

Neonicotinoids, bee disorders and the sustainability of pollinator services

Jeroen P. van der Sluijs¹, Noa Simon-Delso¹, Dave Goulson², Laura Maxim³, Jean-Marc Bonmatin⁴, Luc P. Belzunces⁵

¹ Environmental Sciences, Copernicus Institute, Utrecht University, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands j.p.vandersluijs@uu.nl

² School of Life Sciences, University of Sussex, UK

³ Institut des Sciences de la Communication, CNRS UPS 3088, Paris, France

⁴ Centre de Biophysique Moléculaire, UPR 4301 CNRS affiliated to Orléans University and to INSERM

⁵ INRA, UR 406 Abeilles & Environnement, Laboratoire de Toxicologie Environnementale, CS 40509, Avignon, France

Corresponding author: Jeroen P. van der Sluijs

Abstract

In less than 20 years, neonicotinoids have become the most widely used class of insecticides with a global market share of more than 25%. For pollinators this has transformed the agrochemical landscape. These chemicals mimic the acetylcholine neurotransmitter and are highly neurotoxic to insects. Their systemic mode of action inside plants means phloemic and xylemic transport that results in translocation to pollen and nectar. Their wide application, persistence in soil and water and potential for uptake by succeeding crops and wild plants make neonicotinoids bioavailable to pollinators in sublethal concentrations for most of the year. This results in the frequent presence of neonicotinoids in honeybee hives. At field realistic doses, neonicotinoids cause a wide range of adverse sublethal effects in honeybee and bumblebee colonies, affecting colony performance through impairment of foraging success, brood, larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene etc. Neonicotinoids exhibit a toxicity than can be amplified by various other agrochemicals and they synergistically reinforce infectious agents such as *Nosema ceranae* which together can produce colony collapse. The limited available data suggest that they are likely to exhibit similar toxicity to virtually all other wild insect pollinators. The worldwide production of neonicotinoids is still increasing. Therefore a transition to pollinator-friendly alternatives to neonicotinoids is urgently needed for the sake of the sustainability of pollinator ecosystem services.

Introduction

The introduction to the market in the early 1990s of imidacloprid and thiacloprid opened the neonicotinoid era of insect pest control [1]. Acting systemically, this new class of neurotoxic insecticides is taken up by plants, primarily through the roots, and translocates to all parts of the plant through xylemic and phloemic transport [2]. This systemic property combined with very high toxicity to insects allowed formulating neonicotinoids for soil treatment and seed coating with typical doses from 10 to 200 g ha⁻¹ high enough to

provide long lasting protection of the whole plant from pest insects.

Neonicotinoids interact with the nicotinic acetylcholine receptors (nAChRs) of the insect central nervous system. They act mainly agonistically on nAChRs on the post-synaptic membrane, mimicking the natural neurotransmitter acetylcholine by binding with high affinity [3-8]. This induces a neuronal hyper-excitation, which can lead to the insect's death within minutes [6,9]. Some of the major metabolites of neonicotinoids are equally neurotoxic, acting on the same receptors [10-12] thereby prolonging the effectiveness as systemic insecticide. The binding sites in vertebrate nAChRs in the nervous system are different from those in insects, and in general they have lower numbers of nicotinic receptors with high affinity to neonicotinoids, which are the reasons that neonicotinoids show selective toxicity for insects over vertebrates [9,13].

The main neonicotinoids presently on the market are imidacloprid, thiamethoxam, clothianidin, thiacloprid, dinotefuran, acetamiprid, nitenpyram and sulfoxaflor [12,14,15]. Since their introduction, neonicotinoids have grown to become the most widely used and fastest growing class of insecticides with a 2010 global market share of 26% of the insecticide market [16] and imidacloprid the second most widely used (2008) agrochemical in the world [17]. The worldwide production of neonicotinoids is still increasing [18]. Large-scale use in Europe and US started around 2004. Neonicotinoids are nowadays authorized in more than 120 countries for more than 1,000 uses [19] for the treatments of a wide range of plants including potato, rice, maize, sugar beets, cereals, oil rapeseed, sunflower, fruit, vegetables, soy, ornamental plants, tree nursery, seeds for export, and cotton.

When used as a seed coating, only 1.6 to 20% of the amount of active substance applied actually enters the crop to protect it [20], and the remaining 80 to 98.4% pollutes the environment without any intended action to plant pests. Diffusion and transformation of pesticides in the environment leads to various environmental concentrations and bioavailability, all strongly dependent on the properties of the substance [21]. Because of their high leaching potential, neonicotinoids tend to contaminate surface water and ground water [22-25]. Due to sorption to organic matter in soil and sediments [24,26], the equilibrium partitioning over soil and water varies with soil type and is typically 1 to 3 ($\log P = 0.57$) [25]. In countries where monitoring data are available, high levels of neonicotinoid pollution in surface water have been reported [27-30]. In the Netherlands, 45% of 9037 water samples taken from 801 different locations in a nation-wide routine water quality monitoring scheme over the period 1998 and 2003-2009 exceeded the 13 ng l⁻¹ imidacloprid water quality standard, the median concentration being 80 ng l⁻¹ and the maximum concentration found being 320 µg l⁻¹, which is acutely toxic to honeybees [27]. In the US, neonicotinoids were also found in surface water. In 108 water samples collected in 2005 from playa wetlands on the Southern High Plains, thiamethoxam was found at an average concentration of 3.6 µg l⁻¹ and acetamiprid at 2.2 µg l⁻¹ [30].

Neonicotinoids and their metabolites are highly persistent in soil, aquatic sediments and water. To give an example: Six years after a single soil drench application of imidacloprid, residue levels up to 19 µg kg⁻¹ could be recovered in *Rhododendron* shrub blossoms [31]. Clothianidin has a half-life in soil between 148-

6,900 days [32], and imidacloprid 40-997 days [33]. Consequently, neonicotinoids exhibit a potential for accumulation in soil following repeated applications [23] and can be taken up by succeeding crops up to at least two years after application [34]. Imidacloprid has been detected in 97% of 33 soil samples from untreated fields on which treated corn seeds were used 1 or 2 years before the sampling [34]. Concentrations in these soil samples ranged from 1.2 to 22 $\mu\text{g kg}^{-1}$ [34]. Several studies recovered neonicotinoids in wild flowers near treated fields [35,36]. However, it remains a knowledge gap to what extent the presence in wild flowers results from systemic uptake from polluted soil and water or from direct contamination of the flowers by contaminated dust from seed drilling.

At their introduction, neonicotinoids were assumed to be more efficient than the organophosphates and carbamates that they replaced [37]. As a seed treatment they could be used in much lower quantities and they promised to be less polluting to the environment. It is however not the quantity that is relevant but the potency to cause harm, which results from toxicity, persistence and bioavailability to non-target species. Indeed, soon after the introduction of neonicotinoids, exposure of its residues in pollen, nectar, sowing dust etc., to non-target pollinating insects became clear. This led to various harmful effects [10, 37-43].

Ecosystem services of pollinators

Amongst the wide diversity of pollinating species [44], bees are the most important. Although bee research mostly focusses on the domesticated *Apis mellifera*, over 25,000 different bee species have been identified (FAO: Pollination; URL: <http://www.fao.org/agriculture/crops/core-themes/theme/biodiversity/pollination/en/>). Bees provide a vital ecosystem service, playing a key role in the maintenance of biodiversity and in food and fibre production [45-51]. Pollination comprises an integrated system of interactions that links earth's vegetation, wildlife and human welfare [52]. Of all flowering plants on earth, 87.5% benefits from animal pollination [53]. Globally, 87 of the leading food crops (accounting for 35% of the world food production volume) depend on animal pollination [45]. Pollinator mediated crops are of key importance in providing essential nutrients in the human food supply [54]. The history of apiculture goes back to pre-agricultural times [55,56] and later co-developed with agriculture [57,58]. In addition, wild bees deliver a substantial and often unappreciated portion of pollination services to agriculture and wildflowers [59,60]. Bees and apiary products have a pharmacological [61,62], scientific and technological [63], poetic [64], aesthetic (springs filled with buzzing bumblebees) culinary (e.g. keeping alive traditional cuisine of patisseries with honey) and cultural value.

Global pollinator decline and emerging bee disorders

Long-term declines have been observed in wild bee populations around the world [47, 65-70]. Over the past decades, a global trend of increasing honeybee disorders and colony losses has emerged [71-77]. Winter mortality of entire honeybee colonies has risen in many parts of the world [72-75]. When neonicotinoids were first used, beekeepers started describing different disorders and signs ranging from: bees not returning to the hive, disoriented bees, bees gathered close together in small groups on the ground, abnormal foraging

behaviour, the occurrence of massive bee losses in spring, queen losses, increased sensibility to diseases and colony disappearance [38,40-43,77]. None of these individual signs is a unique effect of neonicotinoids, other causal factors or other agrochemicals could produce similar signs, which complicates the establishment of a causal link.

Scientific research appears to indicate no single cause explaining the increase in winter colony losses. All viruses and other pathogens that have been linked to colony collapse have been found to be present year-round also in healthy colonies [78]. That colonies remain healthy despite the presence of these infectious agents, supports the theory that colony collapse may be caused by factors working in combination. Farooqui [79] has analyzed the different hypotheses provided by science when searching for an explanation of Colony Collapse Disorder (CCD). Research points in the direction of a combination of reciprocally enhancing causes. Among those, the advance of neonicotinoid insecticides has gained more weight in light of the latest independent scientific results [80-82]. In the present article, we synthesise the state of knowledge on the role of neonicotinoids in pollinator decline and emerging bee disorders.

Multiple ways of exposure

Neonicotinoids are authorized for a wide range of agricultural and horticultural plants that flower at different times of the year. The systemic properties of neonicotinoids imply translocation to pollen, nectar, and guttation droplets [34,37,83,84]. The persistency and potential contamination of wild plants and trees surrounding the treated crops [36] and the possibility for traveling far outside the fields via surface and ground water [27] and the potential to contaminate wild plants and crops that take up polluted water, means that pollinating insects are likely to be exposed for much of the year to multiple sources of multiple neonicotinoids in their foraging area, but often at very low doses.

Honeybees' exposure to neonicotinoids can occur through ingestion, contact and inhalation (aerosols). Many possible exposure pathways can exist [85]. Here, we aggregate exposure pathways into: (a) intake of food that contain residues; (b) nesting material (resin, wax etc.); (c) direct contact with spray drift and dust drift during application; (d) contact with contaminated plants, soil, water; (e) use of cooling water in the hive; and (f) inhalation of contaminated air. For bumble bees and other wild bees that nest in soil, contact with contaminated soil is an additional pathway of concern. Leafcutter bees use cut leaf fragments to form nest cells and can thus be exposed to residues in leaves. There are many other conceivable exposure routes, for instance, a bee hive could have been made from timber from trees treated with neonicotinoids and may thus contain residues. However, the best researched exposure pathway is via intake of food. Food with residues can be sub-divided into self-collected raw food (nectar, pollen, water, honeydew, extrafloral nectar, guttation droplets, various other edible substances available in the foraging area etc.), in-hive processed food (honey, beebread, royal jelly, wax etc.), and food supplied by bee keepers (high fructose corn syrup, sugar water, sugar dough, bee candy, pollen, pollen substitutes based on soybean flower and other vegetable protein supplements etc.).

Given the large numbers of crops in which neonicotinoids are used and the large scale of use, there is a huge variability in space and time for each possible exposure pathway as well as in their relative importance for the overall exposure at a given place and time. This is further complicated by the fact that the foraging area of a honeybee colony can extend to a radius of up to 9 km around the hive which is never a homogenous landscape [86]. Additionally, suburban areas have become a stronghold for some wild bee species due to the abundance of floral resources in gardens and parks [87]. Thus, bees may be exposed to systemic insecticides which are widely used on garden flowers, vegetables, ornamental trees, and lawns. The relative importance of exposure pathways will also vary according to bee species as they have different foraging ranges, phenologies, and flight times in a day. This can be exemplified by *Osmia* bees in corn growing areas for which intake of guttation droplets may be more important than for honeybees.

Different categories of honeybees could be exposed in different ways and to varying extents [42]. For example, pollen foragers (which differ from nectar foragers) do not consume pollen, merely bringing it to the hive. The pollen is consumed by nurse bees and to a lesser extent by larvae which are thus the ones that are exposed to residues of neonicotinoids and their metabolites [88]. The exposure of nectar foragers to residues of neonicotinoids and metabolites in the nectar they gather can vary depending on the resources available in the hive environment. In addition, foragers take some honey from the hive before they leave for foraging. Depending on the distance from the hive where they forage, the honeybees are obliged to consume more or less of the nectar/honey taken from the hive and/or of the nectar collected, for energy for flying and foraging. They can therefore ingest more or less neonicotinoid residues, depending on the foraging environment [42]. Oral uptake is estimated to be highest for forager honeybees, winter honeybees and larvae [85].

Little is known about the real exposure to contaminated food for different categories of honeybees in a colony, either in terms of contact with pollen or contact with, and possible consumption of, nectar if needed. For wild bees very few data exist on exposure in the field. The amount that wild bees actually consume in the field has not been measured. EFSA estimated that worker bees, queens and larvae of bumblebees and adult females and larvae of solitary bees are likely to have the highest oral uptake of residues [85].

In 2002, 69% of pollen samples collected by honeybees at various places in France contained residues of imidacloprid and its metabolites [89]. In a systematic sampling scheme covering 5 locations over 3 years, imidacloprid was found in 40.5% of the pollen samples and in 21.8% of the honey samples [90,91]. Based on data from authorization authorities, neonicotinoids residues in nectar and pollen of treated crop plants are estimated to be in the range of below analytical detection limit ($0.3 \mu\text{g kg}^{-1}$) to $5.4 \mu\text{g kg}^{-1}$ in nectar, the highest value corresponding to clothianidin in oilseed rape nectar, and a range of below detection limit ($0.3 \mu\text{g kg}^{-1}$) to $51 \mu\text{g kg}^{-1}$ in pollen, the highest value corresponding to thiamethoxam in alfalfa pollen [85]. A recent review reports wider ranges: for pollen: >0.2 to $912 \mu\text{g kg}^{-1}$ for imidacloprid and <1.0 to $115 \mu\text{g kg}^{-1}$ for thiacloprid [92]. Residues of imidacloprid, dinotefuran, and thiamethoxam plus metabolites in pumpkin

treated with United States label rates reaches average levels up to 122 $\mu\text{g kg}^{-1}$ in pollen and 17.6 $\mu\text{g kg}^{-1}$ in nectar [93]. Up to 346 mg l^{-1} for imidacloprid and 146 mg l^{-1} for thiamethoxam and 102 mg l^{-1} clothianidin and has been found in guttation drops from leaves of plants germinated from neonicotinoid-coated seeds [84,94]. In melon, guttation levels up to 4.1 mg l^{-1} imidacloprid were found 3 d after a top (US) label rate soil application [95]. In a US wide survey of pesticide residues in beeswax, pollen and honeybees during the 2007-2008 growing seasons, high levels of neonicotinoids were found in pollen (included in [92]) but imidacloprid was also found up to 13.6 $\mu\text{g kg}^{-1}$ in wax [96]. In Spain, neonicotinoids were found in beeswax samples from apiaries near fruit orchards: 11 out of 30 samples tested positive in ranges from 11 $\mu\text{g kg}^{-1}$ (acetamiprid) to 153 $\mu\text{g kg}^{-1}$ (thiacloprid) [97].

Little is known on the presence of neonicotinoids in honeydew. Given differences in life span of aphids and bees, concentrations in plant sap too low to kill aphids could translocate to honeydew and could still produce sublethal effects and chronic toxicity mortality in bees and bee colonies.

Acute and chronic effects of lethal and sublethal exposure

Pesticides can produce four types of effects on honeybees: lethal effects and sublethal effects from acute or chronic exposures.

Acute toxicity is expressed as the lethal dose (LD) at which 50% of the exposed honeybees die within 48 hours: abbreviated to 'LD50 (48 hours)'. Neonicotinoids are highly toxic (in the range of ng/bee) to honeybees [98], both when administered orally and by contact. They also have high acute toxicity to all other bee species so far tested, including various *Bombus* species, *Osmia lignaria* and *Megachile rotundata* [99-102]. *Osmia lignaria* is more sensitive to both clothianidin and imidacloprid than is *B. impatiens*, with *Megachile rotundata* more sensitive still [100]. In an acute toxicity test under semi field condition on the Indian honeybee *Apis cerana indica*, clothianidin showed the highest toxicity, followed by imidacloprid and thiamethoxam [103].

For mass-dying of bees in spring nearby and during sowing of corn seeds coated with neonicotinoids there now is a one to one proven causal link with acute intoxication though contact with the dust cloud around the pneumatic sowing machines during foraging flights to adjacent forests (providing honeydew) or nearby flowering fields [104-109]. Such mass colony losses during corn sowing have also been documented in Italy, Germany, Austria and Slovenia [110,111,104]. In response to the incidents, the adherence of the seed coating has been improved owing to better regulations, and an improved sowing-technique has recently become compulsory throughout Europe, [112]. Despite the deployment of air deflectors in the drilling machines or improved seed coating techniques, emissions are still substantial and the dust cloud is still acutely toxic to bees [105,109,111,113-115]. Acute lethal effects of neonicotinoids dispersed as particulate matter in the air seem to be promoted by high environmental humidity which accelerates mortality [105]. Honeybees also bring the toxic dust particles they gather on their body into the hive [106]. Sunny and warm days also seem to favour the dispersal of active substances [35].

Lethal effects from chronic exposure refer to honeybee mortality that occurs after prolonged exposure. In contrast to acute lethal effects, there are no standardised protocols for measuring chronic lethal effects. Therefore, in traditional risk assessment of pesticides they are usually expressed in three ways: LD50: the dose at which 50% of the exposed honeybees die (often, but not always, within 10 days); NOEC (No Observed Effect Concentration): the highest concentration of imidacloprid producing no observed effect; and LOEC (Lowest Observed Effect Concentration): the lowest concentration of imidacloprid producing an observed effect. However, for neonicotinoids and its neurotoxic metabolites, lethal toxicity can increase up to 100,000 times compared to acute toxicity when the exposure is extended in time [10]. There has been some controversy on the findings of that study, which is discussed in detail by Maxim and Van der Sluijs [40,42]. However, the key finding that exposure time amplifies the toxicity of neonicotinoids is consistent with later findings. Micro-colonies of bumblebees fed with imidacloprid showed the same phenomenon [102]: at one tenth of the concentration of the toxin in feed, it took twice as long to produce 100% mortality in a bumblebee microcolony. At a 100 times lower dose, it took ca. four times longer to produce 100% mortality. The measurable shortening of the life span cease to occur only when a dose was administered, for which the (extrapolated) chronic intoxication time would be longer than the natural life span of a worker bumblebee. This implies that the standard 10 day chronic toxicity test for bees is far too short for testing neonicotinoids. Indeed, honeybees fed with $1/10^{\text{th}}$ of the LC50 of thiamethoxam showed a 41.2% reduction of life span [116]. Recent studies have shown that chronic toxicity of neonicotinoids can more adequately be expressed by time to 50% mortality instead of by the 10 day LD50 [117-122]. There is a linear relation between log daily dose and log time to 50% mortality [118,120,121]. In experiments with honeybee colonies similar long term chronic effects have indeed been found with typical times of 14-23 weeks to collapse 25 to 100% of the colonies exposed to imidacloprid contaminated food at $20 \mu\text{g kg}^{-1}$ [123] and 80-120 days for 1 mg kg^{-1} dinotefuran and $400 \mu\text{g kg}^{-1}$ clothianidin [76]. Note that these studies used concentrations that are on the high end of the currently reported ranges of concentrations found in the field. However, such data are sparse and limited to a few crops, so it cannot yet be concluded whether such concentrations are rare or common in the field.

At low concentrations of neonicotinoids, sublethal effects can occur. Sublethal effects involve modifications of honeybee behaviour and physiology (e.g. immune system). They do not directly cause the death of the individual or the collapse of the colony but may become lethal in time and/or may make the colony more sensitive (for example, more prone to diseases), which may contribute to its collapse. For instance, an individual with memory, orientation or physiological impairments might fail to return to its hive, dying from hunger or cold. This would not be detected in standard pesticide tests, which focus on acute mortality. A distinction can be made between acute and chronic sublethal effects. Acute sublethal effects are assessed by exposing bees only once to the substance (by ingestion or by contact), and observing them for some time (variable from one laboratory to another, from several minutes to four days). Chronic sublethal effects are assessed by exposing honeybees more than once to neonicotinoids during an extended period of time (for example, each 24 hours, for 10 days). Both acute and chronic sublethal effects are expressed as NOEC and/or

LOEC (No or Lowest Observable Effect Concentration, respectively) [42].

In an extensive review Desneux et al. found that sub-lethal effects of neonicotinoids exist on neurophysiology, larval development, moulting, adult longevity, immunology, fecundity, sex ratio, mobility, navigation and orientation, feeding behaviour, oviposition behaviour, and learning [124]. All these effects have been reported for pollinators and all have the potential to produce colony level, population level and community level impacts on pollinators.

At field realistic concentrations ($1 \mu\text{g l}^{-1}$) imidacloprid repels pollinating beetles while at concentrations well below the analytical detection limit ($0.01 \mu\text{g l}^{-1}$) it repels pollinating flies [125]. This implies that imidacloprid pollution may disrupt pollination both in polluted nature and in agricultural lands. On honeybees, imidacloprid has no repelling effect at field realistic concentrations: it starts being repellent at $500 \mu\text{g l}^{-1}$ [126]. In some plant protection formulations, neonicotinoids are mixed with bee repellents. However, the persistence of neonicotinoids exceeds that of the repellence and their systemic properties differ. Besides, if bees are effectively repelled and avoid the contaminated flowers, pollination is disrupted because plants are not visited by bees.

Sublethal doses of neonicotinoids impair the olfactory memory and learning capacity of honeybees [127-130] and the orientation and foraging activity [131]. The impact of sublethal exposure on the flying behavior and navigation capacity has been shown through homing flight tests [82,126,132,133]. Exposed to a very low concentration ($0.05 \mu\text{g kg}^{-1}$) imidacloprid honeybees show an initial slight increase in travel distance. However, with increasing concentration, starting at $0.5 \mu\text{g kg}^{-1}$ imidacloprid decreases distance traveled and interaction time between bees, while time in the food zone increases with concentration [134]. Imidacloprid disrupts honeybee waggle dancing and sucrose responsiveness at doses of 0.21 and 2.16 ng bee^{-1} [135].

If honeybee brood is reared at suboptimal temperatures (the number of adult bees is not sufficient to maintain the optimal temperature level), the new workers will be characterised by reduced longevity and increased susceptibility to pesticides (bee-level effect) [136]. This will again result in a number of adult bees insufficient to maintain the brood at the optimal temperature, which may then lead to chronic colony weakening until collapse (colony-level effect).

Sublethal effects seem to be detected more frequently and at lower concentrations when bumblebees (*Bombus terrestris*) have to travel to gather food, even when the distances are tiny. No observable impacts of imidacloprid at field realistic concentrations on micro-colonies of *B. terrestris* provided with food in the nest were found, but when workers had to walk just 20 cm down a tube to gather food they exhibited significant sublethal effects on foraging activity, with a median sublethal effect concentration (EC_{50}) of $3.7 \mu\text{g kg}^{-1}$ [102]. In queenright bumblebee colonies foraging in a glasshouse where food was 3 m from their nest, $20 \mu\text{g}$

kg⁻¹ of imidacloprid caused significant worker mortality, with bees dying at the feeder. Significant mortality was also observed at 10 µg kg⁻¹, but not at 2 µg kg⁻¹ [102]. Bumblebees exhibit concentration dependent sublethal responses (declining feeding rate) to imidacloprid starting at 1 µg l⁻¹ in syrup, while honeybees seemed unaffected [137].

Field-relevant concentrations of imidacloprid, used alone or in mixture with λ-cyhalothrin, were shown to impair pollen foraging efficiency in bumblebee colonies [138]. In an attempt to fulfil colony needs for pollen, more workers were recruited to forage instead of taking care of brood. This seemed to affect brood development resulting in reduced worker production [138]. Bumblebee colonies have been exposed to field realistic levels of imidacloprid (0.7 µg kg⁻¹ in nectar, 6 µg kg⁻¹ in pollen) for two weeks in the laboratory. When subsequently placed back in the field and allowed to develop naturally for the following six weeks, treated colonies showed an 85% reduction in queen production and a significantly reduced growth rate [81]. Effects on bumblebee reproduction occur at imidacloprid concentrations as low as 1 µg l⁻¹ [139] which is highly field-realistic.

It has also been shown that pesticides like imidacloprid act on the hypopharyngeal glands of honeybee nurses by degenerating the tissues [140-142], which induces a shift from nest to field activities. In the native stingless bee *Melipona quadrifasciata anthidioides*, imidacloprid causes impairment of the mushroom bodies which are involved in learning [143]. Imidacloprid and clothianidin have been shown to be potent neuromodulators of the honeybee brain, causing mushroom body neuronal inactivation in honeybees, which affect honeybee cognition and behaviour at concentrations that are encountered by foraging honeybees and within the hive [8]. Sublethal doses of imidacloprid were also found to have cytotoxic activity in the Malpighian tubules in honeybees that make up the excretory and osmoregulatory system [144]. Exposure to thiamethoxam has also been shown to result in morphological impairment of the bee brain and bee midgut [116].

Exposure to neonicotinoid residues lead to a delayed development of honeybee larvae, notably in the first state (day 4 to day 8) [145]. This can favour the development of the *Varroa destructor* parasitic mite within the colony. Likewise, the life span of adult bees emerging from the exposed brood proved to be shorter.

Short and mid-term sublethal effects on individuals or age groups result in long-term effects at the colony level, which follow weeks to months after the exposure, such as honeybee colony depopulation and bumblebee colony queen production [76,81,123,138]. As it has recently been acknowledged, the field tests on which the marketing authorization of the use of neonicotinoids is essentially based were not developed to detect sublethal nor long-term effects on the colony level, and the observations of the performance of colonies after experimental exposure do not last long enough [85]. Major weaknesses of existing field studies are the small size of the colonies, the very small distance between the hives and the treated field and the very low surface of the test field. As a consequence of these weaknesses the real exposures of the honey bees

during these field tests is highly uncertain and may in reality be much smaller than what has been assumed in these field studies. [85]

In addition, the meta-analysis [146] demonstrates that field tests published until now on which European and North American authorizations are based, lack the statistical power required to detect the reduction in colony performance predicted from the dose-response relationship derived from that meta-analysis. For this purpose, the tests were wrongly designed, there were too few colonies in each test groups, and the follow up time monitoring the long term colony level impacts were too short to detect many of the effects described above. Nonetheless, these field studies have been the basis for granting the present market authorizations by national and European safety agencies. The meta-analyses combined data from 14 previous field tests, and subsequently demonstrated that at exposure to field realistic doses, imidacloprid does have significant sublethal effects, even at authorized levels of use, impairs performance and thus weakens honeybee colonies [146].

A further limitation of field studies is their limited reproducibility due to the high variability in environmental conditions in the foraging area of honeybees, which extends up to a 9 km radius around the hive. Observations made in a particular field experiment need not be representative of the range of effects that could occur in real conditions. Due to the large variability of factors that cannot be controlled (e.g. other stressors, soil structure, climate, combination of plants attractive for bees etc.), current field experiments only give information about the particular situation in which they were done.

The challenges of field studies became also clear in the debates over the highly contested field study recently conducted by the Food and Environment Research Agency (FERA) which resorts under the UK Department for Environment, Food and Rural Affairs (DEFRA). This study was set up in response to the *Science* publication that showed that a short term exposure of bumblebees to field realistic imidacloprid concentrations cause a long term 85% reduction in queen production [81]. At three sites 20 bumblebee colonies were exposed to crops grown from untreated, clothianidin-treated or imidacloprid-treated seeds. The agency concluded that “no clear consistent relationships” between pesticide levels and harm to the insects could be found [FERA: URL:

<http://www.fera.defra.gov.uk/scienceResearch/scienceCapabilities/chemicalsEnvironment/documents/reportP2371Mar13.pdf>]. However, it turned out that the control colonies themselves were contaminated with the pesticides tested. Further, thiamethoxam was detected in two out of the three bee groups tested, even though it was not used in the experiment. [147] The major studies that have measured neonicotinoid residues in pollen collected by honeybees clearly show that neonicotinoids are found in pollen all over the year and in all studied regions, not only after the sowing or during the flowering period [89,91,96]. With the present scale of use, it will be very difficult to find a control site where bees cannot come into contact with neonicotinoids.

Given all the major limitations to the reliability of outcomes of field studies, it is recommendable to give more weight in the risk assessment to reproducible results from controlled lab studies and use the ratio

between the environmental concentration and the no effect concentration as the main risk indicator [40,42]. It could perhaps be linked to modelling to explore how and to what the degree the various well-known sublethal effects on individual bees can weaken the colony [148].

A key aspect in honeybee biology is that the colony behaves as a ‘superorganism’ [149]. In a colony, sufficient membership, so that the number of organisms involved in the various tasks to maintain that colony, is critical, not the individual quality of a task performed by an individual bee. Varying between winter and summer, the 10,000 – 60,000 honeybees that typically form a colony function as a cooperative unit, maintaining intraorganismic homeostasis as well as food storage, nest hygienic, defence of the hive, rearing of brood etc. Hence, sublethal effects affecting how many individuals perform specific functions can influence the functioning of the whole colony. In a simplified theoretical modelling approach, colony failure can be understood in terms of observed principles of honeybee population dynamics [150]. A colony simulation model predicts a critical threshold forager death rate above which rapid population decline is predicted and colony failure is inevitable. High forager death rates draw hive bees towards the foraging population at much younger ages than normal, which acts to accelerate colony failure [150].

Synergistic effects: pesticide-pesticide & pesticide-infectious agents

A synergy occurs when the effect of a combination of stressors is higher than the sum of the effect of each stressor alone. When neonicotinoids are combined with certain fungicides (azoles, such as prochloraz, or anilides, such as metalaxyl) or other agrochemicals that block cytochrome P450 detoxification enzymes, their toxicity increases by factor from 1.52 to 1,141 depending on the combination [151,152]. The strongest synergism has been found for triflumizole making thiacloprid 1,141 times more acutely toxic to honeybees[151]. This synergistic effect is the subject of patents by agrochemical companies [152,153].

Synergy has also been demonstrated for neonicotinoids and infectious agents. Prolonged exposure to a non-lethal dose of neonicotinoids renders beehives more susceptible to parasites such as *Nosema ceranae* infections [39, 154-156]. This can be explained either by an alteration of the immune system or by an impairment of grooming and allogrooming that leads to reduced hygiene at the individual level and in the nest, which gives the pathogens more chances to infect the bees. The same mechanism, where the balance between an insect and its natural enemies is disturbed by sublethal exposure to neonicotinoids that impairs grooming, is well known and often used in pest management of target insects [158-161].

Conclusion and prospects

In less than 20 years, neonicotinoids have become the most widely used class of insecticides. Being used in more than 120 countries in more than 1000 different crops and applications, they now account for at least one quarter of the world insecticide market. For pollinators, this has transformed the agrochemical landscape to one in which most flowering crops and an unknown proportion of wild flowers contain varying concentrations of neonicotinoids in their pollen and nectar. Most neonicotinoids are highly persistent in soil,

water and sediments and they accumulate in soil after repeated use. Severe surface water pollution with neonicotinoids is common. Their systemic mode of action inside plants means phloemic and xylemic transport that results in translocation to pollen and nectar. Their wide application, persistence in soil and water and potential for uptake by succeeding crops and wild plants make neonicotinoids bioavailable to pollinators in sublethal concentrations for most of the year. This results in the frequent presence of neonicotinoids in honeybee hives. Neonicotinoids are highly neurotoxic to honeybees and wild pollinators. Their capacity to cross the ion-impermeable barrier surrounding the central nervous system (BBB, blood-brain barrier) [7] and their strong binding to nAChR in the bee's central nervous system are responsible for a unique chronic and sublethal toxicity profile. Neonicotinoid toxicity is reinforced by exposure time. Some studies indicate a non-monotonic [162] dose-response curve at doses far below the LD50. Mass bee dying events in spring from acute intoxication have occurred in Germany, Italy, Slovenia and France during pneumatic sowing of corn seeds coated with neonicotinoids. Bees that forage near corn fields during sowing get exposed to acute lethal doses when crossing the toxic dust cloud created by the sowing machine. At field realistic exposure levels, neonicotinoids produce a wide range of adverse sublethal effects in honeybee colonies and bumblebee colonies, affecting colony performance through impairment of foraging success, brood and larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene etc. Neonicotinoids synergistically reinforce infectious agents such as *Nosema ceranae* and exhibit synergistic toxicity with other agrochemicals. The large impact of short term field realistic exposure of bumblebee colonies on long term bumblebee queen production (85% reduction) could be a key factor contributing to the global trends of bumblebee decline. Only a few studies assessed the toxicity to other wild pollinators, but the available data suggest that they are likely to exhibit similar toxicity to all wild insect pollinators. The worldwide production of neonicotinoids is still increasing. In view of the vital importance of the service insect pollinators provide to both natural ecosystems and farming, they require a high level of protection. Therefore a transition to pollinator-friendly alternatives to neonicotinoids is urgently needed for the sake of the sustainability of pollinator ecosystem services. The recent decision by the European Commission to temporary ban the use of imidacloprid, thiamethoxam and clothianidin in crops attractive to bees is a first step in that direction [163].

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collection and analysis, decision to publish, or preparation of the manuscript.

References and recommended reading

Papers of particular interest, published within the period of review have been highlighted as:

*** of special interest**

**** of outstanding interest**

1. Tomizawa M, & Casida JE: **Neonicotinoid insecticides: highlights of a symposium on strategic molecular designs**. *Journal of agricultural and food chemistry* 2011, **59**:2883-2886.
doi:10.1021/jf103856c
2. Bromilow RH, Chamberlain K, Evans AA: **Physicochemical aspects of phloem translocation of herbicides**. *Weed Science* 1990, **38**:305-314.
3. Buckingham SD, Lapied B, Le Corrionc H, Grolleau F, Sattelle DB: **Imidacloprid actions on insect neuronal acetylcholine receptors**. *J Exp Biol* 1997, **200**:2685-2692.
4. Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Sattelle DB: **Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors**. *Trends Pharmacol Sci* 2001, **22**:573-580.
5. Matsuda K, Shimomura M, Ihara M, Akamatsu M, Sattelle DB: **Neonicotinoids show selective and diverse actions on their nicotinic receptor targets: electrophysiology, molecular biology, and receptor modeling studies**. *Biosci. Biotechnol. Biochem* 2005, **69**:1442-1452.
- ** 6. Belzunces LP, Tchamitchian S, Brunet JL: **Neural effects of insecticides in the honey bee**. *Apidologie* 2012, **43**:348-370.

Excellent review of neural impacts of field exposure of honeybees to sublethal residues of neurotoxic insecticides in pollen and nectar. These impair (a) cognitive functions, including learning and memory, habituation, olfaction and gustation, navigation and orientation; (b) behaviour, including foraging and (c) physiological functions, including thermoregulation and muscle activity. Time is a key factor in insecticide toxicity. Combination toxicity of joint exposure to multiple pesticides urgently requires attention.

- * 7 Tomizawa M: **Chemical Biology of the Nicotinic Insecticide Receptor**. *Advances in Insect Physiology* 2013, **44**:63-99.

Introduction into the molecular basis of binding site interaction and explanation of different binding affinity of neonicotinoids with insect and vertebrate nicotinic acetyl choline receptor. Another key factor explaining the high insect toxicity is its capacity (stemming from hydrophobicity) to penetrate the “ion-impermeable barrier” surrounding the insect nervous system.

- ** 8. Palmer MJ, Moffat C, Saranzewa N, Harvey J, Wright GA, Connolly CN: **Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees**. *Nature Communications* 2013, **4**:1634.
doi:10.1038/ncomms2648

Using recordings from mushroom body Kenyon cells in acutely isolated honeybee brain, it is shown that the neonicotinoids imidacloprid and clothianidin, and the organophosphate miticide coumaphos oxon, cause a

depolarization-block of neuronal firing and inhibit nicotinic responses. These effects are observed at concentrations that are encountered by foraging honeybees and within the hive, and are additive with combined application. Exposure to multiple pesticides that target cholinergic signalling will cause enhanced toxicity to pollinators.

9. Tomizawa M, Casida JE: **Neonicotinoid Insecticide Toxicology: Mecanism of Selective Action.** *Annu. Rev. Pharmacol. Toxicol* 2005, **45**: 247-268.
10. Suchail S, Guez D, Belzunces LP: **Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in Apis mellifera.** *Environmental toxicology and chemistry* 2001, **20**:2482–6.
11. Suchail S, Debrauwer L, Belzunces LP: **Metabolism of imidacloprid in Apis mellifera.** *Pest Management Science* 2004, **60**:291-296.
12. Casida JE: **Neonicotinoid Metabolism: Compounds, Substituents, Pathways, Enzymes, Organisms, and Relevance.** *Journal of agricultural and food chemistry* 2010, **59**:2923-31. doi: 10.1021/jf102438c
13. Liu GY, Ju XL, Cheng J: **Selectivity of Imidacloprid for fruit fly versus rat nicotinic acetylcholine receptors by molecular modeling.** *J. Mol. Model* 2010, **16**: 993-1002.
14. Liu Z., Yao X, Zhang Y: **Insect nicotinic acetylcholine receptors (nAChRs): Important amino acid residues contributing to neonicotinoid insecticides selectivity and resistance.** *African Journal of Biotechnology* 2008, **7**:4935-4939.
- 15 Cutler P, Slater R, Edmunds JF, Maienfisch P, Hall RG, Earley GP, Pitterna T, Pal S, Paul V-L, Goodchild J, Blacker M, Hagmann, L, Crossthwaite AJ: **Investigating the mode of action of sulfoxaflor: a fourth-generation neonicotinoid.** *Pest Management Science* 2013, **69**:607-619. doi:10.1002/ps.3413
16. Casida J E, Durkin K: **Neuroactive insecticides: targets, selectivity, resistance, and secondary effects.** *Annual review of entomology* 2013, **58**:99–117. doi:10.1146/annurev-ento-120811-153645
17. Pollak P: *Fine Chemicals: The Industry and the Business.* John Wiley & Sons; 2011.
18. Shao X, Liu Z, Xu X, Li Z, Qian X: **Overall status of neonicotinoid insecticides in China: Production, application and innovation.** *Journal of Pesticide Science*, **38**:1–9. doi:10.1584/jpestics.D12-037
19. European Food Safety Authority: **Statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe.** *EFSA Journal* 2012, **10**: 2752. doi 10.2903/j.efsa.2012.2752
20. Sur R, Stork A: **Uptake, translocation and metabolism of imidacloprid in plants.** *Bulletin of insectology* 2003, **56**:35-40.
- 21 Van de Meent D, Hollander A, Peijnenburg W, Breure T: Fate and Transport of Contaminants. In: *Ecological Impacts of Toxic Chemicals.* Edited by Sánchez-Bayo F, Van den Brink P, Mann RM. Bentham; 2011.
22. Gupta S, Gajbhiye V, Kalpana T, Agnihotri NP: **Leaching Behavior of Imidacloprid Formulations in Soil.** *Bull Environ Contam Toxicol* 2002, **68**:502–508.
23. Haith DA: **Ecological risk assessment of pesticide runoff from grass surfaces.** *Environmental science*

& *technology* 2010, **44**:6496–502.

24. Selim HM, Jeong CY, Elbana TA: **Transport of imidacloprid in soils: miscible displacement experiments.** *Soil Science* 2010, **175**:375-381.
25. Miranda GRB, Raetano CG, Silva E, Daam MA, Cerejeira MA: **Environmental fate of neonicotinoids and classification of their potential risks to hypogean, epygean, and surface water ecosystems in Brazil.** *Human and Ecological Risk Assessment* 2011, **17**:981-995.
26. Kurwadkar ST, Dewinne D, Wheat R, McGahan DG, Mitchell FL: **Time dependent sorption behavior of dinotefuran, imidacloprid and thiamethoxam.** *Journal of environmental science and health. Part. B* 2013, **48**:237-242. doi 10.1080/03601234.2013.742412
27. Van Dijk T, Van Staalduinen M, Van der Sluijs JP: **Macro-invertebrate decline in surface water polluted with imidacloprid** *PLOS ONE* 2013, **8**:e62374. doi:10.1371/journal.pone.0062374.
28. Starner K, Goh KS: **Detections of the Neonicotinoid Insecticide Imidacloprid in Surface Waters of Three Agricultural Regions of California, USA, 2010-2011.** *Bull Environ Contam Toxicol* 2012, **88**:316–321. doi:10.1007/s00128-011-0515-5
29. Kreuger J, Graaf S: *Pesticides in surface water in areas with open ground and greenhouse horticultural crops in Sweden 2008.* 2010.
30. Anderson TA, Salicea CJ, Erickson RA, McMurry ST, Coxa SB, Smith LM: **Effects of landuse and precipitation on pesticides and water quality in playa lakes of the southern high plains.** *Chemosphere* 2013, **92**:84-90. DOI: 10.1016/j.chemosphere.2013.02.054
31. Doering J, Maus C and Schoening R: *Residues of imidacloprid WG 5 in blossom samples of Rhododendron sp (variety Nova Zembla) after soil treatment in the field application: 2003, sampling 2003 and 2004,* 2004Bayer Cropscience AG. Report No G201806/32.
32. Rexrode M, Barrett M, Ellis J, Gabe P, Vaughan A, Felkel J, Melendez J: *EFED risk assessment for the seed treatment of clothianidin 600FS on corn and canola.* United States Environmental Protection Agency, 20 February 2003.
33. NPIC (National Pesticide Information Center): Imidacloprid technical fact sheet. <http://npic.orst.edu/factsheets/imidacloprid.pdf> (accessed 17 Feb 2013)
34. Bonmatin JM, Moineau I, Charvet R, Colin ME, Fleche C, Bengsch ER: **Behaviour of Imidacloprid in Fields. Toxicity for Honey Bees.** In *Environmental Chemistry - Green chemistry and pollutants in ecosystems* Edited by Springer Berlin; 2005, 483–94.
35. Greatti M, Sabatini AG, Barbattini R, Rossi S, Stravisi A: **Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results.** *Bulletin of Insectology* 2003, **56**:69-72.
- ** 36. Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K: **Multiple Routes of Pesticide Exposure for Honey Bees Living Near Agricultural Fields.** *PLOS ONE* 2012, **7**: e29268. doi: 10.1371/journal.pone.0029268

Bees are exposed in many ways to neonicotinoids in agricultural fields 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. Neonicotinoids were found in soil also of unplanted fields. Dandelions visited by foraging bees growing near these fields contained neonicotinoids: 1.1 to 9.4 $\mu\text{g kg}^{-1}$ clothianidin and 1.1 to 2.9 $\mu\text{g kg}^{-1}$ thiamethoxam.

37. Bonmatin JM, Marchand PA, Charvet R, Moineau I, Bengsch ER, Colin ME: **Quantification of imidacloprid uptake in maize crops.** *Journal of agricultural and food chemistry* 2005, **53**:5336–41. doi:10.1021/jf0479362

38. Maini S, Medrzycki P, Porrini C: **The puzzle of honey bee losses : a brief review.** *Bulletin of Insectology* 2010, **63**:153–160.

** 39. Vidau C, Diogon M, Aufauvre J, Fontbonne R, Viguès B, Brunet J-L, Texier C, Biron DG, Blot N, El Alaoui H, *et al.*: **Exposure to Sublethal Doses of Fipronil and Thiacloprid Highly Increases Mortality of Honeybees Previously Infected by Nosema ceranae.** *PLOS ONE* 2011, **6**:e21550. doi: 10.1371/journal.pone.0021550

Synergism was found between neurotoxic insecticides and *Nosema ceranae* infections: Sublethal doses of fipronil and thiacloprid makes honeybees more prone to *Nosema ceranae* infections and induces increased mortality of nosema-infected bees. This effects does not seem to be linked to a decrease of the bee detoxification system. A step forward in understanding the interactions of factors jointly producing colony depopulation.

40. Maxim L, Van der Sluijs JP: **Uncertainty: cause or effect of stakeholders' debates? Analysis of a case study: the risk for honey bees of the insecticide Gaucho®.** *Sci Total Environ* 2007, **376**:1-17.

41. Suryanarayanan S, Kleinman DL: **Disappearing bees and reluctant regulators.** *Issues in Science and Technology* 2011, **27**:33.

** 42. Maxim L, Van der Sluijs JP: **Seed-dressing systemic insecticides and Honeybees.** In: *Late lessons from early warnings: science, precaution, innovation.* European Environment Agency (EEA); 2013:401-438.

Using the bans of imidacloprid in France for sunflower (1999) and maize (2004) as a case study, the social processes that ultimately lead to application of the precautionary principle are analyzed in a very insightful way. The analysis focuses on the ways in which scientific findings were used by stakeholders and decision-makers to influence policy during the controversy. Eight lessons are drawn about governance of controversies related to chemical risks.

** 43. Suryanarayanan S, Kleinman D: **Be(e)coming Experts: The Controversy Over Insecticides in the Honey Bee Colony Collapse Disorder,** *Social Studies of Science* 2013, **43**:215-240: DOI: 10.1177/0306312712466186

This paper explores the politics of expertise in an ongoing controversy in the United States over the role of neonicotinoids in colony collapse disorder. A set of research norms and practices from agricultural entomology came to dominate the investigation of the links between pesticides and honey bee health. The epistemological dominance of these norms and practices served to marginalize the knowledge claims and policy positions of beekeepers in the colony collapse disorder controversy.

44. Buchmann SL, Nabhan GP: **The Forgotten Pollinators**. Island Press; 1997.
45. Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T: **Importance of pollinators in changing landscapes for world crops**. *Proc. Biol. Sci.* 2007, **274**:303-313. doi:10.1098/rspb.2006.3721
46. Brussaard L, Caron P, Campbell B, Lipper L, Mainka S, Rabbinge R, Babin D, Pulleman M: **Reconciling biodiversity conservation and food security: Scientific challenges for a new agriculture**. *Current Opinion in Environmental Sustainability* 2010, **2**:34-42.
47. Settele J, Penev L, Georgiev T, Grabaum R, Grobelnik V, Hammen V, Klotz S, Kühn I (Eds): *Atlas of biodiversity risk*. Sofia: Pensoft; 2010.
- ** 48. Lautenbach S, Seppelt R, Liebscher J, Dormann CF: **Spatial and Temporal Trends of Global Pollination Benefit**. *PLOS ONE* 2012, **7**:e35954. doi:10.1371/journal.pone.0035954
- The first spatially explicit map of pollination demands at world level identifies the areas that benefit and depend the most on pollination. Based on this maps the dependency of agriculture on pollination is much higher than what has been so far described. Increases in the price of pollination dependent crops were considered as an early warning tool on the abundance of pollinators. Still the effects of international trade of pollination-dependent crops have not been taken into account, in which case the dependency of developed countries would be higher than shown.
49. Gallai N, Salles JM, Settele J, Vaissière BE: **Economic valuation of the vulnerability of world agriculture confronted with pollinator decline**. *Ecological Economics* 2009, **68**:810–821. doi:10.1016/j.ecolecon.2008.06.014
50. Winfree R, Gross BJ, Kremen C: **Valuing pollination services to agriculture**. *Ecol Econ* 2011, **71**:80-88.
51. Maes J, Hauck J, Paracchini ML, Ratamäki O, Hutchins M, Termansen M, Furman E, Pérez-Soba M, Braat L, Bidoglio G: **Mainstreaming ecosystem services into EU policy**. *Current Opinion in Environmental Sustainability*, 2013, **5**:128-134. 10.1016/j.cosust.2013.01.002
52. Kevan PG, Menzel R: **The plight of pollination and the interface of neurobiology, ecology and food security**. *Environmentalist* 2012, **32**:300–310. DOI 10.1007/s10669-012-9394-5
53. Ollerton J, Winfree R, Tarrant S: **How many flowering plants are pollinated by animals?** *Oikos* 2011, **120**: 321–326. doi: 10.1111/j.1600-0706.2010.18644.x
- * 54. Eilers EJ, Kremen C, Smith Greenleaf S, Garber AK, Klein AM: **Contribution of Pollinator-Mediated Crops to Nutrients in the Human Food Supply**. *PLOS ONE* 2011, **6**:e21363. doi:10.1371/journal.pone.0021363
- Pollinator mediated crops account for >90% of vitamin C, 100% of lycopene, almost 100% of the antioxidants β -cryptoxanthin and β -tocopherol, the majority of the lipid, vitamin A and related carotenoids, calcium and fluoride, and a large portion of folic acid. Ongoing pollinator decline may thus put the provision of a nutritionally adequate diet for the global human population at risk.
55. Dams LR: **Bees and honey-hunting scenes in the Mesolithic rock art of eastern Spain**. *Bee World* 1978, **59**:43-53.

56. Pattinson D: Pre-modern beekeeping in China: A short history. *Agricultural History* 2012, **86**(4) 235-255.
57. Bloch G, Francoy TM, Wachtel I, Panitz-Cohen N, Fuchs S, Mazar A: **Industrial apiculture in the Jordan valley during Biblical times with Anatolian honeybees.** *Proc Natl Acad Sci U S A* 2010, **107**:11240-11244.
58. Ebert A: **Nectar for the taking: The popularization of scientific bee culture in England, 1609-1809.** *Agricultural History* 2011, **85**:322-343.
59. Breeze TD, Bailey AP, Balcombe KG, Potts SG: **Pollination services in the UK: How important are honeybees?** *Agriculture, Ecosystems & Environment* 2011, **142**:137-143.
doi:10.1016/j.agee.2011.03.020
60. Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, Bartomeus I, Benjamin F *et al.*: **Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance.** *Science* 2013, **339**:1608-1611. DOI: 10.1126/science.1230200
61. Banskota AH, Tezuka Y, Kadota S: **Recent progress in pharmacological research of propolis.** *Phytotherapy Research* 2001, **15**:561-571.
62. Jull AB, Rodgers A, Walker N: **Honey as a topical treatment for wounds.** *Cochrane Database of Systematic Reviews* 2009, **4**:art.no. CD005083.
63. Srinivasan MV: **Honeybees as a Model for the Study of Visually Guided Flight, Navigation, and Biologically Inspired Robotics.** *Physiol. Rev.* 2011, **91**:413-460
64. Rogers J, Sleigh C: **Here is my honey-machine: Sylvia plath and the mereology of the Beehive,** *Review of English Studies* 2012, **63**:293-310.
65. Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers, P. *et al.* **Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands..** *Science* 2006, **313**:351-354. doi:10.1126/science.1127863
66. Holden C: **Ecology: report warns of looming pollination crisis in North America.** *Science* 2006, **314**:397-397. doi: 10.1126/science.314.5798.397.
67. Goulson D, Lye GC, Darvill B: **Decline and conservation of bumblebees.** *Ann Rev Entomol* 2008, **53**:191-208.
68. Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE: **Global pollinator declines : trends , impacts and drivers.** *Trends in Ecology & Evolution* 2010, **25**:345-353.
doi:10.1016/j.tree.2010.01.007
69. Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL: **Patterns of widespread decline in North American bumble bees.** *PNAS* 2010, **108**:662-667
70. Burkle LA, Marlin JC, Knight TM: **Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence, and Function.** *Science* 2013, **339**. 1611-1615. DOI: 10.1126/science.1232728
71. UNEP: *Global honey bee colony disorders and other threats to insects.* Agriculture United Nations Environmental Program; 2010.

- * 72 van der Zee R, Pisa L, Andonov S, Brodschneider R, Chlebo R, Coffey MF, Crailsheim K, Dahle B, Gajda A, Gray A, *et al.*: **Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008-9 and 2009-10.** *Journal of Apicultural Research* 2012, **51**:100–114. doi:10.3896/IBRA.1.51.1.12

This publication gathers the to-date most harmonised information on colony losses worldwide. Colony bee losses are voluntarily communicated by beekeepers through a common worldwide questionnaire designed by the scientific network COLOSS.

73. VanEngelsdorp D, & Meixner M: **A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them.** *J Invertebr Pathol* 2010, **103**:S80–95. doi:10.1016/j.jip.2009.06.011
74. VanEngelsdorp D, Caron D, Hayes J, Underwood R, Henson M, Spleen A, Andree M, Andree M, Snyder R, Lee K, Roccasecca K, Wilson M, Wilkes J, Lengerich E, Pettis J: **A national survey of managed honey bee 2010-11 winter colony losses in the USA : results from the Bee Informed Partnership.** *Journal of Apicultural Research* 2012, **51**:115–124. doi:10.3896/IBRA.1.51.1.14
- * 75. Taniguchi T, Kita Y, Matsumoto T, Kimura K: **Honeybee colony losses during 2008-2010 caused by pesticide application in Japan.** *Journal of Apiculture* 2012, **27**:15-27

A survey carried out to beekeepers over three consecutive years identified large numbers of bee losses due to acute intoxications to putatively neonicotinoids. The colonies around rice and orange fields were the most affected.

76. Yamada T, Yamada K and Wada N: **Influence of dinotefuran and clothianidin on a bee colony.** *Jpn. J. Clin. Ecol* 2012, **21**:10-23.
77. Maxim L, Van der Sluijs JP: **Expert explanations of honeybee losses in areas of extensive agriculture in France: Gaucho® compared with other supposed causal factors.** *Environmental Research Letters* 2010, **5**:014006. doi 10.1088/1748-9326/5/1/014006
78. Runckel C, Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R, DeRisi JL: **Temporal Analysis of the Honey Bee Microbiome Reveals Four Novel Viruses and Seasonal Prevalence of Known Viruses, Nosema, and Crithidia.** *PLOS ONE* 2011, **6**:e20656. doi: 10.1371/journal.pone.0020656.
- * 79. Farooqui T: **A potential link among biogenic amines-based pesticides, learning and memory, and colony collapse disorder: A unique hypothesis.** *Neurochemistry international* 2012, **62**:122-36. doi: 10.1016/j.neuint.2012.09.020

An insightful discussion of a hypothetical link among biogenic amines-based pesticides (neonicotinoids and formamidines) and their disruptive effects on biogenic amine signaling causing olfactory dysfunction in honeybees. The hypothesis that chronic exposure disrupts neural cholinergic and octopaminergic signaling in honeybees is supported by the fact that abnormality in biogenic amines-mediated neuronal signaling impairs their olfactory learning and memory. This explains why foragers exposed to neonicotinoids fail to return to their hive – a possible cause of CCD.

80. Stokstad E: Agriculture. Field research on bees raises concern about low-dose pesticides. *Science* 2012, **335**:1555. doi: 10.1126/science.335.6076.1555.

- ** 81. Whitehorn PR, O'Connor S, Wackers FL, Goulson D: **Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production.** *Science* 2012, **351**. doi:10.1126/science.1215025

Colonies of bumble bees (*Bombus terrestris*) exposed to field relevant doses of imidacloprid showed significantly lower growth rate and a reduction of 85% of new queen production. This means a severe negative impact on the bumblebees' population.

- ** 82 Henry M, Béguin M, Requier F, Rollin O, Odoux JF, Aupinel P, Aptel J, Tchamitchian S, Decourtye A: **A common pesticide decreases foraging success and survival in honey bees.** *Science* 2012, **336**:348–50. doi:10.1126/science.1215039

Bees exposed to doses of thiamethoxam at 1,34 ng/bee significantly decrease their ability to carry out their homing flight up to levels triggering depopulation dynamics in their colony. RFID tagging technology is used to register the activity of foragers leaving and entering the hive.

83. Stoner KA, Eitzer BD: **Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*).** *PLOS ONE* 2012, **7**:e39114. doi: 10.1371/journal.pone.0039114

84. Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Di Bernardo A, Greatti M, Giorio C, Tapparo A: **Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees.** *J Econ Entomol* 2009, **102**:1808–15.

- * 85. European Food Safety Authority: **Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)** *EFSA Journal* 2012, **10**:2668. doi:10.2903/j.efsa.2012.2668

This non-comprehensive, but rather complete review, includes information about many of the following subjects: (1) a definition of specific protection goals linked to pollinators; (2) exposure paths of bees to pesticides defined in scientific literature; (3) toxicological effects observed; (4) synergistic effects of pesticides and pathologies and among pesticide molecules, (5) an analyses of the different toxicological tests currently carried out for risk assessment. On the basis of the previous points, a new proposal for pesticide risk assessment on bees is proposed.

86. Beekman M, Ratnieks FLW: **Long-range foraging by the honey-bee, *Apis mellifera* L.,** *Functional Ecology* 2000, **14**:490-496.

87. Goulson D, Lepais O, O'Connor S, Osborne JL, Sanderson RA, Cussans J, Goffe L & Darvill B.: **Effects of land use at a landscape scale on bumblebee nest density and survival.** *Journal of applied ecology* 2010, **46**:1207-1215.

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

89. Chauzat M-P, Faucon J-P, Martel A-C, Lachaize J, Cougoule N, Aubert M: **A Survey of Pesticide Residues in Pollen Loads Collected by Honey Bees in France,** *J. Econ. Entomol.* 2006, **99**:253-262.

90. Chauzat MP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon JP: **Influence of pesticide residues on honey bee (*Hymenoptera: Apidae*) colony health in France.** *Environ Entomol* 2009, **38**:514–523.

91. Chauzat, M-P., Martel A-C., Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, et al. (2011). **An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France.** *Environmental toxicology and chemistry* **30**:103-111. doi:10.1002/etc.361
92. Blacquièrè T, Smaghe G, Van Gestel CM, Mommaerts V: **Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment.** *Ecotoxicology* 2012, **21**:973-992. doi10.1007/s10646-012-0863-x
93. Dively GP, Kamel A: **Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators.** *Journal of agricultural and food chemistry* 2012, **60**:4449-4456. doi 10.1021/jf205393x
94. Tapparo A, Giorio C, Marzaro M., Marton D, Soldà L, Girolami V: **Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds.** *Journal of environmental monitoring* : JEM 2011, **13**:1564-1568. doi:10.1039/c1em10085h
95. Hoffmann, EJ, Castle S J: **Imidacloprid in Melon Guttation Fluid : A Potential Mode of Exposure for Pest and Beneficial Organisms.** *Journal of Economic Entomology* 2012, **105**:67–71.
96. Mullin C, Frazier M, Frazier JL, Ashcraft, S, Simonds R, Vanengelsdorp D, Pettis JS: **High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health.** *PLOS ONE* 2010, **5**:e9754. doi:10.1371/journal.pone.0009754
97. Yáñez KP, Bernal JL, Nozal MJ, Martín MT, Bernal J: **Determination of seven neonicotinoid insecticides in beeswax by liquid chromatography coupled to electrospray-mass spectrometry using a fused-core column.** *Journal of chromatography A* 2013. **1285**:110–117. doi:10.1016/j.chroma.2013.02.032
98. Laurino D, Porporato M, Patetta A, Manino A: **Toxicity of neonicotinoid insecticides to honey bees : laboratory tests.** *Bulletin of Insectology* 2011, **64**:107–113.
99. Abbott V, Nadeau JL, Higo H, Winston ML: **Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae).** *Journal of economic entomology* 2008, **101**:784-796.
100. Scott-Dupree CD, Conroy L, Harris CR: **Impact of Currently Used or Potentially Useful Insecticides for Canola Agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymenoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae).** *J Econ Entomol* 2009, **102**:177-182.
101. Gradish AE, Scott-Dupree CD, Shipp L, Harris CR and Ferguson G: **Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae).** *Pest. Man. Sci.* 2010, **66**:142-146.
102. Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G, Smaghe G: **Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior.** *Ecotoxicology* 2010, **19**:207-215. doi:10.1007/s10646-009-0406-2
103. Jeyalakshmi T, Shanmugasundaram R, Saravanan M, Geetha S, Mohan SS, Goparaju A, Balakrishna

Murthy R: **Comparative toxicity of certain insecticides against *apis cerana indica* under semi field and laboratory conditions.** *Pestology* 2011, **35**:23-26.

** 104. Tapparo A, Marton D, Giorio C, Zanella A, Solda L, Marzaro M, Vivan L, Girolami V: **Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds.** *Environmental Science and Technology* 2012, **46**:2592-9. doi: 10.1021/es2035152.

Thanks to a quick analytical method, new potential sources of exposure to pesticides (clothianidin and thiamethoxam) in the environment have been proved: significant amounts of coating particles are emitted by pneumatic drilling machines during corn sowing. Coarse particles seem to sediment closer to the sown field, while fine particles cover longer distances thanks to the wind. The amount of toxic compound released is toxic to bees, and this toxicity is facilitated by humidity.

105. Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A: **Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication.** *Journal of Applied Entomology* 2012, **136**:17–26. doi:10.1111/j.1439-0418.2011.01648.x

106. Girolami V, Marzaro M, Vivan L, Mazzon L, Giorio C, Marton D, Tapparo A: **Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers.** *Journal of Applied Entomology* 2013, **137**:35–44. doi:10.1111/j.1439-0418.2012.01718.x

107. ApeNet: *Effects of coated maize seed on honey bees - Report based on results obtained from the second year (2010) activity of the APENET project.* 2012.

108. Pochi D, Biocca M, Fanigliulo R, Pulcini P, Conte E: **Potential exposure of bees, *Apis mellifera* L., to particulate matter and pesticides derived from seed dressing during maize sowing.** *Bull Environ Contam Toxicol* 2012, **89**:354-61. doi: 10.1007/s00128-012-0664-1

109. Tapparo A, Giorio C, Soldà L, Bogialli S, Marton D, Marzaro M, Girolami V: **UHPLC-DAD method for the determination of neonicotinoid insecticides in single bees and its relevance in honeybee colony loss investigations.** *Analytical and bioanalytical chemistry* 2013, **405**:1007-1014. doi:10.1007/s00216-012-6338-3

110. Gross M: **Pesticides linked to bee deaths.** *Current Biology* 2008, **18**:684.

111. Sgolastra F, Renzi T, Draghetti S, Medrzycki P, Lodesani M, Maini S, Porrini C: **Effects of neonicotinoid dust from maize seed-dressing on honey bees.** *Bulletin of Insectology* 2012, **65**:273-280.

112. European Commission: *Commission Directive 2010/21/EU amending Annex I to Council Directive 91/414/EEC as regards the specific provisions relating to clothianidin, thiamethoxam, fipronil and imidacloprid.* OJ L 65, 13.3.2010

113. Biocca M, Conte E, Pulcini P, Marinelli E, Pochi D: Sowing simulation tests of a pneumatic drill equipped with systems aimed at reducing the emission of abrasion dust from maize dressed seed. *Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes*, **46**:438-448. doi:10.1080/03601234.2011.583825

114. Marzaro M, Vivan L, Targa A, Mazzon L, Mori N, Greatti M, Petrucco Toffolo E, Di Bernardo A,

Giorio C, Marton D, *et al.*: **Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat.** *Bulletin of Insectology* 2011, **64**:119–126.

115. Austria: *Investigations in the incidence of bee losses in corn and oilseed rape growing areas of Austria and possible correlations with bee diseases and the use of insecticidal plant protection products (MELISA).* Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH, Institut für Pflanzenschutzmittel. 2012.
116. Oliveira RA, Roat TC, Carvalho SM, Malaspina O: **Side-Effects of Thiamethoxam on the Brain and Midgut of the Africanized Honeybee *Apis mellifera* (Hymenoptera: Apidae).** *Environmental Toxicology* in press, DOI: 10.1002/tox.21842
117. Sánchez-Bayo F: **From simple toxicological models to prediction of toxic effects in time.** *Ecotoxicology* 2009, **18**:343–354. doi:10.1007/s10646-008-0290-1
118. Tennekes HA: **The Significance of the Druckrey-Küpfmüller Equation for Risk Assessment - The Toxicity of Neonicotinoid Insecticides to Arthropods is Reinforced by Exposure Time.** *Toxicology* 2010, **276**:1–4. doi:10.1016/j.tox.2010.07.005
119. Maus C, Nauen R: **Response to the publication: Tennekes, H.A. (2010): The significance of the Druckrey–Küpfmüller equation for risk assessment—The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time.** *Toxicology* 2011, **280**:176–177. doi:10.1016/j.tox.2010.11.014
120. Tennekes HA: **The significance of the Druckrey–Küpfmüller equation for risk assessment—The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time: Responding to a Letter to the Editor by Drs. C. Maus and R. Nauen of Bayer CropScience AG.** *Toxicology* 2011, **280**:173–175. doi:10.1016/j.tox.2010.11.015
- * 121. Tennekes HA and Sánchez-Bayo F: **Time-Dependent Toxicity of Neonicotinoids and Other Toxicants: Implications for a New Approach to Risk Assessment.** *J Env Anal Toxicol* 2011, **S4**:001. doi:10.4172/2161-0525.S4-001

Neonicotinoids have an irreversible impact on nAChR which implies that a time-to-event model of toxicity is the adequate description of their chronic toxicity profile. Because of time dependence of the toxic effect, the standard risk assessment procedures are not valid in case of exposure to sublethal concentrations of neonicotinoids for long periods of time, so LC50 is a misleading indicator for harm.

122. Mason R, Tennekes H, Sánchez-Bayo F and Uhd Jepsen P: **Immune Suppression by Neonicotinoid Insecticides at the Root of Global Wildlife Declines.** *Journal of Environmental Immunology and Toxicology* 2013, **1**:2-12. DOI: 10.7178/jeit.1
123. Lu C, Warchol KM, Callahan RA: **In situ replication of honey bee colony collapse disorder.** *Bulletin of Insectology* 2012, **65**:99–106.
124. Desneux N, Decourtye A, Delpuech JM: **The sublethal effects of pesticides on beneficial arthropods.** *Annu Rev Entomol* 2007, **52**:81–106. doi:10.1146/annurev.ento.52.110405.091440
125. Easton AH, Goulson D: **The Neonicotinoid Insecticide Imidacloprid Repels Pollinating Flies and Beetles at Field-Realistic Concentrations.** *PLOS ONE* 2013; **8**:e54819.

doi:10.1371/journal.pone.0054819

126. Bortolotti L, Montanari R, Marcelino J, Medrzycki P, Maini S, Porrini C: **Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees.** *Bulletin of Insectology* 2003, **56**:63-67.
127. Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, Cluzeau S, Pham-Delègue MH: **Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*.** *Arch Environ Contam Toxicol* 2005, **48**:242–50. doi:10.1007/s00244-003-0262-7
128. Gauthier, M: State of the art on insect nicotinic acetylcholine receptor function in learning and memory. In: Thany, S.H. (ed.) *Insect Nicotinic Acetylcholine Receptors*, pp. 97–115. Springer, Berlin, 2010.
- * 129. Yang EC, Chang HC, Wu WY, Chen YW: **Impaired Olfactory Associative Behavior of Honeybee Workers Due to Contamination of Imidacloprid in the Larval Stage.** *PLOS ONE* 2012, **7**:e49472. doi: 10.1371/journal.pone.0049472.

A short term sublethal dosage of imidacloprid given to honeybee larvae has a long term effect: it renders the olfactory associative behavior of the adult bees impaired. This may affect the survival condition of the entire colony, even though the larvae survive to adulthood. Also, the brood-capped, pupation, and eclosion rates of the larvae decreases significantly with dose.

130. Williamson SM, Wright GA: **Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees.** *Journal of Experimental Biology* 2013, **216**:1799-1807. doi:10.1242/jeb.083931
131. Yang EC, Chuang YC, Chen YL, Chang LH. **Abnormal Foraging Behavior Induced by Sublethal Dosage of Imidacloprid in the Honey Bee (Hymenoptera: Apidae).** *Journal of Economic Entomology* 2008, **101**:1743–1748. doi 10.1603/0022-0493-101.6.1743
132. Decourtye A, Devillers J, Aupinel P, Brun F, Bagnis C, Fourrier J, Gauthier M: **Honeybee tracking with microchips: a new methodology to measure the effects of pesticides.** *Ecotoxicology* 2011, **20**:429–37. doi:10.1007/s10646-011-0594-4
133. Schneider CW, Tautz J, Grünewald B, Fuchs S: **RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*.** *PLOS ONE* 2012, **7**:e30023. doi:10.1371/journal.pone.0030023
- * 134. Teeters BS, Johnson RM, Ellis MD, Siegfried BD: **Using video-tracking to assess sublethal effects of pesticides on honey bees (*Apis mellifera* L.)** *Environmental Toxicology and Chemistry* 2012, **31**:1349-54. doi: 10.1002/etc.1830

Video-tracking technology has been used to measure effects on behaviour of two pesticides: tau-fluvalinate (topical) and imidacloprid (oral). While at very low dose ($0.05 \mu\text{g kg}^{-1}$) imidacloprid showed an activating effect, starting at $0.5 \mu\text{g kg}^{-1}$ imidacloprid triggered a dose dependent negative effect: an increase in time spent at feeder, while distance travelled and interaction time between bees decreases with dose.

135. Eiri DM, Nieh JC: **A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing.** *J Exp Biol* 2012, **215**:2022-9. doi: 10.1242/jeb.068718

136. Medrzycki P, Sgolastra F, Bortolotti L, Bogo G, Tosi S, Padovani E, Porrini C, Sabatini AG: **Influence of brood rearing temperature on honey bee development and susceptibility to intoxication by pesticides.** *Journal of Apicultural Research* 2010, **49**:52-59.
137. Cresswell JE, Page CJ, Uygun MB, Holmbergh M, Li Y, Wheeler JG, Laycock I, Pook CJ, de Ibarra NH, Smirnoff N, et al.: **Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid).** *Zoology* 2012, **115**:365-71. doi: 10.1016/j.zool.2012.05.003.
- *138. Gill RJ, Ramos-Rodriguez O, Raine NE: **Combined pesticide exposure severely affects individual- and colony-level traits in bees.** *Nature*, 2012, **491**:105-8. doi: 10.1038/nature11585
- Chronic exposure of bumble bees to a neonicotinoid (imidacloprid) and a pyrethroid (λ -cyhalothrin) at field-relevant concentrations reduced the foraging performance and increased worker mortality. This leads to a reduction of brood production and colony success. A synergistic effect of both pesticides is clearly observed.
- * 139. Laycock I, Lenthall KM, Barratt AT, Cresswell JE: **Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*).** *Ecotoxicology* 2012, **21**:1937-45. doi: 10.1007/s10646-012-0927-y / Laycock I, Lenthall KM, Barratt AT, Cresswell JE: **Erratum to: Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*).** *Ecotoxicology* 2012, **21**:1937-45. doi: 10.1007/s10646-012-0927-y
- Environmental relevant concentrations of imidacloprid negatively affects ovary development and fecundity in bumble bees. Dietary imidacloprid at $1 \mu\text{g l}^{-1}$ is capable to reduce brood production by one third. The causal speculations include a feeding reduction linked to toxic exposure and/or alteration of the social communication among bumblebee workers.
140. Smodis Skerl MIS., Gregorc A: **Heat shock proteins and cell death in situ localisation in hypopharyngeal glands of honeybee (*Apis mellifera carnica*) workers after imidacloprid or coumaphos treatment.** *Apidologie* 2010, **41**:73–86
141. Heylen K, Gobin B, Arckens L, Huybrechts R, Billen J: **The effects of four crop protection products on the morphology and ultrastructure of the hypopharyngeal gland of the European honeybee, *Apis mellifera*.** *Apidologie* 2011, **42**:103–116. doi:10.1051/apido/2010043
- ** 142. Hatjina F, Papaefthimiou C, Charistos L, Dogaroglu T, Bouga M, Emmanouil C, Arnold G: **Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo.** *Apidologie* 2013. doi 10.1007/s13592-013-0199-4
- Imidacloprid administered under laboratory conditions to honeybees at $2 \mu\text{g kg}^{-1}$ in sugar solution and $3 \mu\text{g kg}^{-1}$ in pollen pastry has sublethal effects on the development of the hypopharyngeal glands (HPGs) and respiratory rhythm. 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. Imidacloprid 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.
143. van Tome HV, Martins GF, Lima MAP, Campos LAO, Guedes RNC: **Imidacloprid-induced impairment of mushroom bodies and behavior of the native stingless bee *Melipona quadrifasciata***

anthidioides. *PLOS ONE* 2012, **7**:e38406. doi: 10.1371/journal.pone.0038406

144 De Almeida Rossi C, Roat TC, Tavares DA, Cintra-Socolowski P, Malaspina O: **Effects of sublethal doses of imidacloprid in malpighian tubules of africanized *Apis mellifera* (Hymenoptera, Apidae)**.

Microscopy research and technique 2013, **76**:552–558. doi:10.1002/jemt.22199

145 Wu JY, Anelli CM, Sheppard WS: **Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*apis mellifera*) development and longevity**. *PLOS ONE* 2011, **6**:e14720.

10.1371/journal.pone.0014720

* 146. Cresswell, JE: **A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees**. *Ecotoxicology* 2010, **20**:149–57. doi:10.1007/s10646-010-0566-0

This meta-analysis shows that dietary imidacloprid at field realistic doses is able to induce sublethal effects at colony level under either acute or chronic regimes (between 6% and 20% performance reduction). The statistical power of the existing field tests that claim to show no effects of neonicotinoids on honeybee colonies is insufficient to support a claim that such effects don't exist.

147. Cressey D: **Europe debate risks to bees**. *Nature* 2013, **496**:408. doi:10.1038/496408a

148. Becher MA, Thorbek P, Kennedy PJ, Osborne J, Grimm V: **Towards a systems approach for understanding honeybee decline: a stock-taking and synthesis of existing models**. *Journal of Applied Ecology* in press.

149. Moritz RFA, Southwick EE: *Bees as superorganisms: an evolutionary reality*. Springer-Verlag; 1992

150. Khoury DS, Myerscough MR, Barron AB: **A Quantitative Model of Honey Bee Colony Population Dynamics**. *PLOS ONE* 2012, **6**:e18491. doi 10.1371/journal.pone.0018491

151. Iwasa T, Motoyama N, Ambrose JT, Roe RM: **Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera***. *Crop Protection* 2004, **23**:371-378. doi:10.1016/j.cropro.2003.08.018

152. Krohn EA, Synergistic mixtures exhibiting insecticidal and fungicidal action. 2008. US Patent US2008/0261811

153. Jamet EA, Pesticidal mixtures. 2011. US Patent US2011/0046123

** 154 Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP, Le Conte Y: **Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*)**. *Environmental microbiology* 2010, **12**:774–82. doi:10.1111/j.1462-2920.2009.02123.x

This study demonstrates the joint effect of a pathogen microsporidia (*Nosema ceranae*) and imidacloprid on honeybee health. A synergistic effect appears whenever honeybees are exposed to environmental relevant doses of the latter. Significant reduction of the enzyme glucose oxidase, enzyme linked to the social immunity of the colony, was observed in the groups exposed to both stressors.

* 155. Pettis JS, Vanengelsdorp D, Johnson J, Dively G: **Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema***. *Naturwissenschaften* 2012, **99**:153-8. doi: 10.1007/s00114-011-0881-1

Nosema infection increases significantly whenever bee colonies are exposed chronically to sublethal levels

of the neonicotinoid, imidacloprid. 5 and 20 $\mu\text{g kg}^{-1}$ imidacloprid were administered to pollen patties and provided to bee colonies over 5 and 8 weeks.

156. Aufauvre J, Biron DG, Vidau C, Fontbonne R, Roudel M, Diogon M, Viguès B, Belzunces LP, Delbac F, Blot N. **Parasite-insecticide interactions: A case study of *Nosema ceranae* and fipronil synergy on honeybee.** *Nature Scientific Reports* 2012, **2**:326
157. Paula AR, Carolino AT, Paula CO, Samuels RI: **The combination of the entomopathogenic fungus *Metarhizium anisopliae* with the insecticide Imidacloprid increases virulence against the dengue vector *Aedes aegypti* (Diptera: Culicidae).** *Parasites & vectors* 2011, **4**:8. doi:10.1186/1756-3305-4-8.
158. Boucias DG, Stokes C, Storey G, Pendland JC: **The effects of imidacloprid on the termite *Reticulitermes flavipes* and its interaction with the mycopathogen *Beauveria bassiana*.** *Pflan. Nachr. Bayer* 1996, **49**:103–144.
159. Quintella ED, McCoy CW: **Pathogenicity enhancement of *Metarhizium anisopliae* and *Beauveria bassiana* to first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with sublethal doses of imidacloprid.** *Environ. Entomol* 1997, **26**:1173-1182
160. Koppenhöfer AM, Grewal PS, Kaya HK: **Synergism of imidacloprid and entomopathogenic nematodes against white grubs: the mechanism.** *Entomol. Exp. Appl.* 2000, **94**:283–293.
- 161 James RR, Xu J: **Mechanisms by which pesticides affect insect immunity.** *Journal of invertebrate pathology* 2012, **109**:175-182. doi:10.1016/j.jip.2011.12.005
- * 162. Fagin D: **The learning curve.** *Nature* 2012, **490**:5-8.
- Researchers say that some chemicals have unexpected and potent effects at very low doses. The complex interplay of receptor binding and gene reprogramming can generate bizarre dose-response relationships, many of which are still being mapped out. Regulators aren't convinced.
163. Stokstad E: **Pesticides under fire for risks to pollinators.** *Science* 2013, **340**: 674-675.