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June 4, 2013

Don R. Hover
Director
Washington Department of Agriculture
1111 Washington Street SE, 2nd Floor
PO Box 42560
Olympia, WA 98504-2560

Re: Support for Thurston County's request to restrict sale, use and application of neonicotinoid insecticides.

Dear Mr. Hover,

We would like to take this opportunity to support the request for the restriction of the sale, use and application of the neonicotinoid class of insecticides for ornamental uses as submitted by the Thurston County Commissioners. This class of insecticides has been shown to be highly toxic to honey bees, wild bees and other beneficial insects essential to a productive agricultural economy. Beyond Pesticides is very concerned about the status of our nation's pollinators, especially the honey bee. Each year since 2006, commercial beekeepers have reported unprecedented bee losses. Normal losses occur around 17.6%, but this year losses are estimated to be over 30%, with several beekeepers documenting higher than average losses.[1] Olympia Beekeepers Association indicates that its members suffered substantial losses in 2012.

Neonicotinoids, especially clothianidin, imidacloprid and thiamethoxam, have been the focus of several regulatory and legal actions because of their impacts on honey bees. While many entomologists agree that pesticides along with viruses, mites, and other stressors play a role in recent bee losses, the science (see Appendix A) identifies the neonicotinoid class of chemicals as a prime suspect. Most recently, the European Union in May announced a two-year restriction across the continent on the use of these insecticides, citing the risks to bee health and the abundance of scientific literature on the issue. In the U.S., federal agencies, including the U.S. Environmental Protection Agency (EPA) and the U.S. Department of Agriculture (USDA), have initiated interagency review of these insecticides and have not ruled them out as major contributing factors to honey bee decline.

Beyond Pesticides is a plaintiff in a lawsuit that challenges EPA's inaction on the science and identifies statutory grounds for the suspension of the neonicotinoids - clothianidin and thiamethoxam, because of their overwhelming contribution to the decline of honey bee and colony health. To this end, we also

[1] Bee Informed. 2013. Preliminary Results: Honey Bee Colony Losses in the United States, Winter 2012-2013. Available at http://beeinformed.org/2013/05/winter-loss-survey-2012-2013/

urge the Washington State Department of Agriculture (WSDA) to use its authority under state law - based on the science and the failure of the federal registration of neonicotinoids to meet basic honey bee safety standards - to suspend the use of neonicotinoids to protect honey bee health and agriculture in the state of Washington.

What the Science Shows

A. <u>Neonicotinoids are toxic to beees</u>

Neonicotinoids, like imidacloprid and clothianidin, have sublethal effects in honey bees, which include disruptions in mobility, navigation, and feeding behavior.[2] Lethal and sublethal exposures have been shown to decrease foraging activity, along with olfactory learning performance and decreased hive activity.[3] Bees living and foraging near agricultural fields are exposed as a result of multiple mechanisms throughout the spring and summer, and are exposed to foliar and systemic pesticides that studies are reporting cause feeding, navigation and learning behavior disruptions in bees. In fact, a 2013 study reports that sublethal doses of imidacloprid have cytotoxic effects on bee brains and that optic lobes are more sensitive to the insecticide than other regions of the brain of these insects.[4] In a study looking at the acute effects of sublethal doses of clothianidin under field-like conditions at 0.05 -2 ng/bee, a significant reduction of foraging activity and longer foraging flights at doses of ≥0.5 ng/bee during the first three hours after treatment were recorded. [5] A study by Yang et al. reports that honey bees exposed to sublethal doses of imidacloprid show abnormalities in revisiting the feeding site, with some going missing. Returning bees also exhibit a delay in their return trips.[6] A University of California (San Diego) study observed that sublethal doses of imidacloprid induce neurological effects (impaired waggle dancing at colony) that reduce communication and feeding. According to the researchers, waggle dancing can significantly increase colony food intake, and thus a sublethal dose of imidacloprid (0.21 ng $bee^{-1)}$ may impair colony fitness.[7]

B. <u>Neonicotinoid residues contaminate the entire plant</u>

Neonicotinoids are systemic pesticides which mean that their residues are expressed in all parts of the plant, including leaves, pollen and nectar. A 2012 study by entomologist Christian Krupke, PhD, of Purdue University, clarifies some of the mechanisms by which honey bees are exposed to neonicotinoid pesticides. According to the study, in addition to agricultural sources, pesticide residues are found in pollen collected by bees and stored in the hive, in the soil of fields sampled, including unplanted fields, and in other flowering plants (dandelions). According to Dr. Krupke, clothianidin in/on the dandelions could have resulted from translocation from the soil to the flower, from surface contamination of the flowers from dust, or a combination of these two mechanisms.[8] Guttated water of seed-treated plants, which provides a source of water for bees, can also be a source of contamination and exposure.[9] Reetz et al. finds that corn seeds treated with clothianidin resulted in neonicotinoid concentrations up to

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[2] Desneaux, N. et al., 2007. Sublethal Effects of Pesticides on Benefical Anthropods. Annual Review of Entomology, 52:81-106

[3] Decourtye, A. et al., 2004. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicology and Environmental Safety.57: 410-419

[4] de Almeida Rossi C, Roat TC, Tavares DA, Cintra-Socolowski P, Malaspina O. 2013. Brain Morphophysiology of Africanized Bee Apis mellifera Exposed to Sublethal Doses of Imidacloprid. *Arch Environ Contam Toxicol.* DOI 10.1007/s00244-013-9897-1

[5] Schneider CW, Tautz J, Grünewald B, and Fuchs S. 2012. RFID Tracking of Sublethal Effects of Two Neonicotinoid Insecticides on the Foraging Behavior of Apis mellifera. *PLoS One:*7(1):e30023.

[6]Yang, E. C., Chuang, Y. C., Chen, Y. L. and Chang, L. H. 2008. Abnormal Foraging Behavior Induced by Sublethal Dosage of Imidacloprid in the Honey Bee (Hymenoptera: Apidae). *J Economic Entomology.* 101 (6), 1743-1748

[7] Eiri, D.M. and Nieh, J. C. 2012. A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing *J Exp Biol* 215:2022-2029

[8] Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K. 2012. Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields. *PLoS ONE* 7(1): e29268. doi:10.1371/journal.pone.0029268

[9] *Hoffmann, E and Castle, S. 2012. Imidacloprid in Melon Guttation Fluid: A Potential Mode of Exposure for Pest and Beneficial Organisms. J Economic Entomology 105(1):67-71.*

8,000 ng/ mL in the guttated fluid.[10] Guttation drops from plants obtained from commercial seeds coated with thiamethoxam, clothianidin, imidacloprid and fipronil taken from young plants contained high levels of the neonicotinoid insecticides: up to 346 mg/L for imidacloprid, 102 mg/L for clothianidin, and 146 mg/L for thiamethoxam, according to a 2011 study.[11] These residues remain and can expose foraging bees to significant levels of the insecticide.

C. Exposures to neonicotinoids leads to higher susceptibility to pathogens and parasites

Studies have also reported that bees exposed to sublethal does of pesticides are highly susceptible to pathogens that lead to their decline. One 2012 study by USDA researchers discovered that newly emerged bees exposed to sublethal levels of imidacloprid during larval development and indirectly from brood food from nurse bees had higher levels of the gut parasite *Nosema* spp. which is known to adversely affect colony health.[12] According to the study, this suggests that being exposure to pesticides contributes to weakening bees by making them more susceptible to infection. Alaux, et al. reported that the combination of both imidacloprid and *Nosema* caused the highest individual mortality rates and energetic stress, suggesting a synergistic interaction between these agents and, in the long term, a higher susceptibility of the colony to pathogens.[13] Similarly, Vidau, et al observed a significant increase in honeybee mortality when *Nosema ceranae*-infected honeybees were exposed to sublethal doses of insecticides.[14]

D. Neonicotinoids also harm other beneficial organisms

Recent data also supports the harmful effects of neonicotinoids on other beneficial organisms. Imidacloprid residues in surface waters lead to a decline in macro-invertebrate abundance, according to a 2013 study.[15] This study notes that short-term tests with the aquatic worm *Lumbriculus variegatus,* a high mortality was observed at the highest concentrations of imidacloprid in the sediments (1 to 5 mg/kg). At lower concentrations (0.05 to 0.5 mg/kg) effects were observed on growth and behavior of *L. variegatus*. In other tests with the aquatic invertebrates *Chironomus tentans* and *Hyallella Azteca,* chronic low-level exposure (>1.14 $\mu g\ l^{-1}$ for *C. tentans*) to imidacloprid reduced the species survival and growth. Imidacloprid has also been observed to be lethal to earthworms,[16] with larger consequences for soil health and fertility.

Other bees such as bumble bees saw a significant reduction in growth rate and a reduction in the production of new queens when exposed to environmentally relevant levels of imidacloprid.[17] One study observed that bumble bee micro-colonies exhibited a dose-dependent decline in fecundity, with a 33% reduction in brood production environmentally realistic dosages of imidacloprid.[18]

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[10] Reetz, J. et al. 2011. Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees? *Apidologie* 42(5): 596-606.

[11] Tapparo, A. et al. 2011. Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds. *J Environ Monitoring* 13(6): 1564-1568.

[12] Pettis JS, vanEngelsdorp D, Johnson J, Dively G. 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema. Naturwissenschaften.* 99(2):153-8.

[13] Alaux, C. et al. 2010. Interactions between Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environmental Microbiology*. 12(3):774-782

[14] Vidau C, Diogon M, Aufauvre J, Fontbonne R, Viguès B, et al. 2011.Exposure to Sublethal Doses of Fipronil and Thiacloprid Highly Increases Mortality of Honeybees Previously Infected by *Nosema ceranae. PLoS ONE* 6(6): e21550.

[15] Van Dijk, T, Van Staalduinen, M, and Van der Sluijs, J. 2013. Macro-Invertebrate Decline in Surface Water Polluted with Imidacloprid. PLoS ONE 8(5): e62374. doi:10.1371/journal.pone.0062374

[16] Bhattacharya, A; SAHU, Sanjat K. 2013. Environmental impact of Imidacloprid on soil fertility : a case study on Drawida willsi earthworm. *The Clarion*, [S.l.], v. 2, n. 1, p. 21-29.

[17] Whitehorn PR, O'Connor S, Wackers FL, Goulson D. 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science,* 20;336(6079):351-2

[18] Laycock I, Lenthall KM, Barratt AT, Cresswell JE. 2012. Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (Bombus terrestris). *Ecotoxicology*;21(7):1937-45.

The growing use of neonicotinoid in the agricultural and residential sectors has the potential to have catastrophic effects of bee populations. Beekeepers in Washington have been observing higher than averages bee losses. While a restriction on ornamental uses of neonicotinoids may have some effect in protecting bees within non-agricultural foraging areas, the scientific literature supports even broader action to address the impacts of the agricultural use of neonicotinoids (treated seeds) on bees and by extension the state's agricultural economy, especially the impacts on the state's major pollinator-dependent crops such as apples, cherries and peaches.

WSDA is charged with protecting the agricultural economy of the state and should take action as is necessary to ensure its productivity. We encourage WSDA to move forward and implement restrictions of neonicotinoid use in Washington in order to protect the state's bees, beekeeping industry and agriculture.

Respectfully,

Jay Feldman
Executive Director
Beyond Pesticides

<u>Appendix A</u>

Growing Body of Evidence Implicates Pesticides in Declining Bee Health
The following is a sampling of the science that we wish to draw to the WSDA's attention:

2013 European Food Safety Authority Report on Neonicotinoids & Risk Assessment for Bees
This report, presented by the European Food Safety Authority (EFSA), finds that the neonicotinoid class of insecticides poses unacceptable hazards to bees. They concluded that certain crops treated with neonicotinoid chemicals -imidacloprid, clothianidin and thiamethoxam- are of "critical concern" for bee health. The report prompted the European Commission to push EU nations to impose a two year suspension on crops that are attractive to bees, particularly, sunflowers, rapeseed, corn, cotton, and cereal crops.

Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees
Williamson, S and Wright, G. 2013. J Exp Biol 216, 1799-1807
Study reported that prolonged exposure to field-realistic concentrations of the neonicotinoid imidacloprid and the organophosphate acetylcholinesterase inhibitor coumaphos and their combination impairs olfactory learning and memory formation in the honeybee. Bees exposed to imidacloprid, coumaphos, or a combination of these compounds, were less likely to express conditioned proboscis extension towards an odor associated with reward. Bees exposed to imidacloprid were less likely to form a long-term memory.

Bee Health: The Role of Pesticides
Congressional Research Service Report for Congress, 2012
This report, developed by the Congressional Research Service, provides information regarding: 1. changes to managed and wild bee populations- indicating information is limited; 2. factors that are documented to impact bee health including pesticides, pests and diseases, diet and nutrition, genetics, habitat loss, and beekeeper issues, highlighting that there are multiple exposure pathways that may work synergistically; 3. scientific research on the role of pesticides; and finally 4. current research and regulatory activity regarding neonicotinoids, a highly controversial neurotoxin impacting bees.

Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees
Palmer MJ, Moffat C, et al. 2013. Nat Commun; 4:1634.
This study shows that honeybee exposure to two widely used classes of pesticide, neonicotinoid and organophosphate miticides, leads to a shutdown of neurons so that the lobes of the brain fail to communicate with each other. These effects are observed at concentrations that are encountered by foraging honeybees and within the hive, and are additive with combined application.

Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo
Hatjina, F. at al. 2013. Apidologie 44(4):467-480
Study investigated sublethal effects of imidacloprid on the development of the hypopharyngeal glands (HPGs) and respiratory rhythm in honeybees fed under laboratory conditions. The acini, the lobes of the HPGs of imidacloprid-treated honeybees, were 14.5 % smaller in diameter in 9-day-old honeybees and 16.3 % smaller in 14-day-old honeybees than in the same-aged untreated honeybees. midacloprid also significantly affected the bursting pattern of abdominal ventilation movements (AVM) by causing a 59.4 % increase in the inter-burst interval and a 56.99 % decrease in the mean duration of AVM bursts.

Combined pesticide exposure severely affects individual- and colony-level traits in bees
Gill RJ, Ramos-Rodriguez O, Raine NE. 2012. Nature ; 491(7422):105-8.
Researchers show that chronic exposure of bumblebees to two pesticides (neonicotinoid and pyrethroid) at concentrations that could approximate field-level exposure impairs natural foraging

behavior and increases worker mortality leading to significant reductions in brood development and colony success. They also found that worker foraging performance, particularly pollen collecting efficiency, was significantly reduced with observed knock-on effects for forager recruitment, worker losses and overall worker productivity.

Reduction of homing flights in the honey bee Apis mellifera after a sublethal dose of neonicotinoid insecticides

Matsumoto, T. 2012. Bulletin of Insectology 66 (1): 1-9, 2013

Researchers dosed honey bees with sublethal doses four pesticides and released them in the field to determine their success in returning to their hives. The bees were treated with two neonicotinoids (clothianidin and dinotefuran), one pyrethroid (etofenprox) and one organophosphate (fenitrothion) at five different doses. Then the bees were released 500 m from their hives in the field. The proportion of successful homing flights by bees exposed to neonicotinoids and pyrethroid decreased with doses one tenth of their median lethal dose (LD50) or more and one-fourth of their LD50 or more respectively, though the bees exposed to organophosphate did not seem to respond with any declines in homing. The research has implications for the survival of honey bee hives after worker bee exposures.

A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing

Eiri, D.M. and Nieh, J. C. 2012. J Exp Biol 215:2022-2029

University of California at San Diego biologists found in their experiments that honey bees treated with a small, single dose of imidacloprid, comparable to what they would receive in nectar, became "picky eaters." "In other words, the bees preferred to only feed on sweeter nectar and refused nectars of lower sweetness that they would normally feed on and that would have provided important sustenance for the colony," according to author Daren Eiri. "In addition, bees typically recruit their nestmates to good food with waggle dances, and we discovered that the treated bees also danced less." The researchers point out that honey bees that prefer only very sweet foods can dramatically reduce the amount of resources brought back to the colony. Further reductions in their food stores can occur when bees no longer communicate to their kin the location of the food source. "Exposure to amounts of pesticide formerly considered safe may negatively affect the health of honey bee colonies," said lead researcher James Nieh, PhD.

Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production

Whitehorn, P, O'Connor, S, Wackers,F, and Goulson, D. 2012. Science.336 (6079), 351-352

Researchers exposed colonies of the bumble bees to levels of imidacloprid that are realistic in the natural environment, then allowed them to develop naturally under field conditions. Treated colonies had a significantly reduced growth rate and suffered an 85% reduction in production of new queens compared to unexposed control colonies. The study is particularly noteworthy because it shows that bumble bees, which are wild pollinators, are suffering similar impacts of pesticide exposure to "managed" honey bees. While several studies have linked pesticides to declining honey bee health, wild pollinators have not been intensively studied as their economically-relevant cousins. That said, wild pollinators still provide essential services both in agriculture and to a wide-range of wild plants that could not survive without insect pollination.

A Common Pesticide Decreases Foraging Success and Survival in Honey Bees

Henry, M, et al. 2012. Science. 336 (6079), 348-350

In their study, the researchers used Radio-frequency identification (RFID) to test the hypothesis that a sublethal exposure to a neonicotinoid indirectly increases hive death rate through homing failure in foraging honey bees. When exposed to sublethal doses of thiamethoxam, at levels present in the environment, honey bees were less likely to return to the hive after foraging than control bees that were tracked with RFID, but not intentionally dosed with pesticides. Higher risks are observed when the homing task is more challenging. The survival rate is even lower when exposed bees are placed in foraging areas with which they are less familiar.

Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*)

Laycock I, Lenthall KM, Barratt AT, Cresswell JE. 2012. Ecotoxicology. DOI: 10.1007/s10646-012-0927-y

To determine whether environmentally realistic levels of imidacloprid are capable of making a demographic impact on bumble bees, researchers exposed queenless microcolonies of worker bumble bees, *Bombus terrestris*, to a range of dosages of dietary imidacloprid between zero and 125 µg L(-1) and examined the effects on ovary development and fecundity. Microcolonies showed a dose-dependent decline in fecundity, with environmentally realistic dosages in the range of 1 µg L(-1) capable of reducing brood production by one third. In contrast, ovary development was unimpaired by dietary imidacloprid except at the highest dosage. Imidacloprid reduced feeding on both syrup and pollen but, after controlling statistically for dosage, microcolonies that consumed more syrup and pollen produced more brood. The detrimental effects of imidacloprid on fecundity emerge principally from nutrient limitation imposed by the failure of individuals to feed. Findings raise concern about the impact of neonicotinoids on wild bumble bee populations. Authors recognize that to fully evaluate impacts on wild colonies it will be necessary to establish the effect of dietary neonicotinoids on the fecundity of bumble bee queens.

Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds

Tapparo,A, Marton,D et al. 2012. Environmental Science & Technology .46 (5): 2592-2599

The findings of an Italian Research team confirm that high amounts of neonicotinoids are present in the exhaust of corn seed planters and that bees are exposed to these potentially lethal concentrations of the chemical simply by flying through the area during planting. In analyzing bees that were induced to fly through planter exhaust in a field that was being planted, the team found that there were high concentrations of clothianidin and thiamethoxam in and on the bees themselves. These concentrations were “significantly higher” than known lethal doses of the chemicals for honeybees, according to the researchers. The study included analyses of several different kinds of seed coatings as well as seed planting machines, but the team found that no modifications to seeds or planters resulted in any significant decrease in the emission of chemical dust or the contamination levels of the bees themselves.

Multiple routes of pesticide exposure for honey bees living near agricultural fields.

Krupke CH, Hunt GJ, et al. 2012. PLoS One. 7(1): e29268.

Study analyzed samples of honey bees, pollen stored in the hive and several potential exposure routes associated with plantings of neonicotinoid treated maize. Results demonstrate that bees are exposed to these compounds and several other agricultural pesticides in several ways throughout the foraging period. During spring, extremely high levels of clothianidin and thiamethoxam were found in planter exhaust material produced during the planting of treated maize seed. Study also found neonicotinoids in the soil of each field we sampled, including unplanted fields. Plants visited by foraging bees (dandelions) growing near these fields were found to contain neonicotinoids as well. This indicates deposition of neonicotinoids on the flowers, uptake by the root system, or both. Dead bees collected near hive entrances during the spring sampling period were found to contain clothianidin as well. Study also detected the insecticide clothianidin in pollen collected by bees and stored in the hive. When maize plants in our field reached anthesis, maize pollen from treated seed was found to contain clothianidin and other pesticides; and honey bees in our study readily collected maize pollen. These findings clarify some of the mechanisms by which honey bees may be exposed to agricultural pesticides throughout the growing season.

Learning impairment in honey bees caused by agricultural spray adjuvants.

Ciarlo TJ, Mullin CA, Frazier JL, Schmehl DR. 2012. PLoS One. 7(7):e40848.

Olfactory learning is important for foraging honey bees because it allows them to exploit the most productive floral resources in an area at any given time. Impairment of this learning ability may have serious implications for foraging efficiency at the colony level, as well as potentially many social interactions. Three different adjuvant classes (nonionic surfactants, crop oil concentrates, and organosilicone surfactants) were investigated in this study. Learning was impaired after ingestion of 20 µg organosilicone surfactant, indicating harmful effects on honey bees caused by agrochemicals previously believed to be innocuous. Organosilicones were more active than the nonionic adjuvants, while the crop oil concentrates were inactive. Ingestion was required for the tested adjuvant to have an effect on learning, as exposure via antennal contact only induced no level of impairment. Study concludes, organosilicone spray adjuvants may therefore contribute to the ongoing global decline in honey bee health.

Development of biomarkers of exposure to xenobiotics in the honey bee Apis mellifera: Application to the systemic insecticide thiamethoxam.

Badiou-Bénéteau A et al. 2012. Ecotoxicol Environ Saf. 82:22-31.

This study describes the development of acetylcholinesterase (AChE), carboxylesterases (CaE1, CaE2, CaE3), glutathion-S-transferase (GST), alkaline phosphatase (ALP) and catalase (CAT) as enzyme biomarkers of exposure to xenobiotics such as thiamethoxam in the honey bee *Apis mellifera*. The biomarker responses revealed that, even at the lowest dose used, exposure to thiamethoxam elicited sublethal effects and modified the activity of CaEs, GST, CAT and ALP. The researchers consider that the profile of biomarker variation obtained in this study could represent a useful fingerprint to characterize exposure to thiamethoxam in the honey bee A. mellifera. This battery of honey bee biomarkers might be a promising option to biomonitor the health of aerial and terrestrial ecosystems and to generate valuable information on the modes of action of pesticides.

Honey bees (Apis mellifera) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to Nosema (Microsporidia) infection.

Wu JY, et al. 2012. J Invertebr Pathol. 109(3):326-9.

Nosema ceranae and pesticide exposure can contribute to honey bee health decline. This study used bees reared from brood comb containing high or low levels of pesticide residues and placed them in two common colony environments. One colony was inoculated weekly with N. ceranae spores in sugar syrup and the other colony received sugar syrup only. Worker honey bees were sampled weekly from the treatment and control colonies and analyzed for *Nosema* spore levels. Regardless of the colony environment, a higher proportion of bees reared from the high pesticide residue brood comb became infected with N. ceranae, and at a younger age, compared to those reared in low residue brood combs. The data suggest that developmental exposure to pesticides in brood comb increases the susceptibility of bees to N. ceranae infection.

Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema.

Pettis JS, vanEngelsdorp D, Johnson J, Dively G. 2012. Naturwissenschaften. 99(2):153-8.

Researchers exposed honey bee colonies during three brood generations to sub-lethal doses of a widely used pesticide, imidacloprid, and then subsequently challenged newly emerged bees with the gut parasite, *Nosema* spp. The pesticide dosages used were below levels demonstrated to cause effects on longevity or foraging in adult honey bees. *Nosema* infections increased significantly in the bees from pesticide-treated hives when compared to bees from control hives demonstrating an indirect effect of pesticides on pathogen growth in honey bees. We clearly demonstrate an increase in pathogen growth within individual bees reared in colonies exposed to one of the most widely used pesticides worldwide, imidacloprid, at below levels considered harmful to bees. The finding that individual bees with undetectable levels of the target pesticide, after being reared in a sub-lethal pesticide environment within the colony, had higher *Nosema* is significant.

RFID Tracking of Sublethal Effects of Two Neonicotinoid Insecticides on the Foraging Behavior of Apis mellifera.

Schneider CW, Tautz J, Grünewald B, Fuchs S. 2012. PLoS One. 2012;7(1):e30023
Study tested an experimental design using the radiofrequency identification (RFID) method to monitor the influence of sublethal doses of insecticides on individual honeybee foragers on an automated basis. In this experimental approach the authors monitored the acute effects of sublethal doses of the neonicotinoids imidacloprid (0.15-6 ng/bee) and clothianidin (0.05-2 ng/bee) under field-like circumstances. At field-relevant doses for nectar and pollen no adverse effects were observed for either substance. Both substances led to a significant reduction of foraging activity and to longer foraging flights at doses of ≥0.5 ng/bee (clothianidin) and ≥1.5 ng/bee (imidacloprid) during the first three hours after treatment. This study demonstrates that the RFID-method is an effective way to record short-term alterations in foraging activity after insecticides have been administered once, orally, to individual bees.

Exposure to Sublethal Doses of Fipronil and Thiacloprid Highly Increases Mortality of Honeybees Previously Infected by *Nosema ceranae*

Vidau C, Diogon M, Aufauvre J, Fontbonne R, Viguès B, et al. 2011. PLoS ONE 6(6): e21550.
This study explores the effect of *Nosema ceranae* infection on honeybee sensitivity to sublethal doses of the insecticides fipronil and thiacloprid. Honeybee mortality and insecticide consumption were analyzed daily and the intestinal spore content was evaluated 20 days after infection. A significant increase in honeybee mortality was observed when *N. ceranae*-infected honeybees were exposed to sublethal doses of insecticides. Analysis of the honeybee detoxification system 10 days p.i. showed that *N. ceranae* infection induced an increase in glutathione-S-transferase activity in midgut and fat body. The synergistic effect of *N. ceranae* and insecticide on honeybee mortality, however, did not appear strongly linked to a decrease of the insect detoxification system. These data support the hypothesis that the combination of the increasing prevalence of *N. ceranae* with high pesticide content in beehives may contribute to colony depopulation.

Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication

Girolami, V. et al. 2011. J. Appl. Entomol. 136:17–26.
The effect of direct aerial powdering was tested on foragers in free flight near the drilling machine. Bees were conditioned to visit a dispenser of sugar solution whilst a drilling machine was sowing corn along the flight path. Samples of bees were captured on the dispenser, caged and held in the laboratory. Chemical analysis showed some hundred nanograms of insecticide per bee. Nevertheless, caged bees, previously contaminated in flight, died only if kept in conditions of high humidity. After the sowing, an increase in bee mortality in front of the hives was also observed. Spring bee losses, which corresponded with the sowing of corn-coated seed, seemed to be related to the casual encountering of drilling machine during foraging flight across the ploughed fields.

Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees?

Reetz, J. et al. 2011. Apidologie 42(5): 596-606.
This experiment demonstrates that guttated water of plants germinated from seeds dressed with neonicotinoids contains neonicotinoids. Maize seeds treated with clothianidin (Poncho® 0.5 mg/seed and Poncho Pro® 1.25 mg/seed) resulted in neonicotinoid concentrations up to 8,000 ng/mL in the guttated fluid. This concentration decreases rapidly, but remained detectable over several weeks. Seeds treated with Poncho Pro® did not result in higher concentrations in guttated droplets in the first stages of plant development, but the concentration decreased more slowly. Triticale seed treated with imidacloprid contained small quantities of this active agent (up to 13 ng/mL) in the guttated fluid the following spring after overwintering.

Sub-Lethal Effects of Pesticide Residues in Brood Comb on Worker Honey Bee (*Apis mellifera*) Development and Longevity

Wu JY, Anelli CM, Sheppard WS. 2011. PLoS ONE 6(2): e14720. doi:10.1371/journal.pone.0014720

Researchers conducted studies to examine possible direct and indirect effects of pesticide exposure from contaminated brood comb on developing worker bees and adult worker lifespan. Results demonstrate sub-lethal effects on worker honey bees from pesticide residue exposure from contaminated brood comb. Sub-lethal effects, including delayed larval development and adult emergence or shortened adult longevity, can have indirect effects on the colony such as premature shifts in hive roles and foraging activity.

Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds

Tapparo, A. et al. 2011. J Environ Monitoring 13(6): 1564-1568.

In the present study, guttation drops of corn plants obtained from commercial seeds coated with thiamethoxam, clothianidin, imidacloprid and fipronil have been analyzed. The young plants grown both in pots – in greenhouse – and in open field from coated seeds, produced guttation solutions containing high levels of the neonicotinoid insecticides (up to 346 mg L-1 for imidacloprid, 102 mg L-1 for clothianidin and 146 mg L-1 for thiamethoxam). These concentration levels may represent lethal doses for bees that use guttation drops as a source of water. The neonicotinoid concentrations in guttation drops progressively decrease during the first 10–15 days after the emergence of the plant from the soil. Otherwise fipronil, which is a non-systemic phenylpyrazole insecticide, was never detected into guttation drops. Current results confirm that the physiological fluids of the corn plant can effectively transfer neonicotinoid insecticides from the seed onto the surface of the leaves, where guttation drops may expose bees and other insects to elevated doses of neurotoxic insecticides.

Abnormal Foraging Behavior Induced by Sublethal Dosage of Imidacloprid in the Honey Bee (Hymenoptera: Apidae)

Yang, E. C., Chuang, Y. C., Chen, Y. L. and Chang, L. H. 2008. J Economic Entomology. 101 (6), 1743-1748

Synopsis: important issue, they are attracting more and more attention lately. It has been demonstrated that low dosages of the neonicotinoid insecticide imidacloprid may affect honey bee, *Apis mellifera L.*, behavior. In this article, the foraging behavior of the honey bee workers was investigated to show the effects of imidacloprid. Results demonstrated that sublethal dosages of imidacloprid were able to affect foraging behavior of honey bees.

Pesticides and honey bee toxicity – USA

Johnson, R, M et al. 2010. Apidologie 41 (3) 312-331

This review examines pesticides applied to crops, pesticides used in apiculture and pesticide residues in hive products. Authors discuss the role that pesticides and their residues in hive products may play in colony collapse disorder and other colony problems. Although no single pesticide has been shown to cause colony collapse disorder, the additive and synergistic effects of multiple pesticide exposures may contribute to declining honey bee health.

High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health

Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, et al. 2010. PLoS ONE 5(3): e9754. doi:10.1371/journal.pone.0009754

Recent declines in honey bees for crop pollination threaten fruit, nut, vegetable and seed production in the United States. A broad survey of pesticide residues was conducted on samples from migratory and other beekeepers across 23 states, one Canadian province and several agricultural cropping systems during the 2007–08 growing seasons. The 98 pesticides and metabolites detected in mixtures up to 214 ppm in bee pollen alone represent a remarkably high level for toxicants in the brood and adult food of this primary pollinator. This represents over half of the maximum individual pesticide incidences ever reported for apiaries. While exposure to many of these neurotoxicants elicits acute and sublethal reductions in honey bee fitness, the effects of these materials in combinations and their direct association with CCD or declining bee health remains to be determined.

Effects of sublethal concentrations of bifenthrin and deltamethrin on fecundity, growth, and development of the honeybee Apis mellifera ligustica

Dai, P, Wang, Q, et al. 2009. Environl Tox. Chem. 29(3) : 644–649

Bifenthrin and deltamethrin have been widely used as pesticides in agriculture and forestry and are becoming an increasing risk to honeybees. The honeybee, *Apis mellifera ligustica*, is widely recognized as a beneficial insect of agronomic, ecological, and scientific importance. It is important to understand what effects these chemicals have on bees. Effects of two pesticides at sublethal concentrations on fecundity, growth, and development of honeybees were examined with the feeding method for a three-year period (2006–2008). It was shown that both bifenthrin and deltamethrin significantly reduced bee fecundity, decreased the rate at which bees develop to adulthood, and increased their immature periods. The toxicity of bifenthrin and deltamethrin on workers of *Apis mellifera* ligustica was also assessed, and the results from the present study showed that the median lethal effects of bifenthrin and deltamethrin were 16.7 and 62.8?mg/L, respectively.

Synergistic Interactions Between In-Hive Miticides in Apis mellifera

Johnson,R, Pollock, H.S. and Berenbaum, M. 2009. J Economic Entomology 102(2):474-479.

The varroa mite, Varroa destructor Anderson & Trueman, is a devastating pest of honey bees, Apis mellifera L. that has been primarily controlled over the last 15 yr with two in-hive miticides: the organophosphate coumaphos (Checkmite+), and the pyrethroid tau-fluvalinate (Apistan). In this laboratory study, the authors observed a large increase in the toxicity of tau-fluvalinate to 3-day-old bees that had been treated previously with coumaphos, and a moderate increase in the toxicity of coumpahos in bees treated previously with tau-fluvalinate. The observed synergism may result from competition between miticides. These results suggest that honey bee mortality may occur with the application of otherwise sublethal doses of miticide when tau-fluvalinate and coumaphos are simultaneously present in the hive.

Translocation of Neonicotinoid Insecticides From Coated Seeds to Seedling Guttation Drops: A Novel Way of Intoxication for Bees

Girolami, V.; Mazzon, L.; Squartini, A. et al. 2009. J Economic Entomology, 102(5): 1808-1815(8)

The death of honey bees, Apis mellifera L., and the consequent colony collapse disorder causes major losses in agriculture and plant pollination worldwide. The phenomenon showed increasing rates in the past years, although its causes are still awaiting a clear answer. Here, we show that leaf guttation drops of all the corn plants germinated from neonicotinoid-coated seeds contained amounts of insecticide constantly higher than 10 mg/l, with maxima up to 100 mg/l for thiamethoxam and clothianidin, and up to 200 mg/l for imidacloprid. The concentration of neonicotinoids in guttation drops can be near those of active ingredients commonly applied in field sprays for pest control, or even higher. When bees consume guttation drops, collected from plants grown from neonicotinoid-coated seeds, they encounter death within few minutes.

Interactions between Nosema microspores and a neonicotinoid weaken honeybees (Apis mellifera)

Alaux, C. et al. 2010. Environmental Microbiology. 12(3):774-782

Global pollinators, like honeybees, are declining in abundance and diversity, which can adversely affect natural ecosystems and agriculture. The authors tested the current hypotheses describing honeybee losses as a multifactorial syndrome, by investigating integrative effects of an infectious organism and an insecticide on honeybee health. Study demonstrated that the interaction between the microsporidia *Nosema* and a neonicotinoid (imidacloprid) significantly weakened honeybees. In the short term, the combination of both agents caused the highest individual mortality rates and energetic stress. This provides the first evidences that interaction between an infectious organism and a chemical can also threaten pollinators, interactions that are widely used to eliminate insect pests in integrative pest management.

Toxicities of fipronil enantiomers to the honeybee Apis mellifera L. and enantiomeric compositions of

fipronil in honey plant flowers.
Li X, Bao C, et al. 2010. Environ Toxicol Chem. 29(1):127-32.
Fipronil is a chiral phenylpyrazole insecticide that is effective for control of a wide range of agricultural and domestic pests at low application rates. Wide application of fipronil also causes poisoning of some nontarget insects, such as honeybees. In the present study, toxicities of fipronil enantiomers and racemate to the honeybee *Apis mellifera* L. were determined to examine whether using formulations of single or enriched fipronil enantiomer is a possible option to reduce risks to bees. The results indicate that it is unlikely that use of formulations with single or enriched fipronil enantiomer would reduce the risk that fipronil poses to honeybees. Improved fipronil application practices (based on safest timing and bloom conditions) and reduction of overall fipronil usage seem to be more realistic options.
Is Apis mellifera more sensitive to insecticides than other insects?
Hardstone, M.C and Scott, JG. 2010. Pest Manag Sci. 66(11):1171-80.
The goal of this review was to summarize insecticide toxicity data between A. mellifera and other insects to determine the relative sensitivity of honey bees to insecticides. It was found that, in general, honey bees were no more sensitive than other insect species across the 62 insecticides examined. In addition, honey bees were not more sensitive to any of the six classes of insecticides (carbamates, nicotinoids, organochlorines, organophosphates, pyrethroids and miscellaneous) examined. While honey bees can be sensitive to individual insecticides, they are not a highly sensitive species to insecticides overall, or even to specific classes of insecticides. However, all pesticides should be used in a way that minimizes honey bee exposure, so as to minimize possible declines in the number of bees and/or honey contamination.
Exposure to pesticides at sublethal level and their distribution within a honey bee (Apis mellifera) colony.
Smodis Skerl MI, Kmecl V, Gregorc A.2010. Bull Environ Contam Toxicol. 85(2):125-8.
Honey bee colonies were exposed to pesticides used in agriculture or within bee hives by beekeepers: coumaphos; diazinon; amitraz or fluvalinate. Samples of bee workers, larvae and royal jelly were analysed. Amitraz residues in all sampled material were below the level of detection of 10 ng/g. Diazinon was not detected in any of the analyzed samples. The large quantities of fluvalinate found in bee heads and larvae, the coumaphos residues in royal jelly, and additional potential sub-lethal effects on individual honey bees or brood are discussed.
The relevance of sublethal effects in honey bee testing for pesticide risk assessment.
Thompson HM and Maus C. 2007. Pest Manag Sci. 63(11):1058-61.
The option of an evaluation and assessment of possible sublethal effects of pesticides on bees has been a subject of discussion by scientists and regulatory authorities. Effects considered included learning behaviour and orientation capacity. This paper considers whether and, if so, how sublethal effects should be incorporated into risk assessment, by addressing a number of questions: The authors conclude that sublethal studies may be helpful as an optional test to address particular, compound-specific concerns, as a lower-tier alternative to semi-field or field testing, if the effects are shown to be ecologically relevant. However, available higher-tier data (semi-field, field tests) should make any additional sublethal testing unnecessary, and higher-tier data should always override data of lower-tier trials on sublethal effects.
Is it possible to use the honey bee adult as a bioindicator for the detection of pesticide residues in plants?
Mansour SA.1987. Acta Biol Hung.38(1):69-76.
Pesticide residues are usually determined by physical, chemical and biological methods. Theoretically, any organism that is susceptible to a pesticide may be used for its bioassay in any environmental sample. This means that such organism may serve as a bioindicator for the detection of certain pollutants. The susceptibility of honey bees (Apis melifera L.) to many insecticides commonly used in

crop protection led to an attempt to use it as a bioindicator for the determination of residues of some insecticides in plant materials, as well as to detect toxicity hazards to honey bees of some commonly used insecticides. Results of this work which have been recently published may suggest "Yes" to answer the question posed in the title of this subject.

Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior.

Mommaerts V, Reynders S, et al.2010. Ecotoxicology. 19(1):207-15.

Bombus terrestris bumblebees are important pollinators of wild flowers, and in modern agriculture they are used to guarantee pollination of vegetables and fruits. In the field it is likely that worker bees are exposed to pesticides during foraging. Within the context of ecotoxicology and insect physiology, study reports the development of a new bioassay to assess the impact of sublethal concentrations on the bumblebee foraging behavior under laboratory conditions. In general, the experiments showed that concentrations that may be considered safe for bumblebees can have a negative influence on their foraging behavior. Therefore it is recommended that behavior tests should be included in risk assessment tests for highly toxic pesticides because impairment of the foraging behavior can result in a decreased pollination, lower reproduction and finally in colony mortality due to a lack of food.

Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior.

Aliouane Y, et al. 2009. Environ Toxicol Chem. 28(1):113-22.

Laboratory bioassays were conducted to evaluate the effects on honeybee behavior of sublethal doses of insecticides chronically administered orally or by contact. After exposure to fipronil, acetamiprid or thiamethoxam, behavioral functions of honeybees were tested on day 12. Fipronil, used at the dose of 0.1 ng/bee, induced mortality of all honeybees after one week of treatment. In the olfactory conditioning paradigm, fipronil-treated honeybees failed to discriminate between a known and an unknown odorant. Thiamethoxam by contact induced either a significant decrease of olfactory memory 24 h after learning at 0.1 ng/bee or a significant impairment of learning performance with no effect on memory at 1 ng/bee. The only significant effect of acetamiprid (administered orally, 0.1 microg/bee) was an increase in responsiveness to water. Data on the intrinsic toxicity of the compounds after chronic exposure have to be taken into account for evaluation of risk to honeybees in field conditions.

Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee Apis mellifera.

Decourtye A, Devillers J, et al. 2005. Arch Environ Contam Toxicol. 48(2):242-50.

Using a conditioned proboscis extension response (PER) assay, honeybees (Apis mellifera L.) can be trained to associate an odor stimulus with a sucrose reward. In the present study, the effects of sublethal concentrations of nine pesticides on learning performances of worker bees subjected to the PER assay were estimated and compared. Reduced learning performances were observed for bees surviving treatment with fipronil, deltamethrin, endosulfan, and prochloraz. A lack of behavioral effects after treatment with lambda-cyalothrin, cypermethrin, tau-fluvalinate, triazamate, and dimethoate was recorded.

Residues of Pesticides in honeybee (Apis mellifera carnica) bee bread and in pollen loads from treated apple orchards.

Smodis Skerl MI, et al. 2009. Bull Environ Contam Toxicol. 83(3):374-7.

Honey bee (Apis mellifera carnica) colonies were placed in two apple orchards treated with the insecticides diazinon and thiacloprid and the fungicide difenoconazole in accordance with a Protection Treatment Plan in the spring of 2007. Possible sub-lethal effects on individual honey bees and brood are discussed.

The Significance of the Druckrey-Küpfmüller Equation for Risk Assessment - The Toxicity of Neonicotinoid Insecticides to Arthropods is Reinforced by Exposure Time

Tennekes HA. 2010. Toxicology. 276(1):1-4.

The Druckrey-Küpfmüller equation explains why toxicity may occur after prolonged exposure to very low toxicant levels. Recently, similar dose-response characteristics have been established for the toxicity of the neonicotinoid insecticides imidacloprid and thiacloprid to arthropods. This observation is highly relevant for environmental risk assessment. Traditional approaches that consider toxic effects at fixed exposure times are unable to allow extrapolation from measured endpoints to effects that may occur at other times of exposure. Time-to-effect approaches that provide information on the doses and exposure times needed to produce toxic effects on tested organisms are required for prediction of toxic effects for any combination of concentration and time in the environment.