



Pesticide Action Network UK

Sub-lethal and chronic effects of neonicotinoids on bees and other pollinators

This factsheet summarises current knowledge about sub-lethal (i.e. non-fatal) effects of these insecticides and possible impacts of exposure to very low doses over time. It discusses the difficulties in extrapolating results which demonstrate harm in laboratory and semi-field studies to the reality in the field - one of the main controversies in the neonics debate – and implications of the latest research findings.



Credit: Graham White

Subtle but important effects from doses that do not kill bees outright

Sub-lethal toxicity to bees and other pollinators is the most likely exposure scenario in the field from neonicotinoid seed treatments^{1,2} because generally the concentrations that are found in pollen and nectar from seed-treated plants are too low to cause immediate

bee deaths from acute poisoning^{3,4}. However, lethally toxic levels in seed coating dust released at sowing stage have been documented in several countries (see Factsheet 1 on exposure routes) and found in stored pollen in some US hives⁵.

Sub-lethal effects reported in the scientific literature⁶ include a range of behavioural disturbances in honeybees:

- disorientation and difficulties in returning back to the hive (homing ability)
- reduced foraging and travel
- impaired memory and learning
- failure to communicate properly with nest mates

Other effects observed in the lab are delayed development time of bee larvae, reproductive problems and weakened immune systems (see Factsheet 3 on the links with bee disease). For example, imidacloprid is known to affect bees' cognitive behaviour such as the proboscis extension reflex, which relates to how adult workers respond to and remember tastes and odours^{7,8}. These may seem rather insignificant effects but play a critical role in the foraging effectiveness of the entire hive, which could then compromise its viability. Other neonicotinoids and the systemic insecticide fipronil have also been shown to affect bee odour memory and colour recognition in lab tests⁹.

Exposure to neonicotinoids by direct contact, via seed-coating dust or from residues contaminating bee-processed hive materials may cause sub-lethal impacts too. Rearing bee larvae on hive comb contaminated with neonicotinoids led to delayed development of the worker bees¹⁰.

Impaired honeybee health effects have been reported at extremely low doses of neonicotinoids, at concentrations of parts per billion (ppb)^{11, 12, 13, 14}. Abnormal behaviour was exhibited at levels 70 times below the levels causing mortality in standard tests (the LD50, the concentration at which 50% of exposed individuals die in lab toxicity tests)¹⁵.

Researchers on bee declines in Italy conclude that exposure to such doses



impairs a bee's ability to carry out its normal behaviour and hive functions. If exposure is repeated, this can lead to disorientation (inability to return to the nest) and/or death¹⁶.

Chronic exposure and colony level issues

Repeatedly ingesting very low doses of neonicotinoids over long periods (chronic exposure) can certainly end up killing individual bees^{17, 18}. Chronic poisoning at low doses can also cause immunodeficiency and increased susceptibility to diseases in honeybees¹⁹. Standard toxicity testing over a few days only will miss these effects.

Sub-lethal effects on substantial numbers of individual bees can build up to colony level harm, especially if exposure continues for weeks or longer (see also Box 1). Concentrations of pesticides that may be considered safe for bumblebees in terms of individual acute toxicity can have a negative influence on their foraging behaviour, leading to loss of worker survival and nest reproduction²⁰.



Credit: Graham White

Recent French research found that up to 32% of honeybees exposed to sub-lethal levels of the neonicotinoid thiamethoxam failed to return to the hive, effectively doubling the natural loss rate of foraging workers. For colonies living near treated crops where most of their foragers are exposed to neonicotinoids in nectar throughout the spring, these losses could

trigger a marked decline from which the colonies struggle to recover²¹. Similar conclusions about effects on foragers impairing the equilibrium of the entire colony and contributing to chronic weakness or colony decline are also drawn by Italian and US teams^{22,23}. They warn against underestimating the impact of sub-lethal effects yet these have never been properly assessed during government risk assessments (see Factsheet 3).

How do harmful effects observed in the lab relate to field reality?

Lab studies on mortality rates after chronic, sub-lethal exposure present conflicting results and conclusions. Some show mortality at chronic low doses, others did not observe this²⁷. These differences are probably due to variation in the test methods and experimental designs used and in the genetic make-up of different bee populations.

Extrapolating conclusions from lab studies to the field is the major uncertainty, causing controversy about neonicotinoid impacts on bees, especially from treated seed. The pesticide industry, the UK government regulators and some researchers stress that results from lab tests and trials confining individual insects or small colonies of pollinators in cages or tunnels (termed semi-field tests) do not prove that harmful effects actually occur in the field. They frequently point out field studies which fail to show significant harm and criticise lab studies using unrealistically high dose rates.

Box 1. Findings from pollinators other than honeybees

Far less is known about how pesticides may impact on other pollinator species. Tests on bumblebee workers have shown that imidacloprid at low doses can make them less inclined to forage, feed and take much longer time to build up the nest and travel. Strong sub-lethal effects on bumblebee nest reproduction were also noted²⁴. Sub-lethal effects from dietary intake of neonicotinoid has been documented for US species of solitary bees²⁵.

The recent study by Stirling University²⁶ showed that exposed bumblebee colonies had a significantly reduced growth rate and 85% reduction in queen production. Because only large bumblebee colonies produce queens, and only queens survive winter, these impacts could cause population level harm.

While it is true that some studies on neonicotinoid contamination in pollen or hive material have not shown effects on hive health in the field^(e.g. 28,29,30), the issue is complicated by the numerous methodological obstacles to getting clear, unambiguous results from field studies. The feeding, communication, reproduction, hygiene and immune response systems of social insects are highly complex and results can be confused by a wide variety of biological and geographic factors affecting the treated colonies and the untreated control colonies. It is therefore hard to control adequately for these factors in a full field test and be able to analyse the results statistically³¹. Field-level experiments can only give data on the specific hives used and the variability of the factors affecting them makes it hard to draw definitive conclusions that represent the wider situation. What floral resources are available? What levels of pesticide residues are in the pollen and nectar collected? Do the hives all display similar levels of disease and parasites which can affect hive health? Is there considerable mortality or abnormal behaviour observed in the control hives? All these factors can muddy the waters when trying to draw meaningful conclusions.



It's also a problem to prevent the bees from foraging on plants/fields not in the experiment, or to avoid unintended contamination of hives in the untreated controls³². Some studies were not able to guarantee that bees were foraging on contaminated food sources so they haven't actually proved the level of exposure. In one study with negative results³³ less than 15% of maize available to foragers was from the treated fields and bees often avoid maize if other pollen sources available. If bees don't forage much in the treatment plots, it is hard to assess the true effects of the treatment, much less to estimate potential impacts of hundreds of hectares of treated crop³⁴. With a 3km flying radius, a honeybee can forage over an area of 3,240 hectares but several field studies only covered 0.8 hectares of experimentally treated field – just 0.025% of the foraging range. Negative results from hives placed under these unrealistic settings^{35,36,37} need to be treated with caution³⁸.

“Although existing research has documented measurable sub-lethal effects, few field studies have been properly designed or conducted over a long enough period to assess the full risks to bees. Nevertheless, overall evidence points to the fact that neonics are harming bees.”

Xerces Society for Invertebrate Conservation, 2012



Credit: Graham White

The discrepancy between clear lab results and inconclusive or negative field results spurred one researcher to look more carefully at published field trials that have reported no effects on honey bees. Examination of the methods and designs used revealed that half the studies did not have enough statistical power to show sub-lethal effects within conventionally accepted levels of certainty³⁹.

Researchers would need much larger sample sizes to be able to conclude even the largest sub-lethal effects as 'significant' from these studies under scientific norms.

Field-relevant doses?

Some earlier studies clearly used neonicotinoid doses far higher than the average levels now known to contaminate pollen, nectar, hive materials and food stores. Advances in analytical equipment now enable researchers to measure and detect 'trace' levels of neonicotinoids (i.e. at 1-10 parts per billion). More recent studies do use environmentally-relevant doses, i.e. from within these low ppb trace level concentrations documented from field sampling. The Stirling study used pollen concentrations of 6ppb and sugar water concentrations of 0.7ppb representing the levels found in flowering oilseed rape⁴⁰. Strong sub-lethal effects on bumblebees, reduced lifespan and reproduction occurred at 10ppb levels of neonicotinoids and under^{41,42}.

While some studies only show sub-lethal effects at relatively high doses, impaired learning and orientation have been recorded at neonicotinoid doses as low as 0.1 nanogram (ng) per bee^{43,44}. Oral treatment of 0.3ng/bee of the systemic non- neonicotinoid fipronil reduced the number of foraging trips⁴⁵. French studies estimated that foragers can be exposed to as much as 0.6ng per day from sunflowers treated with imidacloprid⁴⁶. Working with data from lab and semi-field studies, Cresswell⁴⁷ estimated that field-realistic doses of imidacloprid in oilseed rape and sunflower could reduce expected performance in adult honey bees by 6-20%. Small effects on performance can ultimately result in declines in colony health⁴⁸.

It is important to consider that multiple exposures to sub-lethal levels may occur for several weeks if treated crop plants constitute the majority of a colony's nectar and pollen resources while in bloom^{49,50}. There is a huge potential exposure for many pollinator species to trace levels of neonicotinoids from treated crops and contaminated non-crop plants, soil and possibly water^{51,52}. An estimated 80 million honey and bumblebees in Britain could ingest neonicotinoids each year foraging on treated oilseed rape⁵³.

Implications of latest findings and blind spots in our understanding

Several recent studies add significant weight to the growing evidence for neonicotinoid effects at very low doses and run counter to the assurances from pesticide manufacturers and regulators

that neonicotinoids pose little or no risk in the field. The Stirling University is the first field study to show harm to bumblebees foraging and developing under natural conditions⁵⁴. Likewise, the French study on honeybees⁵⁵ was able to demonstrate homing failure in the field at realistic doses. The longer term impacts of their documented level of forager loss could mean hives would drop to 5,000 individuals, at the borderline of viability.

Recent US studies have revealed the widespread contamination by neonicotinoids and other pesticides of floral resources and deepened our understanding of other sources of exposure^{56,57}. Other research highlights interactions between different pesticides and bee susceptibility to diseases (discussed in Factsheet 3). The Xerces Society for Invertebrate Conservation report⁵⁸ gives a useful summary of what is known for sure, what can be inferred from research results and highlights 14 gaping holes in current knowledge. PAN North America's report 'Pesticides & Honey Bees: State of the Science'⁵⁹ is also a useful overview of the key research. In Factsheet 8 calls from researchers for better risk assessment and changes in pesticide policy and practice are described, along with other initiatives.

Key points

- The most likely neonicotinoid exposure scenario for pollinators from contaminated pollen and nectar is sub-lethal effects from repeatedly taking in very low doses.



Credit: Graham White

- Sub-lethal effects reported from the lab include behavioural disruptions to honeybee foraging, communication, memory and learning, plus harm to bee brood development, hive reproduction and immune system function.
- Sub-lethal effects on individual bees can build up to colony-level harm, especially if exposure continues for several weeks.
- Very few field studies are adequately designed or conducted for long enough to properly understand the risks to honeybees from low dose neonicotinoid exposure. Even less is known about impacts on other pollinators.
- Two recent field studies have demonstrated for the first time that repeated exposure to the very low doses contaminating pollen and nectar can stunt the growth of bumblebee colonies and disrupt the homing ability of foraging honeybees

References

1. Krupke CH, Hunt GJ, Eitzer BD, Andino G and 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
2. Hopwood, J, Vaughan, M, Shepherd, M, Biddinger, D, Mader, E, Hoffman Black, S and Mazzacano, C. (2012) Are neonicotinoids killing bees? A review of research into the effects of neonicotinoid insecticides on bees, with recommendations for action. Xerces Society for Invertebrate Conservation, USA. www.xerces.org
3. Cresswell, J. (2011) A Meta-analysis of Experiments Testing the Effects of a Neonicotinoid Insecticide (imidacloprid) on Honey Bees. *Ecotoxicology* 20 149-57
4. Blacquière, T, Smagghe, G, van Gestel, CAM and Mommaerts, V. (2012) Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicology*. DOI 10.1007/s10646-012-0863-x
5. Krupke et al., op.cit. 1
6. PANNA (2012) Pesticides and Honey Bees: State of the Science. Submission to the US EPA as a supporting appendix to Emergency Petition to US EPA Seeking Suspension of Registration for Clothianidin. Pesticide Action Network North America, San Francisco.
7. Decourtye, A., Devillers, J., Genecque, E., Le Menach, K., Budzinski, H., Cluzeau, S. and Pham-Delègue, M.-H. (2005) Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. *Arch. Environ. Contam. Toxicol.* 48, 242–250
8. APENET (2011) Unaapi's synthesis and highlighting of the Report on activities and results of the APENET Project "Effects of coated maize seed on honey bees" Year 2011. Union of Italian Beekeeper Associations.
9. *ibid.*
10. Wu, JY, Anelli, CM and Sheppard, WS. (2011) Sub-lethal effects of pesticide residues in brood comb on worker honey bee *Apis mellifera* development and longevity. *PLoS ONE* 6(2):e14720. doi:10.1371/journal.pone.0014720
11. Decourtye A, Devillers J, Cluzeau S, Charreton M, and Pham-Delègue M-H. (2004) Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. *Ecotoxicol Environ Saf* 57 410–419
12. Yang, EC, Chuang, YC, Chen, YL and Chang, LH. (2008) Abnormal foraging behaviour induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J Econ Entomol* 101 1743-1748
13. Johnson, RM., Ellis, MD, Mullin, CA and Frazier, M. (2010) Pesticides and Honey Bee Toxicity – USA. *Apidologie* 41 (3) 312-31.
14. Decourtye, A, Devillers, J, Aupinel, P, Brun, F, Bagnis, C, Fourrier, J and Gauthier, M. (2011) Honeybee tracking with microchips: a new methodology to measure the effects of pesticides. *Ecotoxicology* 20 (2) 429-437
15. Colin ME, Bonmatin JM, Moineau I, Gaimon C, Brun S, Vermandere JP (2004) A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. *Arch Environ Contam Toxicol* 47:387–395
16. APENET op.cit.8
17. Suchail S, Guez D and Belzunces LP. (2001) Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ Toxicol Chem* 20: 2482–2486.
18. Ellis, MD and Teeters, BS. (2011) Assessing the risks of honey bee exposure to pesticides. *American Bee Journal* 151 682-683
19. Halm MP, Rortais A, Arnold G, Tasei JN and Rault S. (2006) New risk assessment approach for systemic insecticides: the case of honey bees and imidacloprid (Gaucho). *Environ Sci Technol* 40 2448–2454.
20. Mommaerts, V, Reynders, S, Boulet, J, Besard, L, Sterk, G and Smagghe, G. (2010) Risk Assessment for Side-effects of Neonicotinoids against Bumblebees with and without Impairing Foraging Behavior. *Ecotoxicology* 19 (1) 207-15.
21. Henry, M, Beguin, M, Requier, F, Rollin, O, Odoux, J-F, Aupinel, P, Aptel, J, Tchamitchian, S and Decourtye, A. (2012) A

In this series

If you would like to find out more about the relationship between pesticides and pollinator declines, all of these leaflets and other info are available via PAN UK's bee webpages at: <http://bees.pan-uk.org>

Bee Declines and the Link with Pesticides. Summary leaflet.

Fact sheets:

1. Different routes of pesticide exposure
2. **Sub-lethal and chronic effects of neonicotinoids on bees and other pollinators**
3. Serious shortcomings in assessing risks to pollinators
4. Different regulatory positions on neonicotinoids across Europe
5. Can restrictions on systemic insecticides help restore bee health?
6. What could farmers do to rely less on neonicotinoids?
7. Opportunities for improving and expanding pollinator habitats
8. Action on neonicotinoid and other bee-toxic pesticides

References continued

common pesticide decreases foraging success and survival in honey bees. *Science Express* 10.1126/science.1215039

22. APENET op.cit.8

23. Teeters, BS, Johnson, RM, Ellis, MD and Siegfried, BD. (2012) Using video-tracking to assess sublethal effects of pesticides on honey bees *Apis mellifera* L. *Environ Toxicol* DOI: 10.1002/etc.1830

24. Mommaerts et al., op.cit. 20.

25. Abbot, VA, Nadeau, JL, Higo, A and Winston, ML. (2008) Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae). *Journal Econ. Entomol* 101 784-796

26. Whitehorn, PR, O'Connor, S, Wackers, FL and Goulson, D. (2012) Neonicotinoid pesticide reduces bumblebee colony growth and queen production. *Science Express* 10.1126/science.1215025

27. Hopwood et al., op.cit.2.

28. Chauzat, M-P, Carpentier, P, Martel, A-C, Bougeard, S, Cougoule, N, Porta, P, Lachaize, J, Madec, F, Aubert, M and Faucon, J-P. (2009) Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. *Environmental Entomol* 38 514-523

29. Cutler, G.C., Scott-Dupree, C.D. (2007) Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. *J. Econ. Entomol.* 100 (3) 765-772

30. Nguyen BK, Saegerman C, Pirard C, Mignon J, Widart J et al (2009) Does imidacloprid seed-treated maize have an impact on honey bee mortality? *J Econ Entomol* 102:616-623

31. Hopwood et al., op.cit.2.

32. Johnson et al., op.cit. 13.

33. Nguyen et al., op.cit.30.

34. Hopwood et al., op.cit.2.

35. Stadler, T, Martinez Gines, D and Buteler, M. (2003) Long-term toxicity assessment of imidacloprid to evaluate side-effects on honey bees exposed to treated sunflower in Argentina. *Bull Insectol* 56 77-81

36. Cutler & Scott-Dupree, op.cit.29.

37. Nguyen et al., op.cit.30.

38. Hopwood et al., op.cit.2.

39. Cresswell, op.cit.3.

40. Whitehorn et al., op.cit.26.

41. Tasei, J-N, Lerin, J and Ripault, G. (2000) Sub-lethal effects of imidacloprid on bumblebees *Bombus terrestris* (Hymenoptera: Apidae) during a laboratory feeding test. *Pest. Manag. Sci.* 56 784-788

42. Mommaerts et al., op.cit. 20.

43. Schmuck, R., Schöning, R., Stork, A. and Schramel, O. (2001) Risk posed to honeybees (*Apis mellifera* L., Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest. Manag. Sci.* 57 225-238

44. APENET op.cit.8

45. Decourtye et al., op.cit. 14.

46. Rortais A, Arnold G, Halm M-P and Touffet-Briens F. (2005) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36 71-83.

47. Cresswell, op.cit.3.

48. Teeters et al., op.cit.23.

49. Ellis & Teeters, op.cit. 18.

50. Henry et al., op.cit.21.

51. Krupke et al., op.cit. 1

52. Hopwood et al., op.cit.2.

53. Cresswell, op.cit.3.

54. Whitehorn et al., op.cit.26.

55. Henry et al., op.cit.21.

56. Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, et al. (2010) High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLoS One* 5: e9754.

57. Krupke et al., op.cit. 1

58. Hopwood et al., op.cit.2.

59. PANNA, op.cit.6.



PAN UK's vital work in the UK and in developing countries

Pesticide Action Network UK is a registered charity dedicated to:-

- Eliminating the most hazardous pesticides,
- Reducing dependence on chemical pesticides,
- Promoting sustainable and equitable food systems and increasing the use of alternatives to chemical pest control in agriculture, urban areas, public health and homes and gardens

In the UK, we campaign for tighter regulatory controls on pesticides and encourage retailers to tackle pesticide problems in their supply chains. We provide advice on alternative ways to control pests and work with local communities to reduce public exposure to pesticides. In the developing world, we raise awareness about pesticide hazards and train farmers in organic and low input agricultural techniques to help them to

make a decent living without putting their own health, their families or their environment at risk.

Populations of bees and other insect pollinators have fallen dramatically in recent years. The reasons for these declines are complex and wide ranging, but there is little doubt that pesticides are playing a key part. PAN UK has prepared these fact sheets to cut through the confusion and provide an up-to date and balanced explanation of the role of pesticides in pollinator declines. To find out more and what you can do, please visit <http://bees.pan-uk.org>

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