates that neonicotinoid insecticides harm multiple bee species, yet substantial knowledge gaps remain. Based on the findings, the Xerces Society for Invertebrate Conservation makes six major recommendations:

**1** The bee safety of currently approved uses of products containing neonicotinoid insecticides should be reassessed and all conditional registrations reexamined and/or suspended until we understand how to manage the risk to bees. The risk from exposure to neonicotinoid insecticides then needs to be evaluated against the risk posed to bees by alternative control measures.

**2** Before registration for a specific crop or ornamental plant species, research facilities should investigate the influences of application rate, application method, target plant species, and environmental conditions on levels of neonicotinoid residues in pollen and nectar.

**3** The US Environmental Protection Agency should adopt a more cautious approach to approving all new pesticides, using a comprehensive assessment process that adequately addresses the risks to honey bees, bumble bees, and solitary bees in all life stages.

4 All neonicotinoid products used by commercial and agricultural applicators should include a clearly stated and consistent (standardized) warning on the label about the hazard to bees and other pollinators, including the unique exposure issues posed by contaminated pollen and nectar. This is particularly important for products marketed for garden and ornamental use.

5 Products marketed to homeowners for use on garden, lawn, or ornamental plants should all have a clear warning label that prominently states, "Use of this product may result in pollen and nectar that is toxic to pollinators."

6 Legislators, regulators, and municipal leaders across the country should consider banning the use of neonicotinoid insecticides for cosmetic purposes on ornamental and landscape plants (as the ban now in force in Ontario, Canada). Approved application rates for ornamental and landscape plants, as well as turf, are often much higher than for farm crops.

**In addition, we urge that the following issues are addressed.**

**Pesticide Risk Assessment and Registration**

- Regulators should evaluate neonicotinoid use and toxicity in mixtures that include fungicides and/or surfactants, including rigorous statistical tests.
- Risk managers need to know how systemic insecticides accumulate in pollen and nectar after repeated use over multiple growing seasons, as well as the sublethal and lethal impacts of these concentrations.
- More data on the lethal and sublethal impacts of neonicotinoids on bees are needed, particularly on those products other than imidacloprid, which to date has been the subject of most studies.
- Regulators, researchers, and pesticide manufacturers should develop more comprehensive laboratory tests that assess the effects of neonicotinoids on multiple life stages of honey bees, bumble bees, and solitary bees during the registration process.
- Regulatory standards for neonicotinoid testing should be changed to require that tests have adequate replication and sample size.
- Testing must be subjected to rigorous statistical analyses so that significant independent variables can be identified.
- Field tests completed during registration should include treated areas of at least 5 acres (2 hectares) and use managed solitary bees such as alfalfa leacutter and blue orchard bees that have a shorter foraging range than honey bees.
- Methods should be developed for ongoing, post-registration assessment of the effects of neonicotinoids on bees at the landscape scale, under real world pest management conditions (e.g., repeated applications on 10s or 100s of acres), over multiple years.
- Regulators should require multi-year tests to examine potential accumulation of residues in soil from repeated annual plantings (e.g., via treated seeds or soil applications) and the impacts of these on ground-nesting bees.

**Risk Management**

- The academic research community should develop IPM protocols that result in recommendations for the lowest effective dose for both specific crops and specific pests, as well as methods for reducing risk to non-target beneficial insects such as pollinators.
- Licensed crop advisors and pesticide applicators should be required to understand the unique risks posed by neonicotinoids to bees and other flower visitors.
- For all foliar applications, every attempt should be made to minimize direct contact with bees and other non-target insects.

**Restrictions on Use**

- Until we know it is safe for bees, the use of neonicotinoids on crops such as apples and blueberries that bloom for a specific period of time each year should not be allowed during or before bloom. Such applications likely increase residue levels in pollen and nectar and increases exposure and risk to bees.
- For crops that bloom continuously or over a long period of time (e.g., squash or tomato), academic IPM professionals should develop clear methods for how neonicotinoids can be used, so that concentrations of these products in crop pollen and nectar stays below sublethal levels.
- Until a ban on cosmetic use of insecticides goes into effect, all neonicotinoid products marketed for non-agricultural use (e.g., homeowner products) should have label restrictions that limit application times, and reduce application rates on plants visited by bees.

# 1 Introduction

Neonicotinoid insecticides became available for use on farms and in gardens and ornamental landscapes in the mid 1990s. They offered great promise for long-term plant protection. Neonicotinoids are systemic insecticides, i.e., they are absorbed by and get inside the treated plant, protecting it from sap-sucking insects and those that chew on it. They were also promoted as being safer for wildlife because they were less toxic to birds and mammals than older classes of insecticides. But because they are within the plant, neonicotinoids also are present in nectar and pollen. This provides a direct threat to bees and other flower-visitors.

There are seven types of neonicotinoids. Only six are found in plant protection products, but there are hundreds of such products on the shelves of garden centers and agricultural supply stores. Neonicotinoids may be applied as a spray, a soil drench, or by direct injection and are used on field and orchard crops, ornamental plants in nurseries and gardens, and on trees in gardens, streets, and parks. They are also used as a seed treatment, a coating that confers protection to even the youngest seedlings. As a result, millions of acres of America's farmlands have been treated, as have uncounted gardens and backyards in the nation's cities and suburbs.

Neonicotinoids have become the subject of public debate, particularly on their impacts on honey bees. Much has been published about these insecticides 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 ways in which pollinating insects are exposed to neonicotinoids, the concentrations at which these insecticides may occur in the environment, and how they affect bees. We also offer an assessment of whether current regulations can adequately manage the effects of neonicotinoids, identify subjects for future research, and make recommendations for protecting bees.


**Now ubiquitous on garden center shelves, neonicotinoids can be applied in much greater concentrations in gardens than on farms, and with fewer restrictions. These products do not carry any warning about hazards to bees or other pollinators. (Photograph: Matthew Shepherd/The Xerces Society.)**

# 2 The Importance of Bees

Insects are a highly diverse group of animals and are abundant in all terrestrial environments. With ninety-five thousand species 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 birds and bats.) Bees are considered the most important group of pollinators in temperate climates. There are approximately 4,000 species of bees in North America (Michener 2007); almost all of these are native.

The European honey bee (*Apis mellifera*) 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). 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 (e.g., 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. Thus, conservation of pollinating insects is critically important to preserving both wider biodiversity and agriculture.

**Pollinators—mostly bees—are needed for more than two-thirds of the world's crop species. Bee pollinated crops are worth around $20 billion each year in the United States. (Photograph © iStockphoto.com/DHuss.)**

# 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 widely used as an insecticide before the Second World War. 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. (See page 5 for examples of uses and products.) A seventh, nitenpyram, is used to treat for fleas and other external parasites of livestock and pets. Because it is unlikely to affect flower-visiting insects, nitenpyram is not discussed further in this report.

Imidacloprid was the first neonicotinoid on the world market, and is the most commonly used (Elbert et al. 2008). Imidacloprid first became available in the United States in 1994, and is currently present in over 400 products on the market (NPIC 2010). With worldwide sales near $1.6 billion, neonicotinoids accounted for at least 17% of the global insecticide market in 2006 (Jeshke and Nauen 2008). In 2009 imidacloprid was among the most used pesticides in California, being applied to hundreds of thousands of acres (CA DPR 2010).

Neonicotinoids paralyze insects by blocking a specific chemical pathway that transmits nerve impulses in the 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 are much less toxic to some birds and many mammals than the older classes of insecticides they are replacing.

These insecticides are systemic, meaning that the chemicals can be absorbed and transported throughout the plant, offering protection against insects that feed on plants. Plants absorb these chemicals through their roots or leaves, and the vascular tissues transport the chemical into stems, leaves, flowers, and even fruit.

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 increasingly used for crop protection (Elbert et al. 2008).

An advantage of neonicotinoids for pest control is that their methods of application (i.e., a range of methods other than spraying) help to reduce direct contact to non-target insects during treatment. However, because these chemicals are systemic and absorbed into plant tissues, insects that rely on nectar, pollen, or other floral resources have increased oral exposure to residues of neonicotinoids or their metabolites. Residues have been recorded in pollen (Laurent and Rathahao 2003; Bonmatin et al. 2003, 2005a), nectar (Schmuck 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 weeds growing within or adjacent to treated fields (Krupke et al. 2012).

Another issue arising from the systemic action of neonicotinoids is that they remain toxic within the plant for longer than other insecticides. Evidence suggests that systemic insecticides may remain in plant tissues for months or even more than a year (e.g., Maus et al. 2005). In addition, 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 148 to 1,155 days (5¼ to 38½ months) depending upon soil types (EPA 2003a). Untreated plants may take up residues of neonicotinoids still present in the soil from previous applications (Bonmatin et al. 2003, 2005b).

**Table 3.1 Half-life in Soil of Neonicotinoids**

| Neonicotinoid | Half-life in Soil (aerobic soil metabolism) |
|---|---|
| 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 (See note below) | 25–100 days[6] |

**Note:** Clothianidin is a primary metabolite of thiamethoxam.

**Sources:** 1. EPA 2002; 2. EPA 2003a; 3. EPA 2004; 4. NPIC 2010; 5. EPA 2003b; 6. Syngenta Group 2005

## 3.2 Systemic Insecticides: 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, insecticide use 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 non-target species. In particular, IPM encourages preventive measures to reduce initial pest build up. These include cultural practices such as planting pest-resistant crop varieties, 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 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 non-toxic options such as pheromone mating disruption as an initial strategy, before resorting to pesticide use. Insecticides are employed as a last resort, ideally using the most targeted products (which may be systemic insecticides).

Routine (calendar-based) spraying and preemptive treatments are contrary to the philosophy of IPM. With only a few exceptions, the increasing prophylactic use of systemic insecticides such as neonicotinoids (Sur and Stork 2003) represents a shift in pest management towards applying chemicals before pest damage has occurred. This approach negates the principles of IPM because insecticides are used before their need is demonstrated, and it complicates the ability to use biological control agents.

**Systemic insecticides may be regularly applied in a preemptive attempt to reduce insect damage to crops, and not as a response to pest insects being on the plants. (Photograph © iStockphoto.com/Balefire9.)**

## Examples of Neonicotinoid Products Used in the United States

| Neonicotinoid | Registered use in the United States | Product trademark names | |
|---|---|---|---|
| | | **Agriculture** | **Turf, Ornamental, Residential** |
| Acetamiprid | Application as foliar spray for leafy vegetables, fruiting vegetables, cole crops, citrus fruits, pome fruits, grapes, cotton, and ornamental plants and flowers. | Assail<br>Tristar | Ortho Flower, Fruit and Vegetable Insect Killer<br>Ortho Rose and Flower Insect Killer |
| Clothianidin | Seed treatment, application as foliar spray or soil drench for a variety of field and tree crops, also for turf and a variety of ornamental trees and flowers | Arena<br>Poncho<br>Clutch<br>Belay | Aloft<br>Arena<br>Bayer Advanced All-in-One Rose & Flower Care granules<br>Green Light Grub Control with Arena |
| Dinotefuran | Application as soil drench or foliar spray to leafy and fruiting vegetables, turf, and ornamental plants.<br>Also used as bait or granules in buildings for cockroach control. | Venom<br>Scorpion | Green Light Tree & Shrub Insect Control with Safari 2 G<br>Safari<br>Transect<br>Zylam 20SG Systemic Turf Insecticide |
| Imidacloprid | Application as seed dressing, soil drench, granules, injection, or spray to a wide range of field and tree crops, as well as ornamental plants, trees, and turf.<br>(Also, topical use on pets for flea control and application to buildings for termite control.) | Admire<br>Gaucho<br>Imicide<br>Provado<br>Macho<br>Malice<br>Sepresto<br>Widow<br>Wrangler | Bayer Advanced 3-in-1 Insect, Disease, & Mite Control<br>Bayer Advanced 12 Month Tree & Shrub Insect Control<br>Bayer Advanced 12 Month Tree & Shrub Protect & Feed<br>Bayer Advanced Fruit, Citrus & Vegetable Insect Control<br>Bayer Advanced All-in-One Rose & Flower Care concentrate<br>DIY Tree Care Products Multi-Insect Killer<br>Ferti-lome 2-N-1 Systemic<br>Hi-Yield Systemic Insect Spray<br>Hunter<br>Knockout Ready-To-Use Grub Killer<br>Lesco Bandit<br>Marathon<br>Merit<br>Monterey Once a Year Insect Control II<br>Ortho Bug B Gon Year-Long Tree & Shrub Insect Control<br>Orhto MAX Tree & Shrub Insect Control<br>Surrender Brand GrubZ Out |
| Thiacloprid | Application as foliar spray to cotton and pome fruit crops. | Calypso | |
| Thiamethoxam | Application as seed dressing, soil drench, injection, granules, or foliar spray to a wide range of field crops, as well as ornamental plants and turf. | Actara<br>Adage<br>Crusier<br>Centric<br>Platinum | Flagship<br>Maxide Dual Action Insect Killer<br>Meridian |

Sources for registered use information (column 2): EPA 2002; EPA 2003a; EPA 2003b; EPA 2004

# 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 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 is not currently considered during the regulation or registration of insecticides by the Environmental Protection Agency (EPA), which registers and monitors pesticides in the United States (EPA 1996).

One factor affecting hazard risk to bees from all routes of 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). This shorter foraging distance may result in a disproportionate risk to small bees that nest near treated crops, because their limited range results in ongoing exposure to neonicotinoids while they gather food or nest materials. In contrast, honey bees and bumble bees may dilute contaminated nectar and pollen by collecting from flowers over a much larger area.

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 not 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 or sublethal effects, or none at all.

***Contaminated pollen and nectar***

Pollen and nectar may be contaminated by neonicotinoids irrespective of how the chemicals are applied. The presence of these products in nectar and pollen delivers the active ingredient directly to bees and other pollinators. Some systemic insecticides can be very persistent, staying in plant tissues for many months or even years, and may build up after repeated applications.

Honey bee larvae are primarily fed royal jelly (a secretion of adult workers), 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). Neonicotinoids have been found in pollen loads brought to hives by honey bees (Chauzat et al. 2006), in pollen stored within honey bee hives (Mullin et al. 2010; Bernal et al. 2010; Krupke et al. 2012), and in honey stored within hives (Chauzat et al. 2009). Larvae of bees native to North America typically feed directly on raw pollen, undiluted nectar, or both (Michener 2007), any of which may contain neonicotinoid residues.

***Direct spray***

Direct contact with foliar spray may be the most obvious exposure route for bees. This may occur when an application is made while bees are actively foraging on flowers or nesting in the ground within a field or orchard, 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 correlates 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.)

***Residue contact***

Exposure to neonicotinoid residues occurs when bees visit flowers or walk on leaves that have been 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).

***Particles released during the planting of treated seeds***

The release of seed coatings during planting as dust drifting onto flowers adjacent to cropland has resulted 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 gluing 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).

The full extent to which this type of exposure may occur is unknown, but millions of acres of treated seed

are planted each year (Krupke et al 2012). To reduce this possible exposure, seeds with high-quality coatings should be used. Even when appropriate seed coatings are used on treated seeds, however, the planting process may expose bees to neonicotinoids (Tapparo et al. 2012).

In the United States, where neonicotinoid seed treatment is used for many annual crops, talc is often added to the seed boxes of planters to aid the flow of the sticky treated 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). Flying bees may also contact aerial insecticidal powders and abraded seed coatings released during seed drilling (Girolami et al. 2012; Tapparo et al. 2012), and be administered lethal doses (Tapparo et al. 2012).

***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 bees in the United States 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, and drift into overgrown habitat or forest edges may contaminate potential bumble bee nesting sites.

***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 bees use existing cavities 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 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. Water sources may be contaminated by chemigation leaks, overspray, drift, or field run-off. A survey of water sources within half a mile of honey bee hives found that some had sublethal levels of imidacloprid (J. Johnson, pers. comm.). Honey bees will also drink from leaking chemigation equipment (D. Biddinger, unpub. data).

***Guttation fluid***

Guttation fluid is the water given off by plants in the morning, as droplets 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).

Although the frequency at which honey bees might actually consume guttations in a field setting is unknown, the risk is considered to be low because honey bees usually collect water when they need to cool their hives (which is much less likely in the morning), and they will only collect guttation fluid if it is the closest source of water.

***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 cotton, sunflower, and pumpkins, and plants found in yards such as morning glory, willows, and black locusts. 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 this.

# 5 Effects of Neonicotinoid Exposure on Bees

## 5.1 Research Study Bias

Research investigating the effects of neonicotinoids on pollinators is restricted to bees, primarily the honey bee. Honey bees are the tested species because they are economically important, readily available in large numbers, and there are existing test protocols. More than forty 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 at least twelve peer-reviewed studies involving neonicotinoids and bumble bees have been published.

To our knowledge, only four studies of effects of neonicotinoids on solitary bees in North America have been published. These have involved two native species, the blue orchard bee and alkali bee, and the introduced alfalfa leafcutter bee, all species that are managed to varying degrees for commercial pollination. The lack of studies concerning effects of neonicotinoids on wild, unmanaged species is of great concern because these bees comprise most of the species in North America and also contribute significantly to crop pollination (e.g., Winfree et al. 2007). 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 (D. Biddinger, unpub. data).

Of the neonicotinoids that have been investigated, imidacloprid is by far the most studied (approximately three-quarters 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 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 studies into the effects of neonicotinoids on bees. Information about impacts on other bees—the majority of species—is lacking. (Photograph © iStockphoto.com/pushlama.)**

## 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 labor division 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's 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 range of agrochemicals, including neonicotinoids.

### 5.2.1 Lethal Toxicity of Neonicotinoids

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). Of these six neonicotinoids, acetamiprid and thiacloprid have structural differences that make them less toxic to honey bees than the other four (Jones et al. 2006; Iwasa et al. 2004). As a result, the contact $LD_{50}$ for thiacloprid in honey bees is 816 times larger than that of imidacloprid (Iwasa et al. 2004).

Unlike many other pesticides, neonicotinoids appear to be more toxic 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 (e.g., olefin-imidacloprid is approximately two times more toxic than imidacloprid) (Suchail et al. 2001). Thiamethoxam actually breaks down into another neonicotinoid, clothianidin (Nauen et al. 2003).

The lethal concentration of imidacloprid needed to kill 50% of a test population (the $LC_{50}$) of honey bees 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 label rates of seed coat applications, some evidence suggests that such residue levels may occur in plants under certain circumstances. As discussed in Section 6.3, soil drenches (Doering et al 2004b; Doering et al. 2005a; Doering et al. 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.

Although it appears unlikely that acute lethal doses of residues are typically found in agricultural settings, there are some unknown factors that still need to be resolved. (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 21–22 gives a comparison of agricultural and garden application rates for apple trees.)

In contrast with acute lethal exposure, chronic exposure in doses much smaller 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 present conflicting results and conclusions. Although several studies demonstrated bee mortality at chronic low doses, others observed no mortality after chronic exposure. One study found no significant differences in the mortality of untreated bees and bees exposed to doses of imidacloprid between 0.002 and 0.02 mg/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) (Dechaume-Moncharmont et al. 2003).

Suchail et al. (2001) found that doses of 0.1, 1, and 10 µg/L (the equivalent of 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 accurate, 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 seed treatment) (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

**Table 5.1 Toxicity of Neonicotinoids**

| Neonicotinoid | Known Toxicity to Honey Bees[1] | | |
|---|---|---|---|
| | | Contact $LD_{50}$ | Oral $LD_{50}$ |
| Acetamiprid | M | 7.1 µg/bee[2]–8.09 µg/bee[3] | 8.85–14.52 µg/bee[3] |
| Clothianidin | H | 0.022 µg/bee[2]–0.044 µg/bee[4] | 0.00379 µg/bee[5] |
| Dinotefuran | H | 0.024 µg/bee[2]–0.061 µg/bee[6] | 0.0076–0.023 µg/bee[6] |
| Imidacloprid | H | 0.0179 µg/bee[4]–0.243 µg /bee[7] | 0.0037 µg/bee[7]–0.081 µg/bee[8] |
| Thiacloprid | M | 14.6 µg/bee[2]–38.83 µg/bee[9] | 8.51–17.3 µg/bee[9] |
| Thiamethoxam | H | 0.024 µg/bee[10]–0.029 µg/bee[2] | 0.005 µg/bee[10] |

**H = highly toxic; M = moderately toxic**

**Toxicity:** Highly toxic: $LD_{50}$ < 2 µg/bee; Moderately toxic: $LD_{50}$ 2–10.99 µg/bee; Slightly toxic: $LD_{50}$ 11–100 µg/bee; Practically non-toxic: $LD_{50}$ >100 µg/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.

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

The information presented below is compiled from studies that investigated the effects of oral doses of neonicotinoids on bees. For 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 below 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.

No data = Data is not available, either because it has not been tested for or because the public does not have access to it for proprietary reasons.

| | Honey bees | Bumble bees | Solitary bees |
|---|---|---|---|
| **Acetamiprid** | | | |
| Lowest reported lethal concentrations | | | |
| Acute exposure * | ≥ 442,500 ppb[1] | No data | No data |
| Chronic exposure | No data | No data | No data |
| Lowest reported sublethal concentrations | | | |
| Acute exposure | 5,000 ppb[2] | No data | No data |
| Chronic exposure | 5,000 ppb[3] | No data | No data |
| **Clothianidin** | | | |
| Lowest reported lethal concentrations | | | |
| Acute exposure | ≥ 190 ppb[4] | No data | No data |
| Chronic exposure | No data | No data | No data |
| Lowest reported sublethal concentrations | | | |
| Acute exposure | 24 ppb[5] | No data | No data |
| Chronic exposure | | No data | 300 ppb[6] |
| **Dinotefuran** | | | |
| Lowest reported lethal concentrations | | | |
| Acute exposure | ≥ 380 ppb[7] | No data | No data |
| Chronic exposure | No data | No data | No data |
| Lowest reported sublethal concentrations | | | |
| Acute exposure | No data | No data | No data |
| Chronic exposure | No data | No data | No data |
| **Imidacloprid** | | | |
| Lowest reported lethal concentrations | | | |
| Acute exposure | ≥ 185 ppb[8] | No data | No data |
| Chronic exposure | 0.10 ppb[9]**; > 20 ppb[10] | 59 ppb[12] | No data |
| Lowest reported sublethal concentrations | | | |
| Acute exposure | No data | No data | No data |
| Chronic exposure | 24 ppb[11] | 10 ppb[12] | 30 ppb[6] |
| **Thiacloprid** | | | |
| Lowest reported lethal concentrations | | | |
| Acute exposure | ≥ 425,500 ppb[13] | No data | No data |
| Chronic exposure | No data | 18,000 ppb[12] | No data |
| Lowest reported sublethal concentrations | | | |
| Acute exposure | No data | No data | No data |
| Chronic exposure | No data | 12,000 ppb[12] | No data |

**Table 5.2 (cont.) Concentrations of Neonicotinoid Insecticides Known to Cause Harm When Ingested by Bees.**

| | Honey bees | Bumble bees | Solitary bees |
|---|---|---|---|
| **Thiamethoxam** | | | |
| Lowest reported lethal concentrations | | | |
| Acute exposure | ≥ 250 ppb[14] | No data | No data |
| Chronic exposure | No data | 120 ppb[12] | No data |
| Lowest reported sublethal concentrations | | | |
| Acute exposure | No data | No data | No data |
| Chronic exposure | 50 ppb[3] | 100 ppb[12] | No data |

* Acute lethal exposure concentrations are based upon reported $LD_{50}$s (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).

** The results of this study have been called into question, so we have included the results from another similar study here as well.

**Sources:** 1. EC 2004; 2. El Hassani et al. 2008; 3. Aliouane et al. 2009; 4. EC 2005; 5. Schneider et al. 2012; 6. Abbott et al. 2008; 7. EPA 2004; 8. Schmuck et al. 2001; 9. Suchail et al. 2001; 10. Schmuck 2004; 11. Decourtye 2003; 12. Mommaerts et al. 2010; 13. EC 2004b; 14. Syngenta Group 2005.

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).

### 5.2.2 Delayed Toxicity and Sublethal Effects

Death is not the only outcome from pesticide contamination. An amount of pesticide too small to kill a bee 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, 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 acute contact or oral sublethal dose of a neonicotinoid indicate that imidacloprid alters learning (Lambin et al. 2001; Guez et al. 2001), motor activity (Lambin et al. 2001; Medrzycki et al. 2003), and memory (Decourtye et al. 2004a), while 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 chronic sublethal doses of neonicotinoids found that imidacloprid impairs learning and foraging (Decourtye et al. 2003; Han et al. 2010) and thiamethoxam decreases sucrose sensitivity and memory (Aliouane et al. 2009). All of these tests used doses above 20 ppb, a concentration greater than published levels of residues in pollen or nectar after seed treatments but within levels found as a result of soil drenches or trunk injections.

All semi-field experiments reviewed involved entire colonies exposed to contaminated pollen or syrup inside tunnels or flight cages. Chronic exposure to syrup contaminated with doses of imidacloprid between 24 and 48 ppb reduced brood production, foraging activity, and food stores (Decourtye et al. 2004b), as well as causing abnormal navigation between feeders and the hive (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 (Yang et al. 2008; Bortolotti et al. 2003). 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 thirteen laboratory and semi-field studies that investigated sublethal effects of imidacloprid on honey bees, Cresswell (2011) found that residues in sunflower and canola nectar (his estimate was between 0.7–10 µg/L [0.7–10 ppb]) after seed treatment reduced adult honey bee performance between 6 and 20%.

Neonicotinoid field studies involving honey bees 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). Researchers placed hives near fields planted with imidacloprid-treated corn or sunflower seeds (Nguyen et al. 2009; Stadler et al. 2003) or clothianidin-treated canola seeds (Cutler and Scott-Dupree 2007) 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); individual bee mortality in front of the hive, colony weight, brood present, and worker longevity (Cutler and Scott-Dupree 2007); and honey production, brood, and colony weight (Stadler et al. 2003). No significant negative effects of imidacloprid or clothianidin seed treatment on honey bee colonies were observed in any of these studies.

To date, field studies involving honey bees have only tested the effects of neonicotinoid seed treatments. Residue levels from seed treatments are much lower (usually less than 20 ppb) than residues from plants treated by methods such as soil drench. Effects of other application rates and methods on honey bee colonies have not been tested, nor has the effect of planting treated seed over multiple years. Similarly, no studies have investigated the impact to bees when crops are planted at scales encountered in commercial farming. Field studies examining residue levels that reflect these treatment conditions are urgently needed.

One important limitation of 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 acres), treated and untreated canola fields were within 300 m (330 yards) 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 do not forage in treatment plots, it is hard to assess effects of treatment, or to determine the impact of hundreds of hectares of treated crop, compared to a single hectare.

Experimental design may also significantly influence results. 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 (Meled et al. 1998; Smirle and Winston 1987; Wahl and Ulm 1983). 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 full year of hive monitoring, it is difficult to know the full effects of neonicotinoids on colony health.

### 5.2.3 Possible Synergism Between Neonicotinoids and Other Agrochemicals

Effects of insecticides on non-target organisms are typically considered in isolation, though they 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. Triflumizole increases the toxicity of the insecticide 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. When applied in a laboratory setting, DMI fungicides increased the toxicity of acetamiprid and thiacloprid as much as 244-fold, but not imidacloprid (Iwasa et al. 2004). However, when honey bees were exposed to foliage treated with acetamiprid and triflumizole under semi-field conditions, no differences in mortality rates were seen (Iwasa et al. 2004). Similarly, Schmuck et al. (2003) 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 DMI fungicides and neonicotinoids is needed.

### 5.2.4 Neonicotinoids and Colony Collapse Disorder

Colony collapse disorder (CCD) is the large-scale loss of European honey bees in the United States first observed during the winter of 2006–2007. 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. As yet, the cause of CCD is unexplained, although it appears that no single factor alone is responsible. Research to date suggests that CCD is a syndrome caused by multiple factors, including pathogens and parasites, 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, though many chemicals have been found in hives (e.g., Mullin et al. 2010). Researchers that 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, insecticide exposure may interact with other factors such as viruses or parasites to weaken colony health and increase susceptibility to CCD (USDA 2010). Recent studies have 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 *Nosema* spores 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. *Nosema* infection may actually increase bee exposure to imidacloprid by causing energetic stress that leads to bees consuming more tainted nectar (Alaux et al. 2010). 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, despite the fact that thiacloprid is less toxic to honey bees than imidacloprid (see $LD_{50}$ information in table 5.1). While neonicotinoids and other agrochemicals do not appear to be the direct cause of CCD, they may be a contributing factor to already stressed colonies. It is increasingly important that future studies focus on interactions of multiple factors suspected of contributing to CCD.

**Commercial beekeepers have lost tens of thousands of hives in the last few years due to colony collapse disorder. There is no evidence that neonicotinoids are a direct cause of this, though they interact with other factors such as *Nosema* parasites. (Photograph: Howard F. Schwartz, Colorado State University, Bugwood.org.)**

## 5.3 Bumble Bees and Neonicotinoids

There are about forty-five species of bumble bees (genus *Bombus*) in North America. They are active from spring to fall—all year in hotter 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 and secretes wax to form brood cells and honey pots for temporary storage of nectar. The queen rears the first generation of bees, but once they are active, she remains inside the nest to lay eggs. The daughter-workers cooperate to raise additional offspring and find food. In this way, the colony increases in numbers throughout the growing season. In the fall, new queens are reared, mate, and overwinter. Other members of the colony die

when winter arrives. 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.

As generalist foragers that visit a wide range of flowers and habitats, bumble bees can 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

Laboratory studies of acute toxicity demonstrate that imidacloprid and clothianidin are highly toxic to bumble bees. Although $LD_{50}$s have not been determined, studies indicate that acute contact exposure to imidacloprid or clothianidin is very harmful (Marletto et al. 2003; Gradish et al. 2009; Scott-Dupree et al. 2009), and an acute oral dose of imidacloprid is highly toxic (Marletto et al. 2003). Clothianidin is apparently slightly more toxic to bumble bees through contact exposure than imidacloprid (Scott-Dupree et al. 2009). Acute toxicity of acetamiprid, dinotefuran, thiacloprid, and thiamethoxam are untested.

The effect of chronic neonicotinoid exposure 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 a dose of 200 ppm died after several hours, and those exposed to doses of 2–20 ppm 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" (Incerti et al. 2003); a third of the bumble bees died within 48 hours of exposure.

### 5.3.2 Delayed Toxicity and Sublethal Effects

Several lab studies have found that bumble bees exhibit sublethal effects after chronic oral exposure to imidacloprid. 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). However, bumble bees may not respond to all neonicotinoids as they do to imidacloprid. One study in which bumble bees were fed clothianidin-contaminated pollen at doses of 6 or 36 ppb found bees did not exhibit any significant sublethal effects (Franklin et al. 2004). Because acetamiprid, dinotefuran, thiacloprid, and thiamethoxam are also used on plants visited by bumble bees, it would be worthwhile to investigate the sublethal impacts of these neonicotinoids as well.

Studies conducted in semi-field conditions, exposing bumble bee colonies to imidacloprid-contaminated nectar or foliage within glasshouses or flight cages, have found results similar to laboratory studies. Mommaerts et al. (2010) provided bumble bee colonies with sugar water containing 2–20 ppb of imidacloprid and placed

**Both nectar and pollen can be contaminated by systemic insecticides. Neonicotinoids are highly toxic to bumble bees. (Photograph: Eric Mader/The Xerces Society.)**

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.

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. Loss of workers and growth of colonies did not appear to be significantly affected by field treatment (Tasei et al. 2001). Although significantly more robust than most field studies in terms of field size, this study had similar deficiencies to some of the honey bee field studies mentioned previously, given that bumble bees are also capable of traveling long distances to find forage. Sunflower pollen was only roughly 25% of the pollen collected, with bees clearly finding alternate pollen sources.

Two field studies conducted by Bayer CropScience AG placed bumble bee colonies near eighteen or thirty ornamental shrubs treated with soil drenches of imidacloprid, and 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 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.

## 5.4 Solitary Bees and Neonicotinoids

North America's native bees are greatly varied in their social behavior, habitat requirements and floral preferences, as might be expected from a group of animals that includes 4,000 species. While some native species form colonies, the majority lead solitary lives. Each female establishes and provisions her own nest, though 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 subterranean 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 they may come in closer contact with residues in contaminated soil or leaves through their nest construction than do honey bees or bumble bees. For example, squash bees have recently been demonstrated to be the primary pollinators of squash and pumpkin plants across much of the United States (J. Cane, pers. comm.). They also nest in the ground at the base of the plants they visit and may easily come in contact with soil-applied systemic insecticides.

There are only four published studies of the impacts of neonicotinoids on solitary bees, and three of these investigated imidacloprid. The bees tested were three managed species, the blue orchard bee (*Osmia lignaria*), the alkali bee (*Nomia melanderi*), and the alfalfa leafcutter bee (*Megachile rotundata*). (The first two are native to North America, the last an introduced Eurasian species.) Three of these studies investigated the effects on mortality in a laboratory setting, while the other investigated sublethal effects in both laboratory and semi-field settings.

Although $LD_{50}$s have not been determined, 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), and blue orchard bees (Scott-Dupree et al. 2009). Acute contact with clothianidin was also toxic to blue orchard bees and alfalfa leafcutter bees (Scott-Dupree et al. 2009). The effects of acute oral, chronic oral, or con-

tact exposure of imidacloprid or clothianidin on solitary bees are unknown, and effects of other neonicotinoids are little studied.

Toxicity of neonicotinoids varies among species of bees. 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. In a related study, imidacloprid was slightly less toxic to Japanese orchard bees (*Osmia cornifrons*) than to honey bees, and acetamiprid was about twelve times more toxic to Japanese orchard bees than to honey bees (D. Biddinger, unpub. data). While acetamiprid is a compound considered to be more bee-safe than other neonicotinoids, like imidacloprid, because it is only moderately toxic to honey bees, it may pose a much higher risk to other bee species such as the blue orchard bee. Toxicity to honey bees is likely not a suitable predictor of toxicity to all bee species.

In a semi-field study designed to understand sublethal effects, 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 contaminated pollen (at the above 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; 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 higher (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 parallel field and laboratory experiments using alfalfa leafcutter bees and clothianidin at low (6 ppb), medium (30 ppb), and high (300 ppb) doses. 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.

Sublethal effects of neonicotinoids on foraging, reproduction, and behavior of adult solitary bees are not known, and studies in these areas would be valuable. Although soil-applied imidacloprid has been found to impair parasitoid wasps seeking underground prey (e.g., Rogers and Potter 2003), effects of neonicotinoids in the soil on ground-nesting bees are entirely unknown and would be a valuable topic of research.

Finally, 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 of most solitary species. As the acreage of neonicotinoid-treated crops increases there are proportionately fewer areas of untreated plants available to bees seeking food. Smaller native bees that have restricted flight ranges may increasingly be confined to treated areas.

**Studies demonstrate that larvae of blue orchard bees take longer to develop in the nest when their food supplies are tainted by neonicotinoid residues. (Photograph: Mace Vaughan/The Xerces Society.)**

# 6 Neonicotinoid Residues

## 6.1 Neonicotinoid 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), it is extremely important to understand the levels at which neonicotinoids 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 influence residue levels. Neonicotinoids have differing characteristics in the soil. For example, 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). 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). In contrast, acetamiprid and thiacloprid degrade quickly in soil (half-lives are estimated at 8 and 27 days respectively) (EPA 2002, 2003b). 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).

Cloyd and Bethke (2011) suggest that residue concentrations in pollen or nectar may vary with plant and flower morphology, but this should be investigated further. Residue concentrations in pollen and nectar also may fluctuate with the age of the plant; there is evidence that residues from seed treatment at label rates increased to an average of 8 ppb during flowering in sunflower heads with time rather than decreasing during development (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, four main neonicotinoid application methods—seed coatings, soil drenching (including chemigation), trunk injections, and foliar sprays—are employed to varying degrees and delivering a range of doses. In this chapter, we review known measurements of neonicotinoid residues that result from these application methods.

## 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 seed treatment are more studied than those resulting from other applications. In fact, seed-treatment residues are often reported in studies as benchmark levels (e.g., Franklin et al. 2004), despite the fact that other methods of application are registered, are commonly used in the United States, and often deliver a higher dose.

- 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 treatment contained 1.9 ppb (Schmuck et al. 2001). Studies of imidacloprid residues in sunflower pollen after seed treatment 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.
- Maximum concentrations of clothianidin residues are reported to be 3 ppb in pollen and 3.7 ppb in nectar from canola seed treated with clothianidin at label rates (Cutler and Scott-Dupree 2007).

**Much of the corn seed planted in the United States is coated with neonicotinoids before sowing. The insecticide remains in the plants, creating a toxic residue in the pollen collected by bees, and dust from sowing contaminates adjacent flowers. (Photograph © iStockphoto.com/BanksPhotos)**

- Krupke et al. (2012) reported residue levels of 3.9 ppb of clothianidin in corn pollen resulting from seed treatment at label rates.
- While residues in nectar resulting from thiamethoxam (e.g., Crusier) seed treatments remain unknown, residues in corn pollen after treatment to corn seed at label rates resulted in 1.7 ppb thiamethoxam (Krupke et al. 2012)

It has been suggested that seed treatments may be less harmful 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 following seed treatment is translocated to the pollen (Laurent and Rathahao 2003). Because imidacloprid and clothianidin residues resulting from seed treatments appear to fall below the no observable adverse effects concentration of 20 ppb, a figure determined by industry scientists (Schmuck et al. 2001; Schmuck and Keppler 2003, as cited by Cutler and Scott-Dupree 2007), it is commonly thought that neonicotinoid residues from seed-treated crops do not reach levels at which they would impact bees, either at lethal or sublethal levels (Maus et al. 2003). However, studies examining repeated use of seed-treatments over time are needed. Bonmatin et al. (2005b) noted that untreated sunflowers absorbed residual imidacloprid from the previous year's treated sunflower plantings. Based upon these findings, annual plantings of seed-treated crops may lead to increased residue levels that may pose more of a risk to bees, since residues from previous seasons remain.

### 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 seed treatments.

- Apple trees ('James Grieve') treated by soil application at label rates had 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 & Hooks 2010).
- Dinotefuran applied with a half rate in water used on pumpkin transplants with the remaining half applied by drip irrigation several weeks later was found in pollen at concentrations of 44–69 ppb and in nectar at 7.1–10.6 ppb (Dively & Hooks 2010).
- Thiamethoxam applied to pumpkins with a half rate in water used on transplants with the remaining half applied by drip irrigation several weeks later was found in nectar at 54.8–90.4 ppb and 7.8–12.2 ppb (Dively & Hooks 2010). Although clothianidin was not applied to the pumpkins, Dively & Hooks (2010) found it to be present in pollen and nectar at about half the levels of thiamethoxam (Dively & Hooks 2010), because clothianidin is a metabolite of thiamethoxam (Nauen et al. 2003).

Soil drench residues in pollen and nectar are much higher than levels reported from seed applications. Imidacloprid, at the levels reported by Dively and Hooks (2010), had sublethal effects on honey bees, bumble bees, and solitary bees (see table 5.2). The highest amounts of imidacloprid in pollen and nectar resulted from split applications, one half of which came during flowering. 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 Hooks 2010); the combined effects of these two compounds on bees are unknown.

### 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. Based on the frequency of application within a growing season (e.g., Provado may be applied to apples at 8 oz. per acre up to five times in a growing season; Provado label 2010), this lack of data on residues in pollen and nectar from foliar applications is disturbing.

- Dinotefuran applied as a foliar spray to pumpkins at label rates (two half-rates separated by three weeks) was found in pollen at concentrations of 36–147 ppb and in nectar at 5.3–10.8 ppb (Dively & Hooks 2010).
- Thiamethoxam applied as a foliar spray to pumpkins at label rates (again, two half-rates separated by three 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 (Dively & Hooks 2010).

## 6.3 Residue Levels From Neonicotinoid Application in Ornamental Landscapes

### 6.3.1 Application by Seed Coating in Ornamental Landscapes

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 seed treatment.

### 6.3.2 Application by Soil Drench in Ornamental Landscapes

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.

- 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).
- Soil applications 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 thirteen months) after treatment (Maus et al. 2004a). Measurements at earlier dates are unknown because they were not taken during this study.
- Shrubs in the genus *Amelanchier* (serviceberry, shadbush) had residue levels of 66–4,560 ppb in blossoms 540 days (eighteen 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 seventeen 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).

### 6.3.3 Application by Trunk Injection in Ornamental Landscapes

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. Studies conducted by Bayer CropScience AG demonstrated that trunk injection resulted in rapid translocation of imidacloprid to blossoms and leaves.

- 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 resulted in higher residues in leaves faster (Maus et al. 2004b).

### 6.3.4 Application by Foliar Spray in Ornamental Landscapes

While pollen and nectar residues resulting from one rate of application in pumpkins have been measured, we know of no measurements of residues in pollen or nectar of ornamental plants after foliar sprays are applied.

Studies of residues in ornamental plants are limited to a few plant species and only one product, imidacloprid. Acetamiprid, clothianidin, dinotefuran, and thiamethoxam are active ingredients in products registered for use in ornamental landscapes, but little is known about residue levels of those compounds in pollen and nectar or ornamental plants. Application during flowering is allowed for products under homeowner registration. This increases risks to pollinators, since it seems that residues occur in higher levels in pollen and nectar when these insecticides are applied during bloom (e.g. Dively and Hooks 2010).

**Treating ornamental shrubs by soil drenches results in long-lasting residues in nectar and pollen. Imidacloprid was found in rhododendron blossoms more than three years after treatment. (Photograph: Matthew Shepherd/The Xerces Society.)**

## 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 cosmetic 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 the case study opposite for details.)

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 (i.e., maintain residue levels in pollen and nectar below no observable effects level [NOEL] concentrations).

# Case Study: Comparison Between Agricultural and Backyard Products

| | Agricultural Use | | Backyard Use |
|---|---|---|---|
| **Product** | 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 |
| **Percent of active ingredient in product (pounds of active ingredient per gallon)** | 42.8% (4.6 lbs. a.i./gal) | 17.4% (1.6 lbs. a.i./gal) | 2.94% (0.26 lbs. a.i./gal) |
| **Rate of application in one season** | Maximum of 10.5 fl. oz./acre | Maximum of 40 fl. oz./acre* | Maximum of 0.5 fl. oz./inch of tree trunk circumference |
| **Amount of active ingredient each tree would receive** | 0.03 oz. a.i./tree Assuming that there are 200 trees per acre in the agricultural setting | 0.04 oz. a.i./tree Assuming that there are 200 trees per acre in the agricultural setting | 0.4875 oz. a.i./tree Assuming that trees are 30" in circumference in a home setting |

* Note that for many apple crop pests (e.g., aphids), applications of Provado are well below 40 fl. oz./acre.

## Agricultural Soil Drench Insecticide: Admire Pro Systemic Protectant™

The Admire Pro Systemic Protectant™ label (accessed January 7, 2010) shows that the product is comprised of 42.8% active ingredient (a.i.; in this case imidacloprid) and 57.2% inert ingredients. The label also reports that this percentage equates to 4.6 lbs. of a.i. per gallon. The amount of a.i. per fluid ounce can be calculated as lbs./gal. ÷ # fl. oz. in a gallon (which is 128):

4.6 lbs./gal. ÷ 128 = 0.0359 lbs. a.i./fl. oz.

Admire can be applied as a soil drench to apples at a maximum rate of 10.5 fl. oz./acre. The amount of imidacloprid applied to each acre of crop can be calculated as lbs. a.i./fl. oz. × fl. oz./acre.

0.0359 lbs./fl. oz. × 10.5 fl. oz./acre = 0.377 lb./acre

This is equal to 6.032 oz./acre of imidacloprid.

Assuming there are 200 trees per acre (a conservative estimate based on the Pennsylvania Tree Fruit Production Guide, http://agsci.psu.edu/tfpg), the amount of imidacloprid each tree receives is:

6.032 oz./acre ÷ 200 = 0.03 oz./tree

## Agricultural Foliar Insecticide: Provado 1.6 Flowable Insecticide™

The Provado 1.6 Flowable Insecticide™ label (accessed March 8, 2010) shows that the product is comprised of 17.4% a.i. (imidacloprid) and 82.6% inert ingredients. The label also reports that this percentage equates to 1.6 lb. of a.i. per gallon. The amount of a.i. per fluid ounce can be calculated as lbs./gal. ÷ # fl. oz. in a gallon (which is 128):

1.6 lbs./gal. ÷ 128 = 0.0125 lbs. a.i./fl. oz.

Provado can be applied as a foliar spray to apples at a maximum rate of 8 fl. oz./acre up to five applications per season (a maximum of 40 fl. oz./acre per season). The amount of imidacloprid applied to each acre of crop can be calculated as lbs. a.i./fl. oz. × fl. oz./acre.

0.0125 lbs. a.i./fl. oz. × 40 fl. oz./acre = 0.5 lb./acre

This is equal to 8 oz./acre of imidacloprid.

Assuming there are 200 trees per acre, the amount of imidacloprid each tree receives is:

8 oz./acre ÷ 200 = 0.04 oz./tree

(Continued on next page.)

## Ornamental or Backyard Soil Drench Insecticide: Bayer Advanced 12 Month Tree & Shrub Insect Control II™

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. (imidacloprid) and 97.06% inert ingredients. We contacted the US EPA to get the weight of a.i. per gallon of concentrate: 0.26 lbs./gal. The amount of a.i. per fluid ounce can be calculated as lbs./gal. ÷ # fl. oz. in a gallon (which is 128):

0.26 lbs./gal. ÷ 128 = 0.00203125 lbs. a.i./fl. oz.

This is equal to 0.0325 oz./fl. oz.

The recommended application rate for 12 Month Tree & Shrub Insect Control II ™ concentrate is 0.5 fl. oz. per 1" of tree trunk circumference. The amount of a.i. applied per tree can be calculated as circumference × fl. oz./inch × oz. a.i./fl. oz. Assuming that a mature apple tree in a backyard has a circumference of 30" (9.5" in diameter), the amount of a.i. applied to the tree is:

30" × 0.5 fl. oz. × 0.0325 oz. = 0.4875 oz./tree

This is approximately 16 times greater than the agricultural rate per tree allowed for Admire and 12 times greater than the rate for Provado.

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:

60" × 0.5 fl. oz. × 0.0325 oz. = 0.975 fl. oz./tree

This is 24 times greater than the yearly application rate of Provado and 32 times greater than that allowed for Admire. 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./acre). Sometimes 3–8 fl. oz. of a.i./acre provides enough aphid control for the whole season. If only 8 fl. oz. of Provado is applied in a season, the amount of imidacloprid is:

0.0125 lbs. a.i./fl. oz. × 8 fl. oz./acre = 0.1 lb./acre

This is equal to 1.6 oz./acre of imidacloprid.

Assuming there are 200 trees per acre, the amount of imidacloprid each tree receives is:

1.6 oz./acre ÷ 200 = 0.008 oz./tree

This would make the relative backyard application rate 60 times greater for a 30" circumference tree (9.5" diameter), and 120 times greater for a tree of 60" circumference (19" diameter).

**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. (Photograph: Matthew Shepherd/The Xerces Society.)**

# 7 Recommendations for Protecting Bees

## 7.1 Improving Pollinator Risk Assessment

Neonicotinoids and other systemic insecticides present unique challenges for pollinator risk assessment. Some of these compounds are used in reduced-risk IPM programs that are arguably better for the people and the environment. However, the translocation of neonicotinoids to pollen and nectar results in direct and potentially long-term exposure to bees. Improved bee toxicology studies are urgently needed to provide an accurate understanding of the unique effects and exposure pathways of these insecticides on bees and to identify ways for minimizing harm. Furthermore, evidence suggests that honey bees are not the best surrogate for bees in general. Thus, that it is important to collect toxicology data on bumble bees and solitary bees. We need to understand what constitutes lethal or sublethal doses for a variety of bee species and use the most sensitive species as the baseline for determining toxicity.

**Solitary bees, such as this mining bee foraging on apple blossom, despite forming the majority of bee species have generally been overlooked in neonicotinoid risk assessments. (Photograph: Matthew Shepherd/The Xerces Society.)**

***Laboratory trials***

Risk assessment should include, at a minimum, testing of acute and chronic oral toxicity for adult and larval honey bees, bumble bees, and a solitary bee species. Acute contact toxicity testing should be conducted for adults of all three bee groups. Data from these tests should result in no observable effects level (NOEL), sublethal, and lethal concentrations or doses for each bee type. Chronic exposure tests should last for either ten days or the duration of bloom for each plant registered for use, whichever is the longer. If testing a social bee species, laboratory studies should control for variability in colony health and the age of individual bees. Tests also should look at potential interactions between products encountered together in the field, such as the combination of neonicotinoids with adjuvents, fungicides, miticides used in honey bee colonies, or other products that are commonly used alongside insecticide treatments.

***Semi-field studies***

Semi-field studies should test acute exposure to spray applications, as well as chronic exposure to contaminated pollen and nectar (if a risk) for honey bees, bumble bees, and a solitary bee species. Residues in treated plants and the time interval between application and appearance of residue in floral resources should also be measured.

***Field studies***

Field studies, where bees are able to move between the treated crop and nearby areas, should demonstrate that bees are adequately exposed to contaminated pollen and nectar. Exposure can be confirmed by monitoring foraging activity on treated plants and the analysis of residues in stored food. Field trials should include sufficient distance between control and treatment plots, and field sizes should reflect real world conditions where the product would be used. Using a minimum, replicated field treatment size of at least 5 acres (2 ha) is an improvement over many existing field studies, but larger areas would be better. Field trials should take advantage of the limited foraging range of managed solitary bees to help model the impact on honey bees at larger scales where field testing is impossible because of honey bees' large foraging area (the foraging area of a typical hive covers more than 8,000 acres [3,240 ha]). Individual bee mortality, foraging activity, and colony health (of social species) should be monitored over a full year (for honey bees), growing season (for bumble bees), or larval development cycle (for solitary bees) to identify possible

delayed effects from things such as stored food being later consumed and fed to honey bee brood. Crop plant species vary in their ability to uptake systemic chemicals. Application rates and methods, and subsequent translocation of neonicotinoid residues into pollen and nectar should be documented for every crop on which the product is registered for use.

***Risk management***

Risk management should take data on NOEL concentrations and data on pollen and nectar residues (exposure) in each registered crop and only allow application rates and methods that result in nectar and pollen residues below NOEL for bees. Risk managers should also regulate the planting of seeds coated with neonicotinoid insecticides so that dust, talc, or other particles from abraded seed dressings do not drift onto adjacent habitat or apiaries (e.g., mandate that all pneumatic planting equipment filter exhaust and vent directly at the ground). Finally, risk managers should eliminate cosmetic use of neonicotinoids on ornamental plants. These products should not be available for use.

## 7.2 Research Recommendations

In addition to improving the design of risk assessment studies, a number of significant knowledge gaps surrounding neonicotinoids and pollinators remain. There is much we do not understand about the movement of neonicotinoids in plants, for example, and yet what we do know about the variable effects of neonicotinoids points to the importance of more research. To ensure that risk assessments can include accurate estimates of exposure, further studies are needed to help us understand how several factors contribute to variation in pollen and nectar residue levels. Future research priorities should include the following.

**Improved knowledge of how neonicotinoids move in plants to provide more detailed guidance for application rate and method.** In particular, more information about the following issues is required:

- Additive effects of repeated neonicotinoid applications.
- Synergistic effects that may arise when neonicotinoids are combined with other agrochemicals, such as fungicides.
- Rates necessary to effectively control pests while maintaining residue levels below the sublethal effect threshold for pollinators. The levels of neonicotinoids expressed in pollen and nectar should not go above NOEL levels.
- Broad studies to demonstrate and then mandate how to eliminate off-site drift of abraded particles from planting seed-treated crops.

**An expanded understanding of how neonicotinoids function within plants.** Specifically, there is limited publicly available information on:

- How residue levels in nectar and pollen vary with different application rates.
- How residue levels vary in plants grown under differing treatment levels, field conditions (e.g., drought), soil types (e.g., sandy vs. loam), and under variable nutrient levels and ground cover.
- How neonicotinoid concentrations within a plant increase over time following repeat applications.
- Which neonicotinoids are most likely to move to pollen and nectar.
- How that movement might vary with type of plant (e.g., herbaceous vs. woody), by functional group (e.g., forbs vs. legumes vs. grasses), and by size of plant.
- How the concentration of neonicotinoids in pollen and nectar may vary with application method (e.g., seed coating, foliar spray, soil drench, or trunk injection). To date, most research has focused on seed coatings, but other application methods are increasingly used.
- How and at what concentrations neonicotinoids move into non-target plants (e.g., contamination of wildflowers in habitat surrounding treated crops or trees).
- Differences in effect and response to contaminated pollen versus contaminated nectar sources. These are two very different metabolic pathways, which might influence how the compound is broken down, and thus, metabolites formed.

**A full understanding of how neonicotinoids affect bees, which are diverse in biology, habitat requirements, and body size.** The following issues should be resolved.

- To be most conservative, it is important to know the concentration in nectar and pollen a bee can consume and see no observable effects. This concentration should then be used to manage regulated use of these products in the field.
- To date, most studies have looked at imidacloprid and honey bees. However, research suggests that different bee species respond differently to different products.

More information on the impacts of products other that imidacloprid to bees other than the honey bee is needed.

- Standardized methods for testing solitary bees and bumble bees are required.
- Both the lethal and sublethal effects of residues in pollen and nectar on bee larvae have been vastly under-examined. Since larvae may be exposed to neonicotinoids in their food, and the sensitivity of larvae to chemicals cannot be extrapolated from that of adults (Alix et al. 2009), toxicity to larvae should be investigated. It is probable that some substances may be more toxic to larvae than to adults or could cause sublethal effects on reproduction, development, or delayed mortality with chronic exposures.
- How chronic low (sublethal) doses interact with bee pests, disease, or nutrition (i.e., pollen diversity in a bee's diet). For example, does a pollen-diverse diet increase the resilience of honey bees or bumble bees to neonicotinoid exposure?
- Since honey bees store food for times of dearth, chemical exposure may be delayed beyond field study timelines. It is critical to understand whether honey bees experience delayed effects of neonicotinoids during periods of adverse weather conditions (e.g., winter or drought) when those stored foods are consumed.
- Due to the significant agricultural contribution of native bees, it is crucial to understand the possible effects of soil residue on ground-nesting bees and of foliar residue on tunnel-nesting bees that use plant materials for nest construction.
- Solitary bee research would benefit from improved testing methods to measure the effects of insecticides on learning, as some solitary species may not respond to proboscis extension reflex (PER) tests (Vorel and Pitts-Singer 2010).

A clearer understanding of the effects of neonicotinoids on other beneficial insect species would also be valuable. Beyond bees, there is a need to understand how neonicotinoids impact other pollinators, such as butterflies, moths, beetles, flies, and wasps. These insects make minor contributions to crop pollination, but serve important roles within crop systems and other ecosystems (e.g., as larvae, syrphid flies may be predators). Additionally, insects such as butterflies are valued in residential and ornamental landscapes where neonicotinoid use is increasingly common. Natural enemies of pests and other beneficial insects may be differentially affected by neonicotinoids due to their diverse natural histories. We need to better understand the levels of exposure to beneficial insects, whether through contaminated floral resources, contaminated prey, or residues in places such as soil or leaf litter.

# 8 Conclusions

Because they are safer for wildlife and people, neonicotinoids have been widely adopted for pest control. With the widespread and growing use of neonicotinoids in agricultural, commercial, and residential landscapes, bees are exposed to residues on plants or in nectar or pollen. Though studies are few, data demonstrates that the level of residue exposure in pollen and nectar may be high enough to harm bees, particularly in plants treated at ornamental (non-crop) rates. Although existing research has documented measurable sublethal effects, few field studies have been properly designed or conducted over a long enough period of time to assess the full risks to bees. Nevertheless, the overall evidence points to the fact that neonicotinoids are harming bees.

The use of systemic neonicotinoids stands in contrast to some of the principles of Integrated Pest Management. Thanks to application methods such as seed treatments and their long-term persistence, the use of neonicotinoids negates mitigation strategies typically employed to reduce harm to bees. Nighttime spraying, not spraying during bloom, and relocating honey bee hives simply become irrelevant to pollinator protection wherever long-residual systemic insecticides are used. Fundamental IPM practices, such as pest monitoring to determine when action is appropriate, are also negated by prophylactic treatment of seeds, early-season turf treatments, or seasonal trunk injections into woody plants to control pests that might not even show up.

Research to date indicates that neonicotinoid residues in 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 are not expected to reach levels high enough to cause sudden mortality of bees. But chronic exposure (i.e., at low concentrations over a long period of time) may put bees at risk. Research shows that bees experience detrimental sublethal effects such as changes in foraging behavior or delayed development at the residue levels recorded under some applications. In contrast, residue levels in some ornamental plants far exceed the level of lethal concentration for honey bees, and during industry-run studies dead bumble bees were found under treated shrubs, which suggests that non-agricultural use of neonicotinoids poses high risks to bees.

Along with a risk to pollinators, the use of systemic insecticides poses a risk to other benign and beneficial flower visitors that prey upon crop pests, recycle organic matter, feed other wildlife such as songbirds, or simply contribute to a more beautiful and interesting world.

With pollinators and global biodiversity in decline, and with worldwide neonicotinoid use expanding, more robust risk assessments are critically needed. Applications of neonicotinoids should be limited until we have data on how neonicotinoid use on a specific plant may be managed to provide pest protection without exposing beneficial insects to sublethal or lethal levels in nectar and pollen. Without clear evidence that they are not causing long-term harm to non-target species such as pollinators, the use of neonicotinoids should be restricted to applications that will not affect these vital insects.

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# 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 without causing them harm (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).

# Conversions

| Symbol | Unit | Value |
|---|---|---|
| kg | kilogram | $10^3$g |
| g | gram | 1g |
| mg | milligram | $10^{-3}$g |
| µg | microgram | $10^{-6}$g |
| ng | nanogram | $10^{-9}$g |
| L | liter | 1L |
| mL | milliliter | $10^{-3}$L |
| µL | microliter | $10^{-6}$L |
| ppm | parts per million | n/a |
| ppb | parts per billion | n/a |

| Unit | Equivalent value |
|---|---|
| 1 ppm | 1 µg/g |
| | 1 mg/kg |
| | 1 µg/mL* |
| | 1 mg/L* |
| | 1000 ppb |
| 1 ppb | 1 µg/kg |
| | 1 ng/g |
| | 1 ng/mL* |
| | 1 µg/L* |
| | 0.001 ppm |

* 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.


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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