Gardeners Beware:

Bee-Toxic Pesticides Found in "Bee-Friendly" Plants Sold at Garden Centers Nationwide

Friends of the Earth

BeeAction.org

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Any errors or omissions in this report are the responsibility of Friends of the Earth.

About Friends of the Earth:

Friends of the Earth – U.S., founded by David Brower in 1969, is the U.S. voice of the world's largest federation of grassroots environmental groups, with a presence in 74 countries. Friends of the Earth works to defend the environment and champion a more healthy and just world. Through our more than 40-year history, we have provided crucial leadership in campaigns resulting in landmark environmental laws, precedent-setting legal victories and groundbreaking reforms of domestic and international regulatory, corporate and financial institution policies. **www.FoE.org**

Friends of the Earth's BeeAction Campaign is working to protect bees and other pollinators which are critical to food production and healthy ecosystems. **www.BeeAction.org**

Executive summary

Bees and other pollinators are essential for the two-thirds of the food crops humans eat everyday. However, the health and productivity of honey bees, bumblebees, and other pollinators are in great peril, and populations are dwindling worldwide. Concerned citizens have responded by planting "bee-friendly" gardens to provide urban foraging grounds. Unfortunately, as our new study shows, many of the bee-attractive nursery plants sold at top retailers in the U.S. contain persistent, systemic neonicotinoid insecticides that have been shown to impair the health and survival of bees and other vulnerable pollinators.

Although population losses have been linked to multiple factors—including Varroa mite infestations, pathogens, malnutrition and habitat degradation — a strong and growing body of scientific evidence suggests that neonicotinoid pesticides are a major contributing factor. Neonicotinoids, manufactured by Bayer CropScience and Syngenta, are the fastest-growing class of synthetic pesticides. The neonicotinoid imidacloprid — introduced in 1994 — is the most widely used insecticide in the world. Neonicotinoids are used as seed treatments on more than 140 crops, with virtually all corn, soy, wheat, and canola seeds planted in the U.S. being pretreated with neonicotinoids.

Neonicotinoid pesticides can poison bees directly, but even low-level exposure can lead to sublethal effects such as a compromised immune system, altered learning, and impaired foraging, effectively exacerbating the lethality of infections and infestations.

Neonicotinoids are systemic pesticides that are taken up through roots and leaves and distributed throughout the entire plant, including pollen and nectar. These pesticides can poison bees directly, but even low-level exposure can lead to sublethal effects such as a compromised immune system, altered learning, and impaired foraging, effectively exacerbating the lethality of infections and infestations. Unfortunately, home gardeners have no idea they may actually be poisoning pollinators through their efforts to plant bee-friendly gardens.

Friends of the Earth conducted a pilot study to determine the extent of neonicotinoid contamination of common nursery plants purchased at retail garden centers in cities across the U.S. This is the first investigation of neonicotinoid insecticide concentrations in "bee-friendly" nursery plants sold to consumers at garden centers in cities across America. The findings indicate that bee-friendly nursery plants sold at U.S. retailers may contain systemic pesticides at levels that are high enough to cause adverse effects on bees and other pollinators — with no warning to consumers.

The plants included in this pilot study were purchased from major nursery outlets and garden centers including Home Depot, Lowe's, and Orchard Supply Hardware in three different locations across the country: the San Francisco Bay area of California; the Washington, DC area; and the Twin Cities area of Minnesota. The collected plant samples were

submitted to an independent accredited analytical laboratory to identify specific neonicotinoids and quantify their concentrations in whole plant material.

Findings include:

- Neonicotinoid residues were detected in seven out of thirteen samples (54 percent) of commercial nursery plants. In the samples with detections, concentrations ranged from 11 to 1,500 micrograms per kilogram (µg/kg or parts per billion) of plant material.
- The high percentage of contaminated plants and their neonicotinoid concentrations suggest that this problem is widespread, and that many home gardens have likely become a source of exposure for bees.
- For the samples with positive detections, adverse effects on bees and other pollinators are possible, ranging from sublethal effects on navigation, fertility, and immune function to bee death.

This pilot study points to the need for further studies in order to provide a statistical picture of the scope of nursery plant contamination with neonicotinoid insecticides. Larger sample sizes with sufficient plant material to directly measure pollen and nectar concentrations of neonicotinoids in plants treated with both foliar and soil applications would help to clarify some of the questions raised by this preliminary work. Additional studies that measure the distribution of neonicotinoid pesticides in different plant parts over time for different pesticides, plants and soil types are also necessary to enable prediction of pesticide concentrations in pollen and nectar.

The bulk of available scientific literature suggests that neonicotinoids are a substantial contributing factor to the decline of pollinator populations. As a result of this growing body of evidence, the European Commission recently announced a suspension on the use of three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) on flowering plants attractive to bees in European Union countries, effective December 1, 2013. Unfortunately, U.S. EPA has been slow to adequately address the threats to pollinators posed by neonicotinoids, delaying any meaningful action until 2018 when these chemicals are scheduled to enter the Registration Review process.

Although U.S. EPA has not yet taken action, there is still much that can be done to protect bees. Friends of the Earth U.S. is asking consumers, retailers, suppliers, institutional purchasers and local, county, state and federal regulators and policymakers to take action to avoid neonicotinoid pesticides to help protect bees and other pollinators.

Recommendations for garden retailers:

- Do not sell off-the-shelf neonicotinoid insecticides for home garden use.
- Demand neonicotinoid-free vegetable and bedding plants from suppliers and do not sell plants pre-treated with these pesticides.

- Offer third-party certified organic starts and plants.
- Educate your customers on why your company has made the decision to protect bees and other pollinators.

Recommendations for wholesale nursery operations supplying retailers:

- Use only untreated seeds for plants grown from seed.
- Do not use neonicotinoid insecticide soil drenches, granules, or foliar treatments when growing vegetable and bedding plants.
- Offer neonicotinoid-free and organic vegetable and bedding plants to your customers and label them as such.
- Inform your customers about why your nursery operation made the choice to limit the use of neonicotinoid pesticides.
- If quarantine regulations require use of systemic insecticides on certain plants that are hosts for invasive pests, treat only those plants, and minimize the number of treatments. Use pest exclusion systems wherever possible to avoid having to treat plants.

Recommendations for home gardeners and institutional purchasers (such as schools, universities, private companies, hospitals, and others):

- Stop using all neonicotinoid insecticides on your property and facilities (e.g. landscaping around parking lots, grounds and gardens) and only plant neonicotinoid-free plants.
- Ask landscaping companies that service your grounds and trees to not use neonicotinoids or pretreated plants.

Recommendations for cities, counties and states:

- Stop using all neonicotinoid insecticides on city- and county-owned property, including schools, parks and gardens.
- Require that bee-toxic pesticides be prominently labeled as such in displays of these chemicals at hardware stores and nurseries.
- Ban the use of neonicotinoids and other insecticides for cosmetic purposes on ornamental and landscape plants, like the ban now in force in Ontario, Canada.

Recommendations for the U.S. EPA:

- Cancel cosmetic and other unnecessary uses of neonicotinoid pesticide products.
- Require a bee hazard statement on the label of all pesticides containing systemic pesticides toxic to pollinators, not just the foliar use products.
- Prioritize the systemic insecticides for Registration Review starting in 2013, and ensure inclusion of the independent science on the short- and long-term effects of pesticides on pollinators.
- Expedite the development and implementation of valid test guidelines for sublethal effects of pesticides on pollinators and

require data from these studies for all currently registered and any new pesticides.

Recommendations for congress:

- Support and pass H.R. 2692, the Save America's Pollinators Act, introduced by Representatives John Conyers (D, Mich.) and Earl Blumenauer (D, Ore.). This legislation will suspend seed treatment, soil application, or foliar uses of certain neonicotinoid pesticides on bee-attractive plants until:
  - all of the scientific evidence is reviewed by US EPA, and
  - field studies can be done to evaluate both short- and long-term effects of these pesticides on pollinators.

Recommendations for consumers:

- **Take action:** Join the Friends of the Earth BeeAction campaign at www.BeeAction.org and sign our petition to garden retailers asking that they stop selling neonicotinoid treated plants and products that contain neonicotinoids. You can also contact your member of Congress and encourage them to support the Save America's Pollinators Act. You can find an action kit and bee-friendly garden tips at www.BeeAction.org.
- **Raise your voice locally:** Let your local nursery manager know that you will only purchase plants free of neonicotinoids and ask the manager to communicate your request to their corporate headquarters and suppliers who grow the plants they sell. Find a sample letter and more ideas for action at www.BeeAction.org.
- **Grow bee-safe:** Purchase organic plant starts or grow your plants from untreated seeds in organic potting soil for your home vegetable and flower gardens.

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- **Practice bee-safe pest control:** Avoid the use of systemic bee-toxic pesticides in your garden (see Appendix A) and use alternative approaches such as providing habitat to attract beneficial insects that prey on pest insects in your garden. If pest pressure is too high, use insecticidal soaps or oils and other eco-friendly pest control products. For more tips and links to more resources for pollinator and eco-friendly gardening, visit www.BeeAction.org.
- **Do not buy products that contain neonicotinoids:** Read the label and avoid using off-the-shelf neonicotinoid insecticides in your garden. These products contain acetamiprid, clothianidin, imidacloprid, and thiamethoxam as active ingredients. See Appendix A at the end of this report for a list of common off-the-shelf neonicotinoid plant treatments and the neonicotinoids they contain.
- **Do a clean sweep:** See if you have these products at home and dispose of them properly or take them back to the store where you bought them.

I. Introduction and background

Bees in trouble

Bees are essential to the production of one out of every three bites of food we eat.[1, 2] In fact, 71 of the 100 crops that provide 90 percent of the world's food—from almonds to tomatoes and strawberries—are pollinated by bees.[3] Honeybees, in particular, contribute nearly $20 billion to the U.S. economy[4] and $217 billion to the global economy.[5, 6] Yet evidence is mounting that the health and productivity of these critical pollinators, along with many wild pollinators, is declining rapidly.

In the mid 1990s, beekeepers in France, then in the U.S. and elsewhere experienced high colony losses, both overwintering losses and colony collapse during the spring and summer, when colonies should be thriving. In the U.S., beekeepers noticed their colonies mysteriously collapsing, with adult bees disappearing and leaving the queen, honey and capped brood in the nearly empty hives. This phenomenon has been dubbed "Colony Collapse Disorder" or CCD.[7, 8]

In the winter of 2012-2013, beekeepers from Texas to California consistently reported colony failures of between 40–50 percent, a 78 percent increase in losses over the previous winter.[9] In July 2013, 37 million bees were reported dead across a single farm in Ontario.[10] Some farmers are facing shortages of bees necessary to pollinate their crops, and the cost to farmers of renting bees for pollination services has increased by up to 20 percent in some cases.[11] Bumblebees, as well as many other wild pollinators have also recently experienced dramatic declines.[12]

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These die-offs are adversely affecting all of us, not just beekeepers, with the food supply dependent on honey bees and other wild pollinators. With roughly 80 percent of all flowering plants reliant on pollinators to reproduce, bee losses could contribute to losses of a host of other important species.

Key factor: The world's most popular pesticide

Pests,[13] diseases, loss of forage and habitat[14] and changing climate[15] have all been identified as possible contributing factors to colony losses. However, a growing body of evidence points to exposure to pesticides, specifically a class of neurotoxic pesticides called neonicotinoids introduced in the mid-1990s, as a key factor in colony losses and the decline of bees and other essential pollinators.[16] While all

of these factors may contribute to the disappearance of pollinators and hive failure, exposure to neonicotinoids is a common thread that has been shown to increase pollinator vulnerability and decrease natural resilience to external stressors such as pests and pathogens.[17, 18, 19, 20]

Neonicotinoids are the fastest-growing class of synthetic pesticides and are manufactured primarily by Bayer CropScience and Syngenta. Indeed, Bayer CropScience's imidacloprid is the most widely used insecticide in the world. Neonicotinoids are used as seed treatments on more than 140 crops varieties, with virtually all corn, soy, wheat, and canola seeds planted in the U.S. being pretreated with neonicotinoids. These insecticides have a variety of uses beyond agriculture, from lawn maintenance and landscaping, to termite and flea control. They are systemic pesticides that are taken up through the roots and leaves of the plant and distributed throughout the entire plant. Other systemic pesticides—including insecticides, herbicides, and fungicides—are also commonly used, but neonicotinoids have received the most study in terms of their effects on bees and are the most widely used of the systemic pesticides.

While most insecticides are toxic to pollinators, this family of insecticides stands apart from the rest, posing both immediate and long-term risks to bees and other pollinators. New research shows that neonicotinoids are not only capable of killing bees outright, attacking their nervous systems, but low levels of exposure have been shown to disrupt foraging abilities, navigation,[18] learning, communication and memory,[17] and suppress the immune systems of bees,[21] making them more vulnerable to disease and pests. Neonicotinoids are persistent, lasting for years in the soil, as well as systemic, permeating the entire plant and later released in pollen, nectar and other plant fluids.[22]

Neonicotinoid pesticides aren't just harming honey bees, they have also been shown to kill other helpful insects critical to sustainable food production and healthy ecosystems, such as wild bees, bats, butterflies, dragonflies, lacewings, and ladybugs.[23, 24] Further, this class of pesticides may also be severely impacting bird populations[25] as well as earthworms, mammals, amphibians, and aquatic insects.[26] Outbreaks of infectious diseases in honey bees, fish, amphibians, bats and birds in the past two decades have coincided with the increasing use of systemic insecticides, specifically several neonicotinoids, with research suggesting a cause and effect link.[27]

The recent mass death of bumblebees in Oregon—the largest-ever reported incident of bumblebee death in the U.S.—illustrates the problem of neonicotinoids. In June, more than 50,000 bumblebees, representing roughly 300 colonies, were found dead or dying in a Target store parking lot in Wilsonville, OR. The culprit was a neonicotinoid pesticide, dinotefuran, applied to nearby trees.[28] The pesticide was applied for cosmetic reasons. That same week, hundreds of bees were found dead after a similar cosmetic pesticide application in the nearby town of Hillsboro. In the wake

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of these incidents, the Oregon Department of Agriculture restricted the use of 18 insecticide products containing dinotefuran until December 24, 2013, while it completes an investigation into these poisonings.[29]

Spurred by recent events, Representatives John Conyers (D, Mich.) and Earl Blumenauer (D, Ore.) introduced the "Save America's Pollinators Act" H.R. 2692, legislation that will suspend seed treatment, soil application, or foliar uses of certain neonicotinoid pesticides on bee-attractive plants until all of the scientific evidence is reviewed by US EPA, and field studies can be done to evaluate both short- and long-term effects of these pesticides on pollinators.[30]

EPA delays while pollinators suffer

The evidence that neonicotinoids are a key factor in pollinator decline is compelling, which is why these insecticides have been restricted in several European countries including France, Germany and Italy starting in 2009. In January, 2013, the European Food Safety Authority (EFSA) published a scientific review[31] stating that neonicotinoids pose an unacceptably high risk to bees, and the industry-sponsored science upon which regulatory agencies' claims of safety have relied is inadequate for assessing the potential impacts on pollinators.[32] EFSA recommended that the three most used pesticides— imidacloprid, clothianidin, and thiamethoxam—should not be used on crops attractive to bees.[31]

As a result of EFSA's recommendation, the European Commission voted to enforce a continent-wide two-year suspension on the use of neonicotinoids imidacloprid, clothianidin, and thimethoxam on flowering plants, effective December 1, 2013.[33] This regulatory action represents the first and only wide-reaching restriction on these pesticides due to science-based concerns of toxicity toward honey bees and other pollinator populations.

Meanwhile, the United States Environmental Protection Agency (EPA) has yet to take substantial action on the threats to pollinators posed by neonicotinoids.[34] While some neonicotinoids are fully registered, others were allowed to enter the market under a "conditional registration." The conditional registration loophole has allowed hundreds of pesticides—more than 60 percent of those used in the U.S.—to be used commercially without adequate safety data.[35, 36] In some cases, these temporary approvals were implemented in the face of objections by EPA's own scientists.[37] Despite mounting scientific evidence linking these pesticides to colony losses, and more than a million public comments urging swift action on neonicotinoids to protect bees, the EPA has delayed action until its review of neonicotinoids is complete in 2018. As a result, these chemicals remain on the market.

Due to a growing scientific and public concern and a successful campaign by Friends of the Earth England, Wales, Northern Ireland (EWNI) and allies, a majority of the UK's

largest home improvement retailers—including Homebase, B&Q and Wickes—have made public commitments to no longer sell products containing pesticides linked to declining bee populations. Friends of the Earth U.S. and allies have just launched a campaign at BeeAction.org, calling on U.S. retailers to take similar actions in absence of meaningful action by the U.S. EPA.

Bee-toxic pesticides hiding in "bee-friendly" gardens

Many home gardeners, "urban homesteaders" and beekeepers have responded to the global bee and pollinator crisis by planting bee-friendly gardens, creating habitat and forage for wild pollinators and domesticated honey bees alike.[38] Due to their efforts, many urban gardens have become a haven for wild pollinators and honeybees.

However, as this report shows, gardeners may be unwittingly purchasing toxic seedlings and plants attractive to pollinators for bee-friendly gardens, only to poison them in the process.

Neonicotinoids sold to consumers as plant treatments and in pre-treated nursery plants

Neonicotinoids aren't just used in commercial agriculture, but are commonly found in systemic plant treatments for roses and a variety of other plants attractive to bees and other pollinators. These pesticide products are sold in garden centers and other retailers under names including Merit and GrubEx (a list of commonly available systemic plant treatments and neonicotinoid pesticides is provided in Appendix A).[39] U. S. EPA requires the foliar use products to have a bee hazard warning statement on the label, but the granular and soil drench "systemic plant treatments" intended for soil applications are not labeled to

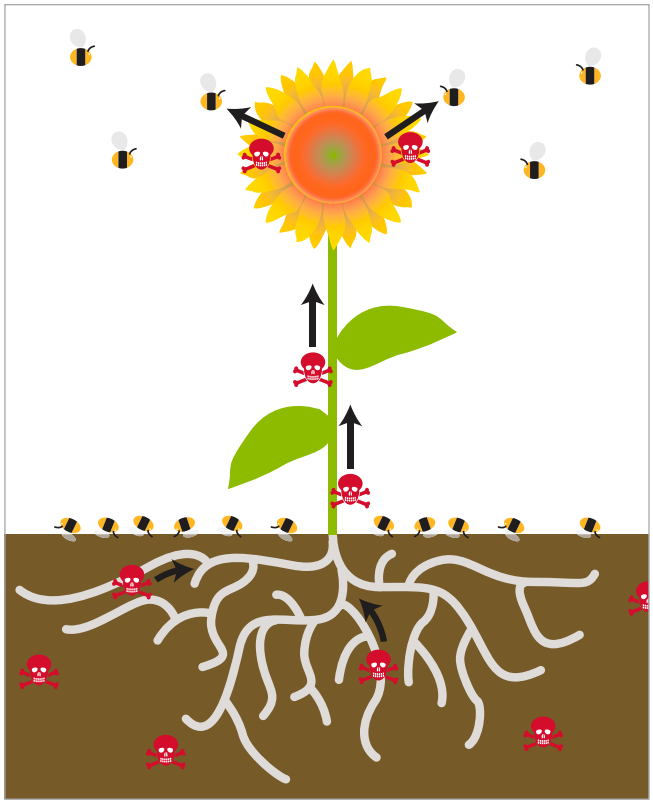
indicate their effects on non-target, beneficial insects like bees, lacewings, butterflies, and ladybugs. In addition, many of the seedlings and plants sold in nurseries and garden stores across the U.S. have been pre-treated with neonicotinoids at much higher doses than are used on farms, where levels of neonicotinoid use are already raising concerns among beekeepers and researchers studying the decline of pollinator populations.

In certain extreme cases, such as an infestation of disease-carrying invasive insects, federal and state laws mandate the treatment of nursery plants with neonicotinoids and other insecticides to prevent the spread of pests capable of disabling an entire crop sector. For example, the California Department of Food and Agriculture (CDFA) and U.S. Department of Agriculture (USDA) have implemented quarantine requirements to reduce the role that retail sales of citrus and

other host plants play in the spread of Asian citrus psyllid (ACP), which carries a disease lethal to citrus trees known as Huanglongbing disease (HLB).[40] Any host plants within or moving into a CDFA-established quarantine zone must receive a combination insecticide treatment consisting of a foliar pyrethroid and a soil drench containing a systemic insecticide, mostly in the form of a neonicotinoid.[41] Notwithstanding this requirement, the results of our study show that many nursery bedding and vegetable plants not listed as hosts for ACP or other regulated pests are still being treated with one or more neonicotinoids prior to sale — with no disclosure to people who are purchasing the plants.

Systemic insecticides are distributed throughout plants

Nurseries commonly apply neonicotinoids as soil injections, granular or liquid soil treatments, foliar sprays (applied to leaves), and seed treatments. Water-soluble pesticides such as neonicotinoids are readily absorbed by plant roots and transported systemically in the plant's vascular system to other portions of the plant, including roots, pollen, leaves, stems, and fruit.[42] This systemic action results in the exposure of beneficial, non-target insects such as bees to potentially lethal doses of neonicotinoids.



Systemic pesticides are absorbed from the soil by the roots and transported to other parts of the plant

Residue levels in plant tissues vary widely depending on the application rate, plant variety, soil composition, and water solubility of the particular pesticides.[43] In past laboratory studies, the highest concentrations of imidacloprid (a relatively water-soluble member of the neonicotinoid class) from seed and soil treatments were observed in the leaves of younger plants with lower concentrations in older plants, roots, apex leaves, fruit, and flowers.[44, 45] Neonicotinoids also have the potential to be transported with irrigation water horizontally through soil and into neighboring plants.

The method of pesticide application also affects the amount of residue taken up by the plant, with soil drenches at recommended application rates resulting in higher concentrations than seed treatments.[44] Foliar applications of neonicotinoids are absorbed through the leaves into the internal plant tissue and tend to remain localized in the treated area.[46] This attribute of foliar sprays stands in contrast to soil applications, where systemic insecticide residues are distributed and concentrated in various tissues of the plant following treatment.[47]

Nursery plants are treated at higher application rates than agricultural crops

Across the U.S. approximately 90 million acres of corn and 74 million acres of soybeans are planted from neonicotinoid-treated seeds. Bees can be exposed through dust during planting, as well as pollen and nectar in mature

plants. Although there are more acres of neonicotinoid-treated agricultural crops, nursery plants are treated at much higher application rates and represent a more potent source of exposure. A single corn plant grown from an imidacloprid-treated seed will have access to 1.34 milligrams (mg) of imidacloprid from the soil it is grown in. In contrast, the recommended label application rate for a perennial nursery plant in a three-gallon pot is 300 mg of imidacloprid, an amount that is 220 times more imidacloprid per plant.

Neonicotinoids persist from one season to the next

Plants treated with neonicotinoids continue exuding these pesticides in pollen and nectar for months to years after initial treatment. Neonicotinoids applied to soil and as seed treatments are found in soils, plant tissues, pollen, nectar, and even surface water long after the application. This persistence is a common property of neonicotinoids and is characterized by a measurement called "half-life," which is the time required for half of the pesticide to degrade.

A good general guideline is that the time required for more than 95 percent of a compound to degrade will take five half-lives. For example, imidacloprid, a neonicotinoid and one of the most widely used insecticides worldwide, has a reported soil half-life of 48 to >365 days depending on the soil type, exposure to sunlight, and the amount of vegetation present.[49, 50] With this degradation rate, it could take well over five years for the imidacloprid to degrade after application.

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Imidacloprid and other neonicotinoids released from seed treatments are likely to persist in the soil near the treated seed and become incorporated into later generations of plants. One study found imidacloprid in soils up to 82 days after planting,[51] while another study reported 23 percent of the original imidacloprid being present in the growing soil after 97 days.[52] Further, many of the degradation products are themselves toxic to pollinators and also persistent in the environment.

How pollinators are exposed to neonicotinoids

Pollen, nectar, and water are all sources of pollinator exposure to harmful insecticides. Worker bees foraging on contaminated plants and drinking from contaminated water sources ultimately carry these harmful insecticide residues back to the hive. These contaminated materials are then used as food for the colony, delivering a potentially lethal dose of toxic insecticides to other worker bees, drones, the queen, and sensitive larvae.

II. Multiple bee-toxic pesticides found in bee-friendly nursery plants

The widespread agricultural use of neonicotinoids is a common exposure pathway for bees; however, cosmetic use of these pesticides in gardens, lawns, and landscapes may be an important factor in declining bee and wild pollinator health. Nursery plants are typically treated with systemic insecticides, either by foliar or soil treatments or by use of treated seeds to kill pest insects that feed on the plant. Systemic pesticides are absorbed through the roots or leaves of the plant and transported to various plant tissues. While this phenomenon is well established, no quantitative information is available on the levels of neonicotinoids found in consumer nursery plants sold at garden retailers and how these levels in the environment might affect pollinator health. Unfortunately, pollinator friendly nursery plants sold to unsuspecting consumers carry neither a list of pesticides used, nor do they carry a warning that these pesticides could harm pollinators.

In an effort to make this information accessible to researchers, retailers, policymakers and home gardeners, Friends of the Earth has undertaken a pilot study of neonicotinoid levels in common nursery plants purchased at retail garden centers in cities across the U.S.

Within this report, we have outlined sampling results that provide insight into the level of contamination found in representative nursery plants. We have also outlined how neonicotinoid insecticides contaminate various plant materials (stems, leaves, pollen and nectar), the damaging effects of this contamination on the health of bees and other pollinators, and what various stakeholders can do to help protect the welfare of these critically important insects.

The widespread agricultural use of neonicotinoids is a common exposure pathway for bees; however, cosmetic use of these pesticides in gardens, lawns, and landscapes may be an important factor in declining bee and wild pollinator health.

Sampling and analysis

The plants used in this study were purchased from major retail outlets in three different regions of the United States:

1. West Coast – San Francisco Bay Area, CA (Home Depot and Orchard Supply Hardware)
2. East Coast – Washington, DC Area (Home Depot and Lowe's)
3. Midwest – Minneapolis, MN Area (Home Depot)

In each location, pollinator friendly plants (flowers and vegetables) were purchased for neonicotinoid residue analysis. Only soft-stemmed (non-woody) flowering plants known to attract both pollinators and pest insects (aphids, etc.) were selected for this study. Popular vegetable plants attractive to pests and pollinators, such as tomato and summer squash starts, were also included.

Multiple plants of the same kind, purchased at the same store and location, were combined to provide sufficient material for each individual sample.

Within one day of purchase, the plants were prepared for neonicotinoid analysis, employing a rigorous protocol to avoid cross-contamination between samples. Each plant was cut at the base of the stem, above the roots and level of the soil. A composite sample of stems, leaves, and flowers was prepared; soil and roots were excluded. Plant materials from multiple potted plants of the same kind were combined to provide sufficient material for the analysis of a single sample. As a result, 26 individual plants were analyzed as part of the 13 composite samples submitted for analysis. An accredited analytical laboratory analyzed all prepared samples for imidacloprid, clothianidin, thiamethoxam, thiacloprid, acetamiprid, and dinotefuran using AOAC method 2007.91, with a 10 µg/kg (ppb) detection limit for the pesticides of interest. The analysis did not include any pesticide degradation products. For more details on the experimental procedures, see Appendix B.

Results

Based on the analysis of a composite of leaves, stems and flowers, seven out of thirteen (or 54 percent) of the composite plant samples in this study tested positive for one or more of the neonicotinoid pesticides, acetamiprid, clothianidin, dinotefuran, imidacloprid, or thiamethoxam. The full data set is presented in Figure 1 and Table 2. Imidacloprid was found most frequently, with residues detected in five of the seven samples that tested positive. Only one Bay Area sample (a tomato plant) tested positive; however, this sample had the highest concentration of imidacloprid of any of the samples.

While the majority of samples contained only one neonicotinoid, two samples (Salvia from DC and MN) tested positive for two residues, and a Gaillardia plant from MN showed measurable levels of three different neonicotinoids. This result provides some insight into how nurseries use these insecticides. There are very few insecticide products containing multiple neonicotinoids as active ingredients and none containing three different neonicotinoids,[39] so these plants were possibly treated multiple times during their short lifespan. Clothianidin, a breakdown product of thiamethoxam, is also likely to be observed in thiamethoxam-treated plants.

Plant samples were individually packaged before shipping to the lab.

In order to capture the cumulative toxicity of the plants with multiple neonicotinoids, we developed a method to express all toxicity in units of imidacloprid equivalents. The neonicotinoids all have high acute toxicity to bees, and all act by the same mechanism of action that interferes with the proper functioning of the nervous system. Clothianidin is the most acutely toxic and acetamiprid the least (see Table 1). To assess cumulative toxicity, we created Relative Potency Factors (RPFs) for each pesticide using the oral LD_{50} values (the dose of neonicotinoid at which 50 percent mortality of test bees is observed following oral exposure), where the RPF is equal to the ratio of the oral honey bee LD_{50} of each insecticide relative to the LD_{50} of imidacloprid.

Using this method, we obtained a cumulative neonicotinoid concentration for each plant sample in terms of imidacloprid equivalents. For details regarding the RPF approach, please see Appendix B.

Comparison of measured residues in nursery plants to other studies

The levels of neonicotinoids found in the nursery samples tested in this pilot project are comparable to those found in other studies of treated plants (see Table 3). Overall, concentrations depend on the type and age of the plant, the part of the plant analyzed, the soil type, the length of time between treatment of the plant and measurement of insecticide concentrations, and the treatment method (soil, foliar, or seed treatment). For the nursery plant samples tested, we do not know how or when the plants were treated. Concentrations in plants are likely to change over time, either increasing if more pesticide is available from the soil or decreasing as the plant grows. These data provide a snapshot of neonicotinoid residue levels in young starter plants available in retail nurseries. A larger study would help clarify these details.

> Unfortunately, pollinator friendly nursery plants sold to unsuspecting consumers carry neither a list of pesticides used, nor do they carry a warning that these pesticides could harm pollinators.

Data from a representative sample of available published studies are presented in Table 3, providing a comparison of concentrations of imidacloprid and/or thiamethoxam in various plants treated under different conditions. Imidacloprid concentrations ranged from a high of 6,600 µg/kg for buckwheat flowers grown in pots treated at the recommended

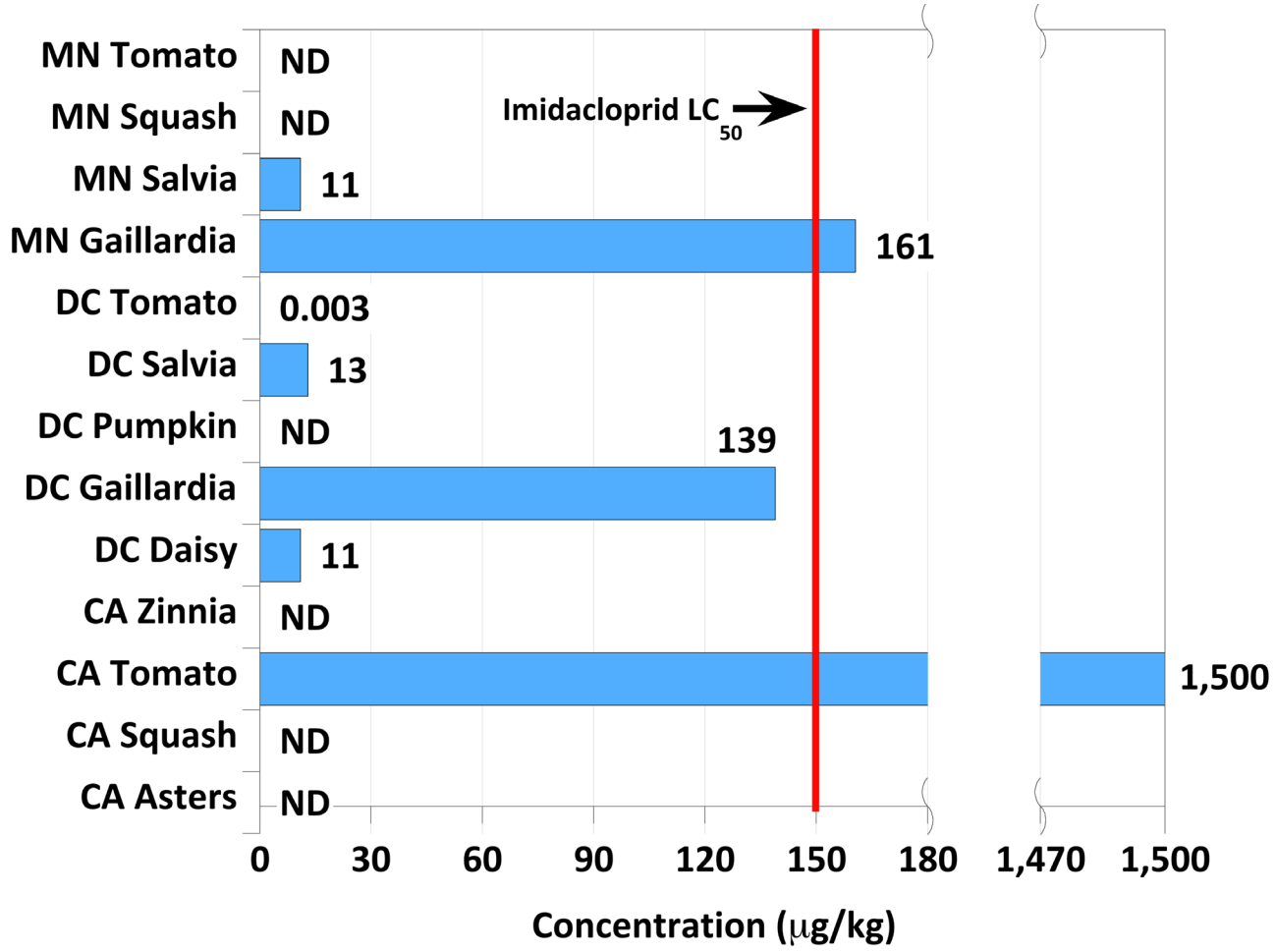
Table 1. Relative Acute Toxicity of Neonicotinoid Insecticides to Honey Bees

Pesticide	Oral LD_{50} (µg/bee)	Oral LC_{50} (µg/L)	Relative Potency Factor
Acetamiprid	14.53	558,846	0.0003
Clothianidin	0.0037	142	1.06
Dinotefuran	0.023	885	0.17
Imidacloprid	0.0039	150	1.00
Thiamethoxam	0.005	192	0.78

application rate, to a low of 1 µg/kg in the stems of mature sunflower plants.

Whole plants (leaves, stems and flowers) were analyzed in this study, but pollinators only eat the nectar and pollen, which typically have lower concentrations of neonicotinoids than other parts of the plant. Comparison studies (Table 3) indicate that concentrations of soil-applied imidacloprid (applied at label-recommended rates) in pollen, nectar, and fruit in squash and tomato plants range from approximately 0.1–15 percent of the concentration in the whole plant.[44, 53] In contrast, a model developed to calculate the distribution of imidacloprid in tomatoes from soil applications estimates the concentrations in tomato

Figure 1. Total toxicity per sample in imidacloprid equivalents



Tests reveal that seven out of 13 samples of nursery plants available at retail outlets contained detectable levels of neonicotinoid insecticides at levels ranging from 11 to 1,500 µg/kg. All toxicity is expressed in units of imidacloprid equivalents to account for the cumulative bee toxicity of plants containing multiple insecticides. Three of the corrected whole-plant residue concentrations either exceed or approach the LC_{50} of imidacloprid (the concentration of imidacloprid in nectar at which 50% of test bees died after one feeding, indicated in this chart as the red line). If those concentrations were found in nectar or pollen, then bees would be exposed to sublethal or even lethal doses of the insecticide. See text for further explanation.

fruits at approximately half of that in stems and one-third of the concentration in roots.[54] From the available comparison data, it is not possible to predict concentrations in pollen and nectar for all plants in all life stages using residues measured in whole plants. However, University of Minnesota studies indicate that application of label-recommended rates of imidacloprid to buckwheat and milkweed—two very attractive plants to pollinators—produced nectar concentrations of 16 and 26 µg/kg, respectively, at 21 days after treatment.[55] Indeed, these levels were sufficiently lethal to kill a large fraction (the precise kill rate depended on the species and experimental conditions) of ladybugs allowed to feed on treated plants compared to a control group. The measured residues are 11–17 percent of the oral LC_{50} for honey bees.

Table 2. Results from nursery plant sampling in California, Minnesota and the District of Columbia

Sample Description[a]	Plants per Sample	Neonicotinoid	Observed Concentration (µg/kg)[b]	Imidacloprid Equivalent Toxicity (µg/kg)[b]	Total Neonic Concentration in Imidacloprid Equivalents (µg/kg)[b]
CA Asters	1	--	ND		
CA Squash	3	--	ND		
CA Tomato	4	Imidacloprid	1,500	1,500	1,500
CA Zinnia	1	--	ND		
DC Daisy	1	Imidacloprid	11	11	11
DC Gaillardia	1	Dinotefuran	820	139	139
DC Pumpkin	2	--	ND		
DC Salvia	1	Dinotefuran	12	2	
		Imidacloprid	11	11	13
DC Tomato	1	Acetamiprid	12	0.003	0.003
MN Gaillardia	1	Clothianidin	36	38	
		Imidacloprid	21	21	
		Thiamethoxam	130	101	161
MN Salvia	4	Acetamiprid	54	0.014	
		Imidacloprid	11	11	11.014
MN Squash	3	--	ND		
MN Tomato	3	--	ND		

ND = not detected
a = Plants were obtained from Home Depot in Richmond, CA, Richfield, MN, and Washington, DC; Orchard Supply Hardware in El Cerrito, CA; and Lowe's in Alexandria, VA.
b = µg/kg = micrograms per kilogram.

Table 3. Neonicotinoid residues in plants from other studies

Plant	Pesticide	Concentration (µg/kg)	Study Description	Reference
Buckwheat	Imidacloprid Flowers Nectar (2004)	 6,600 16	Pots were treated with soil granules of Marathon 1G® 14 days after emergence at label recommended rate. Nectar sampled 21 days after treatment.	24, 55
Milkweed	Imidacloprid Nectar (one application) Nectar (two applications)	 26 53	Pots were treated with soil drench of Marathon 1G® at label recommended rate. Nectar sampled 21 days after treatment.	55
Clover	Clothianidin Nectar	 89–319 Avg = 171 (N = 5)	Nectar extracted one week after application from 100-flower samples of clover from plots treated at the highest label rate (0.40 lb clothianidin/acre) with Arena 50 WDG.	56
Squash-1	Imidacloprid Whole plant Flower base Stamens Thiamethoxam Whole plant Flower base Stamens	 47 10 15 154 10 19	Seed-hole (11 cm diameter) spray application of label-recommended rates of an imidacloprid-containing product (Admire Pro®) or a thiamethoxam-containing product (Platinum®).	44
Squash-2	Imidacloprid Whole plant Flower base Stamens Thiamethoxam Whole plant Flower base Stamens	 218 31 46 362 22 31	Transplants were treated using drip irrigation with label-recommended rates of an imidacloprid-containing product (Admire Pro®) or a thiamethoxam-containing product (Platinum®) five days after transplanting.	44
Sunflower	Imidacloprid Leaves Seeds Flower head Pollen Stem	 520 28 18 13 1	Sunflowers grown from Gaucho (imidacloprid) treated seeds at the commercial loading of 1 mg of imidacloprid active ingredient per seed. Plant tissues were analyzed after two-thirds of the florets were blooming.	57
Sugarbeet	Imidacloprid Leaves	 4,500	Plants grown from seeds treated at the commercial loading rate of 90 g imidacloprid per hectare. Concentrations correspond to foliage sampled 40 days after sowing.	51
Tomato	Imidacloprid Leaves Fruit	 7,400 63	15-day-old tomato plants were transplanted to 1-L pots containing imidacloprid-contaminated soil (0.33 mg/L of soil). Foliage and fruits sampled 60 days after transplantation.	53

ND = not detected
a = Plants were obtained from Home Depot in Richmond, CA, Richfield, MN, and Washington, DC; Orchard Supply Hardware in El Cerrito, CA; and Lowe's in Alexandria, VA.
b = µg/kg = micrograms per kilogram.

III. How could contaminated flowers and vegetable plants affect bees?

This analysis confirms the presence of neonicotinoids in common garden plants sold to unsuspecting consumers at garden centers nationwide. The measured concentrations represent a lower bound estimate of the total toxicity to pollinators because the analysis did not include any pesticide degradation products, some of which are comparable to the parent compound in toxicity. Sufficiently high concentrations of neonicotinoids can kill bees; lower concentrations can still impair pollinator behaviors, reproduction, and immune function.

Acute effects

Comparison of the imidacloprid-equivalent concentrations measured in nursery plants to the acute honey bee LC_{50} for imidacloprid* (150 µg/kg) reveals one sample with ten times this concentration and two others approaching it. [58,59] It is not possible to precisely determine what dose the bees would be receiving in the pollen and nectar of these plants, but at the levels observed, it is possible that consumption of pollen and nectar could lead to a significant impairment of bee health and even death.

Sufficiently high concentrations of neonicotinoids can kill bees; lower concentrations can still impair pollinator behaviors, reproduction, and immune function.

*Acute oral LC_{50} (in µg/kg) was calculated using the acute oral LD_{50} (in µg/bee) from the US EPA EcoTox Database[57] and the amount of sucrose solution ingested by a bee in an LC_{50} test (26 mg). Specifically, LC_{50} = LD_{50} / 26 mg.[58] The resulting LC_{50} (in µg/mg) is corrected to µg/kg using a conversion factor of 1,000,000 mg/kg. For additional details, see Appendix B.

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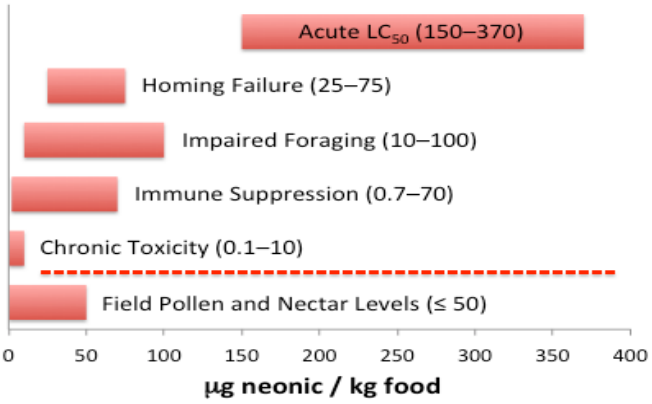
Sublethal effects and chronic toxicity

All of the samples with detections could potentially cause sublethal effects and mortality in pollinators following chronic exposure. Beyond acute pollinator mortality from bees receiving a lethal dose, neonicotinoids contribute to impairment in immune response, learning and memory, hive communications, and reproduction at doses far below those that cause bee kills (Figure 2). Although not all of the mechanisms of toxicity are fully known, many of these effects stem from the ability of neonicotinoids to interfere with the proper functioning of the insect nervous system.[60]

Because the neonicotinoid pesticides are systemic and persistent, exposures to low levels of neonicotinoids in pollen and nectar over an extended period of time (weeks to months) is a serious consideration. Toxicological studies show that neonicotinoids can produce effects at even very low concentrations, provided the exposure time is sufficiently long.[61] In one study, dietary exposure to field-realistic levels of 1 picogram (pg = 0.000000000001 gram) of imidacloprid per day resulted in bee mortality within 10 days.[62] Bumblebee colonies

foraging for six days on clover in turf treated with a clothianidin grub-control product at label rates not only experienced bee kills, but the surviving bees produced no queens.[56] Chronically exposed bumblebee hives have also shown reduced colony growth rates and substantial (as high as 85 percent) reduction in the production of new queens, creating an enormous barrier to colony propagation.[19] Not surprisingly, chronic exposure to low doses of neonicotinoids is also lethal to other non-target insects, as well as natural predators of the pest insects targeted by these systemic pesticides.[63, 64]

Figure 2: Concentrations of neonicotinoids associated with effects on bees



Neonicotinoids are highly toxic to bees, but even at low doses, they can impair colony health. Concentrations of neonicotinoids are given in µg/kg (µg/kg = parts per billion). Homing behavior study based on thiamethoxam exposure. All other studies based on imidacloprid exposure. Chronic toxicity refers to increased bee mortality associated with long-term, low-level exposure.

Behavioral and learning impairment caused by neonicotinoid exposure are equally deleterious to long-term bee survival and colony success. A recent study demonstrated that field level (10 µg neonicotinoid / g plant material) exposure to the neonicotinoid imidacloprid adversely affected the pollen-collecting efficiency of worker bees, leading to reductions in brood development and colony success.[66] In addition, homing failure following low-level thiamethoxam exposure was observed in 10–30 percent of bees, depending on their familiarity with a particular foraging region.[18] Other effects of neonicotinoid exposure on pollinator behavior include reduced activity levels,[67] short- and long-term memory impairment,[17, 68] and diminished ability to recruit foragers through waggle dancing.[69]

Because the neonicotinoid pesticides are systemic and persistent, exposures to low levels of neonicotinoids in pollen and nectar over an extended period of time (weeks to months) is a serious consideration. Bumblebee colonies foraging for six days on clover in turf treated with a clothianidin grub-control product at label rates not only experienced bee kills, but the surviving bees produced no queens.

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Although bee kills are visible impacts of systemic insecticides, exposure to levels of neonicotinoids that don't cause immediate bee death can still damage colonies through the less apparent effects on the immune system (making the bees more vulnerable to disease), learning and memory (affecting the bees ability to find food and return to the hive), and reproduction (reducing queen fertility[66]).

Mechanistic studies have correlated these observed behavioral anomalies to physiological changes related to neonicotinoid exposure. For example, smaller hypopharyngeal glands (used by nurse bees to produce the royal jelly that is fed to young larvae) were observed in honeybees that consumed sugar and pollen treated with imidacloprid during development.[70] Scans of isolated honeybee brains also show that imidacloprid blocks neuronal firing in the cells responsible for learning and memory at concentrations likely encountered by foraging bees.[71]

Systemic pesticides also weaken the immune response in bees. In particular, the relationship between Nosema (a unicellular parasite) infestation and neonicotinoid exposure has recently been investigated. In three independent studies, a statistically significant increase in mortality rates was observed in test groups simultaneously infected with Nosema and exposed to neonicotinoids (imidacloprid or thiacloprid) relative to those only infected with Nosema or exposed to imidacloprid alone.[21, 72, 73] In addition to increased individual mortality rates, the combination of stress factors adversely affected the ability of worker bees to disinfect larvae and promote immunity.[21] Although this synergistic effect is not completely understood, it is clear that neonicotinoid exposure exacerbates Nosema infections.

IV. Conclusion

This pilot study represents the first investigation of neonicotinoid insecticide concentrations in "bee-friendly" nursery plants sold to consumers at garden centers in cities across the U.S. The high percentage of contaminated plants and their neonicotinoid concentrations suggest that this problem is widespread, and that many home gardens have likely become a source of exposure for bees. Although pollen and nectar were not directly analyzed, comparison of our sampling results to published data indicate that adverse effects on bees and other pollinators are possible. Potential effects on bees due to neonicotinoid exposure range from impaired navigation, reduced fertility, and immune suppression to bee death.

These results underscore the need for further studies in order to provide a statistical picture of the scope of nursery plant contamination with neonicotinoid insecticides. Larger sample sizes with sufficient plant material to directly measure pollen and nectar concentrations of neonicotinoids in plants treated with both foliar and soil applications would help to clarify some of the questions raised by this preliminary work. Additional studies that measure the distribution of neonicotinoid pesticides in different plant parts over time for different pesticides, plants and soil types are also necessary to enable prediction of pesticide concentrations in pollen and nectar and the environmental fate of those residues introduced into our gardens.

As this pilot study demonstrates, consumers may unwittingly be purchasing bee-attractive plants that have been pretreated with neonicotinoid pesticides that may be harming or killing bees and other threatened pollinators essential to food production and ecosystem health.

V. Recommendations for reducing risks to pollinators

As this pilot study demonstrates, consumers may unwittingly be purchasing bee-attractive plants that have been pretreated with neonicotinoid pesticides that may be harming or killing bees and other threatened pollinators essential to food production and ecosystem health. Unfortunately for bees, other pollinators, and for all of us, the now common cosmetic use of neonicotinoid pesticides in gardens, lawns, and landscapes may be an important factor in declining bee and wild pollinator health.

Unfortunately for bees, other pollinators, and for all of us, the now common cosmetic use of neonicotinoid pesticides in gardens, lawns, and landscapes may be an important factor in declining bee and wild pollinator health.

BeeAction Campaign: "Bee" part of the global movement!

Due to a successful campaign by Friends of the Earth England, Wales, Northern Ireland (EWNI) and allies, a majority of the UK's largest garden retailers, including Homebase, B&Q and Wickes, have made public commitments to no longer sell products containing pesticides linked to declining bee populations. Friends of the Earth U.S. and allies have just launched a campaign at BeeAction.org, calling on U.S. garden retailers to take similar actions in absence of meaningful action by the U.S. EPA. Through the campaign, we are joining our sister organization Friends of the Earth England, Wales, Northern Ireland and other allies, beekeepers, farmers, gardeners, scientists, parents, educators and many others in a global movement to save bees and other pollinators and speed the essential transition to sustainable, just, ecological agriculture. Thousands of people already joined our campaign and demanded that top retailers stop selling these bee-killing pesticides.

We are also asking consumers, retailers, suppliers, institutional purchasers and local, county, state and federal regulators and policymakers to take action to restrict neonicotinoid pesticides to help protect bees and other pollinators.

Recommendations for garden retailers:

- Do not sell off-the-shelf neonicotinoid insecticides for home garden use.
- Demand neonicotinoid-free vegetable and bedding plants from suppliers and do not sell plants pre-treated with these pesticides.
- Offer third party certified organic starts and plants.
- Educate your customers on why your company has made the decision to protect bees and other pollinators.

Recommendations for wholesale nursery operations supplying retailers:

- Use only untreated seeds for plants grown from seed.
- Do not use neonicotinoid insecticide soil drenches, granules, or foliar treatments when growing vegetable and bedding plants.

- Offer neonicotinoid-free and organic vegetable and bedding plants to your customers and label them as such.
- Inform your customers about why your nursery operation made the choice to limit the use of neonicotinoid pesticides.
- If quarantine regulations require use of systemic insecticides on certain plants that are hosts for invasive pests, treat only those plants, and minimize the number of treatments. Use pest exclusion systems wherever possible to avoid having to treat plants.

Recommendations for home gardeners and institutional purchasers (such as schools, universities, private companies, hospitals, and others):

- Stop using all neonicotinoid insecticides on your property and facilities (e.g. landscaping around parking lots, grounds and gardens) and only plant neonicotinoid-free plants.
- Ask landscaping companies that service your grounds and trees to not use neonicotinoids or pretreated plants.

Recommendations for cities, counties and states:

- Stop using all neonicotinoid insecticides on city- and county-owned property, including schools, parks and gardens.
- Require that bee-toxic pesticides be prominently labeled as such in displays of these chemicals at hardware stores and nurseries.
- Ban the use of neonicotinoids and other insecticides for cosmetic purposes on ornamental and landscape plants, like the ban now in force in Ontario, Canada.[74]

Recommendations for the U.S. EPA:

- Cancel cosmetic and other unnecessary uses of neonicotinoid pesticide products.

- Require a bee hazard statement on the label of all pesticides containing systemic pesticides toxic to pollinators, not just the foliar use products.
- Prioritize the systemic insecticides for Registration Review starting in 2013, and ensure inclusion of the independent science on the short- and long-term effects of pesticides on pollinators.
- Expedite the development and implementation of valid test guidelines for sublethal effects of pesticides on pollinators and require data from these studies for all currently registered and any new pesticides.

Recommendations for congress:

- Support and pass H.R. 2692, the Save America's Pollinators Act, introduced by Representatives John Conyers (D, Mich.) and Earl Blumenauer (D, Ore.). This legislation will suspend seed treatment, soil application, or foliar uses of certain neonicotinoid pesticides on bee-attractive plants until:
  - all of the scientific evidence is reviewed by US EPA, and

- field studies can be done to evaluate both short- and long-term effects of these pesticides on pollinators.

Recommendations for consumers:

- **Take action:** Join the Friends of the Earth Bee Action campaign at www.BeeAction.org and sign our petition to garden retailers asking that they stop selling neonicotinoid treated plants and products that contain neonicotinoids. You can also contact your member of Congress and encourage them to support the Save America's Pollinators Act. You can find an action kit and bee-friendly garden tips at www.BeeAction.org.
- **Raise your voice locally:** Let your local nursery manager know that you will only purchase plants free of neonicotinoids and ask the manager to communicate your request to their corporate headquarters and suppliers who grow the plants they sell. Find a sample letter and more ideas for action at www.BeeAction.org.
- **Grow bee-safe:** Purchase organic plant starts or grow your plants from untreated seeds in organic potting soil for your home vegetable and flower gardens.
- **Practice bee-safe pest control:** Avoid the use of systemic bee-toxic pesticides in your garden (see Appendix A) and use alternative approaches such as providing habitat to attract beneficial insects that prey on pest insects in your garden. If pest pressure is too high, use insecticidal soaps or oils and other eco-friendly pest control products. For more tips and links to more resources for pollinator and eco-friendly gardening, visit www.BeeAction.org.
- **Do not buy products that contain neonicotinoids:** Read the label and avoid using off-the-shelf neonicotinoid insecticides in your garden. These products contain acetamiprid, clothianidin, imidacloprid, and thiamethoxam as active ingredients. See Appendix A at the end of this report for a list of common off the shelf neonicotinoid plant treatments and the neonicotinoids they contain.
- **Do a clean sweep:** See if you have these products at home, dispose of them properly or take them back to the store where you bought them.

Appendix A: Common names of neonicotinoid-containing products used on ornamental plants in nurseries or sold to consumers for home garden use

There are approximately 300 insecticide products containing neonicotinoid insecticides as active ingredients used on ornamental plants in either nursery or home garden settings. The specific active ingredients include acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam. Some products contain these chemical names in the product name. Many other products contain neonicotinoids, but do not have the active ingredient in the product name. These product names are included in the table below. Some of these same products go by several different distributor names, such as the Ortho™ brand or other brand names. Inspect the label of any insecticide labeled as "systemic" for the presence of neonicotinoid active ingredients. To protect pollinators, avoid using these products.

Insecticide Product Name	Active Ingredient(s)
ALIAS	Imidacloprid
ALLECTUS	Imidacloprid, bifenthrin
ALOFT	Clothianidin, bifenthrin
ARENA	Clothianidin
ASSAIL	Acetamiprid
ATERA	Imidacloprid, bifenthrin
AURA	Imidacloprid
BITHOR	Imidacloprid, bifenthrin
BOUNTY	Imidacloprid
CARAVAN	Thiamethoxam, azoxystrobin
CORETECT	Imidacloprid
DERBY	Thiamethoxam, lambda-cyhalothrin
DINO	Dinotefuran
DOMINION	Imidacloprid
EQUIL ADONIS	Imidacloprid
FLAGSHIP	Thiamethoxam
FLOWER, ROSE & SHRUB CARE	Clothianidin, imidacloprid, tebuconazole
GAUCHO	Imidacloprid
GRUB-NO-MORE	Imidacloprid
GRUBEX	Imidacloprid
GRUBOUT	Imidacloprid
HAWK	Imidacloprid
I MAXXPRO	Imidacloprid
IMA-JET	Imidacloprid
IMI INSECTICIDE	Imidacloprid
IMID-BIFEN	Imidacloprid, bifenthrin
IMIDA-TEB GARDEN SC	Imidacloprid, tebuconazole
IMIDAPRO	Imidacloprid

Insecticide Product Name	Active Ingredient(s)
IMIGOLD	Imidacloprid
LADA	Imidacloprid
LANCER GOLD	Imidacloprid, acephate
MALICE	Imidacloprid
MALLET	Imidacloprid
MANTRA	Imidacloprid
MARATHON	Imidacloprid
MERIDIAN	Thiamethoxam
MERIT	Imidacloprid
NUPRID	Imidacloprid
OPTIGARD FLEX	Thiamethoxam
PASADA	Imidacloprid
POINTER INSECTICIDE	Imidacloprid
PRONTO	Imidacloprid
PROTHOR	Imidacloprid
ROTAM	Imidacloprid
SAFARI	Dinotefuran
SAGACITY	Dinotefuran
SCORPION	Dinotefuran
STARKLE	Dinotefuran
TANDEM	Thiamethoxam, lambda-cyhalothrin
TRIMAX	Imidacloprid
TRIPLE CROWN INSECTICIDE	Imidacloprid, bifenthrin, zeta-cypermethrin
TRISTAR	Acetamiprid
TURFTHOR	Imidacloprid
WRANGLER	Imidacloprid
XYTECT	Imidacloprid

Appendix B: Methods of sampling, sample analysis, and data analysis

This project involved the determination of neonicotinoid insecticide residues found in the tissues of flowering plants (both ornamental and fruit/vegetable producing) commonly purchased at commercial garden centers.

Sampling

Plants were purchased from large commercial garden centers, including Home Depot (San Francisco Bay area, California; Washington, DC area; and Minneapolis area, Minnesota), Lowe's (DC), and Orchard Supply Hardware (California). Four to five plants were sampled per location, and typically consisted of at least two ornamental flowers and two vegetable starts.

Once purchased, the plants were cut at the base of the stem, above the roots and soil. The workspace was protected with a clean plastic sheet, and gloves were worn when handling plant materials. All plant material above the level of soil was included in the package for neonicotinoid multiresidue analysis; roots and dirt were not included. To avoid cross contamination between samples, a new plastic sheet and pair of gloves were used for each new sample, and scissor blades used to cut the plants were wiped down multiple times with rubbing alcohol wipes.

Following sample preparation, the samples were placed in a Ziploc® bag in an insulated shipping container with cold packs to limit degradation of the plant material and pesticide residues. The samples were shipped cold overnight to the lab and stored in the refrigerator until analysis.

Sample preparation

An accredited independent analytical laboratory prepared all submitted samples for quantitative analysis according to AOAC Official Method 2007.01, Pesticide Residue in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. An exact mass (approximately 50 g) of each sample was first subjected to QuEChERS extraction using a buffered acetonitrile extraction solution (1 percent acetic acid in acetonitrile) and magnesium sulfate (MgSO4) to enable partitioning of the acetonitrile layer from the water in the sample. Dispersive solid phase extraction (d-SPE) was then performed to remove organic acids, excess water, and other components. Extracts were analyzed using high performance liquid chromatography (HPLC) with mass spectrometry (MS), as described below in the following section.

Analysis

A Waters Acquity UHPLC System equipped with a Waters analytical column and Xevo Tandem Mass Spectrometer was employed for

multiresidue neonicotinoid residue analysis of the extracted plant tissues. Calibration curves for all neonicotinoids included in the screen were constructed to determine the concentrations of any neonicotinoids detected during analysis. The limit of quantification (LOQ) for all screened neonicotinoids using this protocol was 10 µg/kg (ppb). Prior to analysis, sample extracts (described above in "Sample Preparation") were allowed to warm to room temperature and diluted 10X by diluting 100 µL of sample extract in 900 µL of solvent. Following LC-MS/MS analysis, all compounds detected in a sample were positively identified and confirmed. The following criteria were met for confirmation: (1) the retention time of the compound in the sample must match the retention time for that compound in the standard, and (2) the ratio of the response for the compound's two distinct ion transitions must be within 30 percent of the values established during initial calibration.

Quality assurance / quality control

Both extraction blank and matrix spike samples were prepared and analyzed for quality control/quality assurance. The same reagents, volumes, and laboratory manipulations as those for sample preparation were employed in preparing the blank in order to demonstrate that the extraction batch is devoid of any interference from glassware or reagents that could produce a false positive. The matrix spike consists of a sample that is fortified with the QC stock solution of neonicotinoids included in the analysis (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, thiamethoxam) to demonstrate acceptable recovery in matrix.

Unmarked trip blank and trip spike samples were also provided to the contracted laboratory for quality assurance purposes. Analysis of the trip blank revealed no neonicotinoid residues, confirming that the method of preparing and packaging, as well as the laboratory's analysis was free of unintended contamination. Likewise, the neonicotinoid concentrations determined for the trip spike were within acceptable limits of percent recovery. Details regarding the calculated and expected concentrations of neonicotinoid residues in the trip blank and trip spike samples are provided below in Table B-1.

Determination of total plant toxicity in imidacloprid equivalents

The analytical results of the study of nursery plants indicated the presence of more than one neonicotinoid pesticide in some of the plants sampled. In order to account for the total neonicotinoid toxicity of the pesticides in the plants, we developed Relative Potency Factors (RPFs) based on oral LD_{50} values for the five neonicotinoid insecticides found in this study. Toxicity was expressed in units of imidacloprid toxicity. The observed neon-

Table B-1. Results of LC-MS/MS analysis of trip blank and spiked samples

Sample	Neonicotinoid	Expected (µg/kg)	Reported (µg/kg)	Percent Recovery (%)
Trip Blank	ND	0	0	– –
Trip Spike	Acetamiprid	20	12	60
(Gaillardia)	Imidacloprid	98	66	67
	Thiamethoxam	200	160	80

ND = None detected

Photo: creative commons Hayes Valley Farm/Flickr

icotinoids include acetamiprid, clothianidin, dinotefuran, imidacloprid, and thiamethoxam. Oral LD_{50} values for the five neonicotinoids were available from the US EPA Office of Pesticide Programs Pesticide Ecotoxicity Database, as shown in Table 1 in the report.[58]

The creation of RPFs is based on the assumption of a common mechanism of action for mortality caused by the neonicotinoid insecticides. All of these chemicals bind to the nicotinic acetylcholine receptors (nAChR), blocking their function.[75] The RPF methodology is similar to US EPA's use of RPFs for organophosphorus (OP) pesticides based on cholinesterase inhibition.[76]

Described below is the stepwise procedure for estimating the toxicity of the observed plant residue levels in terms of LC_{50} values for imidacloprid in foods consumed by bees.

1. Oral LD_{50} values (in µg/bee) were obtained from US EPA EcoTox database for acetamiprid, clothianidin, dinotefuran, imidacloprid, and thiamethoxam
2. The oral LD_{50} of imidacloprid was divided by the LD_{50} of each neonicotinoid to obtain an Imidacloprid Relative Potency Factor for chemical x:

$$RPF_x = \frac{LD_{50\,(imidacloprid)}}{LD_{50(x)}}$$

The calculated RPFs are shown in Table 1 in the report.

1. The observed neonicotinoid concentrations in plants were transformed to Imidacloprid-Equivalent Toxicity, where the concentration of each neonicotinoid in each plant sample was expressed as a concentration equivalent to the same amount of imidacloprid:

$$Imidacloprid\ Equiv\ Toxicity\ (\mu g/kg) = Neonicotinoid_x\ Conc.\ (\mu g/kg) \times RPF_x$$

2. For samples having multiple neonicotinoid residues, the Imidacloprid Equivalent Toxicity values for each neonicotinoid were summed to provide the Total Toxicity per Plant in Imidacloprid Equivalents. For those having only one residue, the Total Toxicity value is equivalent to the Imidacloprid Equivalent Toxicity.
3. Dividing Total Toxicity per Plant in Imidacloprid Equivalents by the LC_{50} of imidacloprid normalizes the Total Toxicity Per Plant relative to the acute dose of imidacloprid that is lethal to bees. The oral LC_{50} for imidacloprid was determined using the equation of Fischer et al.[59] in which the reported oral LD_{50} is divided by the amount of a 50 percent (weight/volume) sucrose solution ingested by a bee in an oral acute toxicity test (26 mg), and converted to parts per billion (or µg/kg):

$$LC_{50}\left(\frac{\mu g\ pesticide}{kg\ of\ nectar}\right) = \left(\frac{LD_{50}\left(\frac{\mu g\ pesticide}{bee}\right)}{26\ mg\ nectar}\right) \times 1{,}000{,}000\ \frac{mg}{kg}$$

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