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SCIENTIFIC REVIEWS

Neonicotinoid insecticides. Banned by the European Union in 2018 after scientific studies concluded that harm honey bees

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Abstract

The European Union voted in April 2018 to ban three controversial neonicotinoid insecticides on all crops grown outdoors because of their adverse effects on pollinators, especially honey bees. The decision caused quite a stir in the farming community because neonicotinoid insecticides had certain advantages in crops like soya. For centuries, gardeners have been using home-made mixtures of tobacco leaves and water as a natural pesticide to kill insect pests. The first commercially available compound was imidacloprid and has been in use since the early 1990s. Neonicotinoids and Fipronil account for approximately 1/3 of the insecticide market worldwide. The success of neonicotinoids as insecticides in agriculture was the result of no known pesticide resistance in target pests, especially boring insects and roof-feeding insects that cannot easily controlled by foliar sprays. Neonicotinoids are licensed for use in more than 120 countries and have a global market value of $2·6 billion, with imidacloprid alone comprising 41% of this market and being the second most widely used agrochemical in the world. In the last 20 years studies investigated if neonicotinoids have contributed to yield increases in farming or whether neonicotinoids offer economic benefits compared to alternatives. Most results were negative. In the last decade much of the controversy over the use of neonicotinoids has focussed on their effects on honey bees. Neonicotinoids are routinely used to dress seeds of oilseed rape, sunflower and maize, and these crops are major forage sources for both managed honeybees and wild pollinators in arable landscapes. Being systemic, small concentrations of neonicotinoids are found in both pollen and nectar of seed-treated crops. Neonicotinoids are also routinely applied as foliar sprays to fruit crops such as raspberries (mainly thiacloprid), which are visited by both managed and wild pollinators. In 27 April 2018 the European Commission expanded a controversial ban of three neonicotinoid insecticides because of the threat they pose to pollinators. Already, from 2013 the EU placed a moratorium on 3 neonicotinoids forbidbiting their use in flowering crops that appeal to honey bees and other pollinators. The last decision was greeted with trepidation by farming associations which fear economic harm. There is no ban of neonicotinoids in other countries. This review presents all the facts from scientific studies on neonicotinoids.
Introduction

Tobacco water extracts with high concentrations of nicotine have been used for several centuries to control insects in various agricultural countries and on a small scale as a natural organic pesticide. It is no wonder that for centuries, gardeners have been using home-made mixtures of tobacco and water as a natural pesticide to kill insect pests. Nicotine extracted from tobacco (Nicotiana spp.) and other members of the nightshade (Solanaceae) family of plants has been used for centuries to kill sucking insects such as aphids, thrips and spider mites. Nicotine sulfate is considered as a botanical pesticide and allowed in organic gardening, but is no longer available commercially because of its toxicity. Tobacco leaves were first utilized as an insecticide in 1690, as a wash having been applied to pear trees in France to control the pear lacebug and in 1763 was applied as a remedy for plant lice. In 1773 tobacco extracts were used against plant lice and red spider in England. Also, there are records that tobacco was used as an insecticide in USA in 1814 against sucking insects.¹

![Chemical structure of Nicotine](image1)

**Figure 1.** Farmers in regions with tobacco, used to substitute chemical pesticides with natural extracts from tobacco leaves. Finely chopped tobacco leaves were soaked in water for 24 hours, then filtered and the liquid was used for spraying plants. On the left women making pesticides from tobacco plant (Kenya Institute of Organic Farming, Nairobi, Kenya).
Neonicotinoids are widely used insecticides in agriculture

Scientific research on Neonicotinoid insecticides lasted many years and the first products for application in agricultural fields started in the 1980s. The first commercially available compound was imidacloprid and has been in use since the early 1990s. Neonicotinoids and Fipronil (a broad use insecticide that belongs to the phenylpyrazole chemical family) account for approximately 1/3 of the insecticide market worldwide. The annual world production of the archetype neonicotinoid, imidacloprid, was estimated to be ca. 20,000 tons active substance in 2010. The success of neonicotinoids as insecticides in agriculture was the result of no known pesticide resistance in target pests. Neonicotinoids had low toxicity and physicochemical properties that offered many advantages over previous generations of insecticides (i.e., organophosphates, carbamates, pyrethroids, etc.). Also, neonicotinoids were less toxic to farmers and consumers consuming food products. Neonicotinoid have become the most widely used group of insecticides worldwide. Applications ranging from plant protection (crops, vegetables, fruits), veterinary products, and biocides to invertebrate pest control in fish farming. Commercially there are available for agricultural applications 7 neonicotinoid compounds as insecticides worldwide. The most important are Imidacloprid and Thiacloprid (developed by Bayer CropScience), Clothianidin (Bayer CropScience and Sumitomo), Thiamethoxam (Syngenta), Acetamiprid (Nippon Soda), Nitenpyram (Sumitomo), and Dinotefuran (Mitsui Chemicals). An 8th compound, Sulfoxaflor has recently come onto the market in China and the USA (Dow Agro Sciences).  

All neonicotinoids are systemic insecticides. Systemic pesticides are water-soluble, so they can easily move throughout a plant as it absorbs water and is distributed throughout its tissues, stem, leaves, roots, and any fruits or flowers. Neonicotinoids act by binding strongly to nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects, causing nervous stimulation at low concentrations, but receptor blockage, paralysis and death at higher concentrations. Neonicotinoids bind more strongly to insect nAChRs than to those of vertebrates, so they are selectively more toxic to insects.
Neonicotinoid insecticides are generally toxic to insects in minute quantities; for example, the LD$_{50}$ (Lethal Dose that kills 50% of individuals) for ingestion of imidacloprid and clothianidin in honeybees is 5 and 4 ng (nanogram = $10^{-9}$ g), per insect, respectively. The toxicity of neonicotinoids is at very low concentration. For comparison is approximately 1/10,000th of the LD$_{50}$ for the well known polychlorinated insecticide DDT (dichlorodiphenyltrichloroethylene).$^6$

The most important feature of neonicotinoids is that they are water soluble and are readily absorbed by plants via either their roots or leaves and then are transported throughout the tissues of the plant. This provides many advantages in pest control, for they protect all parts of the plant. Neonicotinoids are effective against boring insects and root-feeding insects, both of which cannot easily be controlled using foliar sprays of non-systemic insecticide compounds. Concentrations of neonicotinoids in plant tissues and sap between 5 and 10 ppb, which are very low concentrations (parts per billion), are generally regarded as sufficient to provide protection against pest insects.$^7,^8$

Neonicotinoids in the USA and in W. Europe are predominantly used as seed dressings for a broad variety of crops such as oilseed rape, sunflower, cereals, beets and potatoes (primarily imidaclolprid, clothianidin and thiamethoxam). Studies
showed that in the United Kingdom (UK), use as a seed dressing accounted for 91% of all neonicotinoid use in farming in 2011. Seed dressing was the most common use of neonicotinoids in 60% of cases worldwide, because do not require further action from the farmer. Neonicotinoids protect all parts of the crop for several months following sowing, and they are also regarded as providing better targeting of the crop than spray applications.

Neonicotinoids have many different insecticidal applications. They are commonly used as foliar sprays on horticultural crops such as soft fruits and on some arable crops such as soya, and they are sold for garden use as a spray on flowers and vegetables. They are used in bait formulations for domestic use against cockroaches and ants and also as granular formulations for the treatment of pasture against soil-dwelling insect pests. They can be applied as a soil drench or in irrigation water to defend perennial crops such as vines, and they can be injected into timber to combat termites or into trees to protect them against herbivores, where a single application can provide protection for several years. Finally, they are commonly used in topical applications on pets such as dogs and cats to control external parasites.

Figure 2. Neonicotinoids are used as foliar sprays on horticultural crops (soft fruits and on some arable crops such as soya). Neonicotinoids are used in gardens as spray on flowers and vegetables. Their advantages of low toxicity to vertebrates, high toxicity to insects, flexible use and systemic activity led to neonicotinoids swiftly becoming among the most widely used insecticides globally.
Neonicotinoids in the last decade are used more than any other class of insecticides and comprise approximately 25% of all insecticides used. They are licensed for use in more than 120 countries and have a global market value of ~$2·6 billion, with imidacloprid alone comprising 41% of this market and being the second most widely used agrochemical in the world.¹⁰

Some scientists argued that over-reliance on systemic insecticides for pest control is inflicting serious damage to the environmental services that underpin agricultural productivity. A number of alternative models, which are far better for the environment without increasing costs or risks for farmers. The widespread adoption of neonicotinoids as seed dressings has led to a move away from integrated pest management (IPM), a philosophy of pest management predicated on minimizing use of chemical pesticides via monitoring of pest populations, making maximum use of biological and cultural controls, applying chemical pesticides only when needed and avoiding broad-spectrum, persistent compounds.¹³

**Are there economic benefits from the use of neonicotinoids?**

Many studies have provided evidence that the use of neonicotinoids can provide effective control of a broad range of insect pests and bring economic benefits to farmers. These studies are reviewed in reference ¹⁰.

An 11 year study investigated economic return from the oilseed rape seed coatings with imidacloprid. A large-scale pesticide usage and yield observations from oilseed rape with those detailing honey bee colony losses over an 11 year period, and reveal a correlation between honey bee colony losses and national-scale imidaclorpid (a neonicotinoid) usage patterns across England and Wales. The study provided the first evidence that farmers who use neonicotinoid seed coatings reduce the number of subsequent applications of foliar insecticide sprays and may derive an economic return.¹⁴

But from most studies is less clear to what extent the widespread adoption of neonicotinoids has contributed to yield increases in farming or whether neonicotinoids offer economic benefits compared to alternatives. In the other hand, yields per hectare of almost all arable crops have increased markedly over the last
decades as a result of many changes, including improved crop varieties, widespread use of artificial fertilizers, better irrigation, new agronomic techniques and the development of successive generations of pesticides. However, the pace of yield increases has slowed, and yield increases in the last 20 years in developed countries have been modest. Some crops such as oilseed rape showing no increased yields with the introduction of neonicotinoids. Another example, in the UK, yields of oilseed rape were the same pre-1994 (when no neonicotinoids were available) as they are today, when close to 100% of crops are treated. Where yield increases have occurred in recent years, it is hard to disentangle the contribution of neonicotinoids from the effects of other changes in agronomic practices.\textsuperscript{15,16}

\begin{figure}
\centering
\includegraphics[width=\linewidth]{figure3.jpg}
\caption{Neonicotinoid insecticides are widely used on oilseed rape in the UK to combat pests like the peach potato aphid, \textit{Myzus persicae}; A), which can carry damaging viruses such as \textit{Turnip yellows virus} (TuYV; B), and cabbage stem flea beetle (\textit{Psylliodes chrysocephala}) (C). Oilseed rape represents a mass flowering nectar and pollen resource in the UK landscape for important pollinators such as the honey bee (D). Images A, C and D, Courtesy Food and Environment Research Agency; Image B, Courtesy Dr Stevens M, Rothamsted Research.}
\end{figure}
In the USA an interesting review on neonicotinoid use was compiled by the Center for Food Safety, (a national non-profit public interest and environmental advocacy organization, Washington DC, USA). The report of CFS was published under the title: “Heavy Costs, Weighing the Value of Neonicotinoid insecticides in Agriculture” (March 2014).\textsuperscript{17} After reviewing a large number of studies the CFS summarized the conclusions in an Executive Summary. The basic question that the report tackles was: Are neonicotinoid insecticidal seed treatment products beneficial or not? The authors of the CFS report reviewed 19 scientific studies from scientific journals that studied the relationship between neonicotinoid treatments and actual yields of major US crops: canola, corn, dry beans, soybeans, and wheat. The report concluded that numerous studies show neonicotinoid seed treatments do not provide significant yield benefits in many contexts. European reports of crop yields being maintained even after regional neonicotinoid bans corroborate this finding. Also, several independent experts agreed that neonicotinoids are massively overused in the USA. Additionally, Neonicotinoids have acute and sublethal effects on honey bees and other pollinators and are considered a major factor in colony bee collapse. “Pre-sterilizing” field (neonicotinoids used as seed coatings) has rendered integrated pest management (IPM), in which pesticides are only used if economic pest damage thresholds are exceeded, obsolete for many major field crops. The CFS recommends to Environmental Protection Agency (EPA) to evaluate future insecticide registration applications and comply with EPA’s mandate to account for both benefits and costs, the agency should: The CFS asked the EPA to take into account: honey bee colony impacts and resulting reduced yields of pollinated crops, reduced production of honey, financial harm to beekeepers and loss of ecosystem services.\textsuperscript{17}

Studies on neonicotinoids investigated the benefits and adverse effects in various crops. In USA and Canada, Seagraves and Lundgren (2012) compared yield of either imidacloprid or thiamethoxam seed dressings on soya with untreated controls and found no difference in yield in either of the two years of their study, but populations of beneficial natural enemies were depressed in treated plots. In this system, the evidence would suggest that the cost of seed treatment (\textasciitilde$30/ ha)
is not being recouped by the farmer.\textsuperscript{18} This study is very similar with other studies on soya cultivation that investigated the effects of usage of neonicotinoids. All of them found no yield benefits or yield benefits below those which could be achieved more economically using foliar insecticides applied only when pests exceeded a threshold. This is a big argument by the supporters of restrictions of insecticide use, that neonicotinoids are applied irrespective of the presence of insects, thus increasing the cost to the farmer.\textsuperscript{19-21}

Another study (2005-2006, Nebraska) evaluated the efficacy of neonicotinoid seed treatment to reduce soybean aphid population in the field and controlled conditions. In 2006 aphid numbers were significantly higher than 2005, reaching approximately 1,200 aphids per plant in the untreated plots. Aphid injury significantly reduced yield and individual seed size in 2006. Imidacloprid significantly reduced aphid densities but not below the economic threshold.\textsuperscript{22}

Crop scientists recommend for best management practices to use precaution in applying neonicotinoids. Although seed treatment with neonicotinoids has advantages when compared to application of other pesticides, they are highly toxic to bees. They recommend, not to use seed treated with neonicotinoids unless there is a specific pest problem. Also, they recommend to avoid planting on windy days when any dust will blow the dust into the environment, particularly if wind is blowing toward bee hives, flowering trees or standing water sources used by bees.\textsuperscript{23}

Wheat seed dressing is practiced in various countries with neonicotinoids. Studies of the efficacy of imidacloprid dressing of winter wheat in North America suggest that yield benefits are small (compared to unprotected, control crops) and often exceeded by the cost of the insecticide.\textsuperscript{24} In contrast, in Western Australia, scientists demonstrated that application of an imidacloprid seed dressing to spring wheat is cost-effective compared to using no pest control, but that using foliar applications of alpha-cypermethrin (which is much cheaper) provided a significantly higher economic return.\textsuperscript{25}
**Neonicotinoids and insect resistance**

Insect resistance after prolonged exposure is a potential consequence of consistent exposure to any pesticide. When growers repeatedly plant neonicotinoid-treated seeds in fields where no economic levels of target pests occur, the rate at which resistance will occur accelerates. In addition, foliar neonicotinoids applied to soybean during the season will further increase pressure on pests to evolve resistance. Researchers have documented neonicotinoid resistance in several key pest species in other cropping systems. Despite the current scale of resistance, neonicotinoids remain a major component of many pest control programmes, and resistance management strategies, based on mode of action rotation, are of crucial importance in preventing resistance becoming more widespread.26

From 1991, when neonicotinoids use started, the insecticides have proved relatively resilient to the development of resistance, especially when considering aphids such as *Myzus persicae* and *Phorodon humuli*. Stronger resistance has been confirmed in some populations of the whitefly, *Bemisia tabaci*, and the Colorado potato beetle, *Leptinotarsa decemlineata*. Resistance in B- and Q-type B. tabaci appears to be linked to enhanced oxidative detoxification of neonicotinoids due to overexpression of monooxygenases. No evidence for target-site resistance has been found in whiteflies.27

Another study found that imidacloprid resistance in Colorado potato beetle was detected for the first time in the midwestern USA. Neonicotinoid resistance in summer-generation adults was higher than in overwintered adults from the same locations. By 2009, 95% of the populations tested from the NW and MW USA had significantly higher LD$_{50}$ values for imidacloprid than the susceptible population. Scientists concluded that increasing resistance to neonicotinoid insecticides raises concerns for the continued effective management of Colorado potato beetles in potatoes and highlights the need for more rigorous practice of integrated pest management methods (IPM).28

Neonicotinoid resistance was tested with rice brown planthopper, *Nilaparvata lugens* Stål (primary insect pest of cultivated rice) in Asia. Resistance to
neonicotinoid insecticides (particularly imidacloprid) has been reported as an increasing constraint in recent years. The study found that 10 of the 12 samples collected during 2005 to be susceptible to imidacloprid, but two late-season samples from India showed reduced mortality at both diagnostic doses. All 13 strains collected in 2006 showed reduced mortality at both doses when compared with the susceptible strain. Scientists concluded that data demonstrate the development and spread of neonicotinoid resistance in *N. lugens* in Asia and supported reports of reduced field efficacy of imidacloprid.\(^{29}\)

Bed bug population resistance to pyrethroidsf forced farmers in the USA to use combinations of pyrethroids and neonicotinoids, although there were concerns of evolution of resistance in the mixture. Laboratory assays were used in a study to measure the toxicity of topical applications of 4 neonicotinoids (acetamiprid, imidacloprid, dinofuran, thiamethoxam). The study showed high levels of resistance.\(^{30}\)

A recent article (2018) argued that although neonicotinoids in the last 15 years are used widely by farmers in all developed countries and in the USA, little is known about how large-scale deployment affects pest populations over long periods. The research reports a positive relationship between the deployment of neonicotinoid seed-dressings on multiple crops and the emergence of insecticide resistance in tobacco thrips (*Frankliniella fusca*), a polyphagous insect herbivore that is an important pest of seedling cotton but not for soybean or for maize. Using a geospatial approach, scientists studied the relationship between neonicotinoid resistance measured in 301 *F. fusca* populations to landscape-scale crop production patterns across nine states in the southeastern U.S. cotton production region, in which soybean, maize and cotton are the dominant crops. The results of the research linked the spatiotemporal abundance of cotton and soybean production to neonicotinoid resistance in *F. fusca* that is leading to a dramatic increase in insecticide use in cotton. Results demonstrated that cross-crop resistance selection has important effects on pests and, in turn, drives pesticide use and increases environmental impacts associated with their use.\(^{31}\)
Tobacco thrips, *Frankliniella fusca* (Hinds), is one of the many species of thrips which is a highly voracious insect and despite the name eat a great variety of green plants. Because this insect is highly polyphagous and the window of insecticide exposure is short, neonicotinoid resistance was expected to pose a minimal risk. However, reports of higher than expected *F. fusca* seedling damage in seed-treated cotton fields throughout the Mid-South and Southeast USA production regions suggested neonicotinoid resistance had developed. To document this change, *F. fusca* populations from 86 different locations in the eastern United States were assayed in 2014 and 2015 for imidacloprid and thiamethoxam resistance to determine the extent of the issue in the region. Approximately 57% and 65% of the *F. fusca* populations surveyed had reduced imidacloprid and thiamethoxam sensitivity respectively. Estimates of neonicotinoid resistance indicate an emerging issue for management of *F. fusca* in the eastern United States.32


Many pesticides have gradually lost their effectiveness over time because pests have developed resistance, which reduces the field performance of these pesticides. Many environmental agencies in developed countries are concerned about resistance issues with neonicotinoids. They insist on managing the development of pesticide resistance, in conjunction with alternative pest management strategies and Integrated Pest Management (IPM) programs, Neonicotinoids were put into use (1994) and proved very effective against one of
the most insecticide-resistant insects, the Colorado potato beetle. But as with earlier pesticides, the Colorado potato beetle started developing resistance to neonicotinoids by 2005, and this adaptation was common by 2012.\textsuperscript{33}

**Persistence of neonicotinoids in soils**

Another concern with neonicotinoids insecticides (when used for seed dressing to provide accurate targeting of the crop) was the amount of applied chemical lost in soil as dust during sowing. Research showed that of the uptake of neonicotinoid seed dressings into the target crop between 1.6-20% of the active ingredient is absorbed by the crop.\textsuperscript{34}

During the last years there was concern among scientists for the seed-coating neonicotinoid insecticides that were used on corn crops worldwide. Corn crop production was increased worldwide for renewable energy biodiesel. Unfortunately, the increased use of neonicotinoid insecticides have been blamed for the honeybee decline in the last years. Studies showed that of the 80–98% of the active ingredient in seed dressings, which is not absorbed by the crop, a small proportion (<2%) is lost as dust during sowing. The neonicotinoid insecticides was introduced in the late 1990s, European beekeepers have reported severe colony losses in the period of corn sowing (spring). The atmospheric emission of particulate matter containing the insecticide by drilling machines, has been quantitatively studied. Using optimized analytical procedures, quantitative measurements of both the emitted particulate and the consequent direct contamination of single bees approaching the drilling machine during the foraging activity have been determined. Experimental results show that the environmental release of particles containing neonicotinoids can produce high exposure levels for bees, with lethal effects compatible with colony losses phenomena observed by beekeepers.\textsuperscript{35}

Studies showed that aerial dust (of insecticide) in crop fields can be sufficient to cause direct mortality in honeybees flying nearby and is deposited on field margin vegetation at concentrations ranging from 1 to 9 ppb. Chemical analysis showed high quantities of neonicotinoid insecticide in dead honey bees earlier exposed to dust in the field.\textsuperscript{36,37}
The bulk of the active ingredient of neonicotinoids enters the soil (90%) when used for seed coating. Neonicotinoids are also water soluble thus polluting water tables. Their half-life in soil varies greatly among active compounds depending with soil type. For the most commonly used seed treatments, reported half-lives in soil typically range from 200 to in excess of 1,000 days (range 28–1250 days for imidacloprid; 7–353 days for thiamethoxam).2,38

Pollution by neonicotinoids of other environments

Loss of neonicotinoids from agricultural soils is via degradation or leaching in soil water. The pattern of loss is commonly biphasic, with an initial rapid phase followed by a much slower second phase, probably reflecting sorption of a proportion of the active ingredient of the insecticide onto soil particles which then slows dissipation. Simulation in the laboratory of rainfall resulted in 79% leaching of neonicotinoid insecticides.39

Leaching of neonicotinoids from soil after application is lower and sorption is higher in soils with high organic matter content.40,41 Scientists are concerned with increased leaching of neonicotinoids before they are bound to soil after heavy rainfall in agricultural areas. Loss of neonicotinoids by leaching inevitably will increase substantially concentrations in groundwater and run-off immediately after application, especially with rainfall and in steep slopes where the soil organic content is low.42-44 Study showed that another factor that increases leaching of neonicotinoids from soil sorption sites is dissolved organic carbon which appears in studies to compete with neonicotinoid insecticides.45

Analytical measurements of neonicotinoids residues detected the insecticides in groundwater, streams, storm-water ponds and tidal creeks.46,47

A study with 75 samples of surface water detected the principal neonicotinoid imidacloprid in 89% of water samples taken from rivers, creeks and drains in California (in agricultural regions), with 19% of samples exceeding the USA EPA guideline concentration of 1.05 ppb (parts per billion). The results indicated...
that imidacloprid commonly moves offsite and contaminates surface waters at concentrations that could harm aquatic organisms following use under irrigated agriculture conditions in California.48

A similar study was contacted in the Netherlands and detected up to 200 ppb in groundwater, streams and ditches.49 However, neonicotinoid insecticides are absent from many groundwater and run-off samples collected in areas where they are deployed. This may be because they are only present for a short period after application and so are likely to be missed by most sampling.2

Adverse effects of neonicotinoid insecticides on pollinators

After 2000 many research groups investigated the adverse effects of neonicotinoids on pollinators, especially honey bees (nectar-foraging bees). Some of these studies encountered significant uncertainties. The first problem was about the magnitudes of both lethal and sublethal effects on honey bees caused by trace neonicotinoids. Lethal effects, were carried out initially in the laboratory and the single oral dose that kills 50% of dosed individuals, or LD50, was found to be about 100 times higher than the estimated daily ingestion of neonicotinoids by nectar-foraging honey bees.50

These toxicological results suggested that systemic insecticide neonicotinoids in the field are not lethal to honey bees. However, scientists argued that single doses under laboratory conditions may be unrealistic, because mass flowering crops, such as oilseed rape, bloom over several weeks and foraging bees are likely to ingest nectar repeatedly. Honey bees have suffered increased mortality after multiple ingestions of trace dietary neonicotinoid at field-realistic levels. A study found discrepancies between acute and chronic toxicity by imidacloprid in honey bees.51

But other studies contested these results. An extensive literature survey did not find evidence for the reported high difference between the acute and the chronic dietary toxicity of imidacloriprid and its plant metabolites to honeybees. The majority of data indicated a dietary no observed lethal-effect concentration 0.04
and 0.02 mg/L in 50% sucrose solution, respectively, for an acute and a chronic dietary exposure of honeybees to either imidacloprid or its plant metabolites. No increased treatment-related mortality or behavioural abnormalities were recorded in four independent research facilities during a 10-day dietary exposure of honeybees of different ages to sucrose solutions spiked with the respective metabolites at 0.0001, 0.001, and 0.010 mg/L 50% sucrose solution.\textsuperscript{52}

\textbf{Figure 5.} A field study (UK, Hungary, Germany) has found exposure to crops treated with neonicotinoid seed coatings reduced overwintering success of honeybee colonies in two of three countries examined and in the third country (Germany) saw no impact. Co-author Professor Richard Pywell, Science Area Lead, Sustainable Land Management at the Centre of Ecology & Hydrology, said, \"Neonicotinoids remain a highly contentious issue with previous research on both honeybees and wild bees inconclusive\" (\textit{Science}, 356:1393-1395, 2017).

In laboratory studies of sublethal effects, the emergence of a clear picture of the potency of imidacloprid previously has been hindered by the wide variety of performance indices that have been measured and inconsistency in experimental outcomes among studies. A quantitative survey of the literature on laboratory and semi-field studies of lethal and sublethal responses of honey bees to doses to the most used imidacloprid in agricultural fields, showed that trace of dietary intake of each of several neonicotinoids affected honey bees.\textsuperscript{53}

Most studies focused on imidacloprid because it is extensively used in agriculture. A comprehensive review on potential risks of imidacloprid (used for seed dressing) on honey bees published in 2004. The studies in this review (field
and semifield trials) were carried out by different scientists and were briefly described and their results discussed. The acute oral LD$_{50}$ (Lethal Dose 50%) of imidacloprid to honey bees was found in the majority of laboratory studies between 40.9 and > 81 ng/bee (nanograms, $10^{-9}$ g) with NOED (No-observed Effect Dose) values between 1 and 5 ng/bee. From the reviewed studies a field-relevant NOAEC (No-observed Lethal Effect Concentration) of 20 ppb was concluded for honey bees. Analytical studies on nectar and pollen samples of sunflower, rape and corn plants have shown that residue levels of imidacloprid and toxicologically relevant imidacloprid metabolites are typically well below 5 ppb. When comparing the NOAEC with the reported residue data, it becomes evident that imidacloprid seed-dressings will pose only a negligible risk to honey bees. This conclusion was supported by the findings of more than 30 semifield and field studies conducted in various regions of the world. But it must be added that there were only qualitative methods employed in these studies.$^{54}$

Dr. Cresswell in 2011, subjected the data from published laboratory studies to standard quantitative techniques of meta-analysis which is a well established methodology. He excluded anomalous studies and fitted dose–response relationships to estimate the expected magnitudes of the effects on honey bees of field-realistic doses of trace dietary imidacloprid. The meta-analysis of 14 published studies comprised measurements on 7,073 adult individuals and 36 colonies, fitted dose–response relationships estimate that trace dietary imidacloprid at field-realistic levels in nectar will have no lethal effects, but will reduce expected performance in honey bees by between 6 and 20%. Statistical power analysis showed that published field trials that have reported no effects on honey bees from neonicotinoids were incapable of detecting these predicted sublethal effects with conventionally accepted levels of certainty. These findings raise renewed concern about the impact on honey bees of dietary imidacloprid.$^{55}$

A paper in 2013 presented an overview (by collecting data from a series of papers) of the impact of neonicotinoids on pollinators due to their wide application, their systemic mode, persistence in soil and water, and bioavailability at sublethal concentrations for most of the year. The neonicotinoids with their systemic mode
of action inside plants are transported in the phloemic and xylemic parts that results in translocation to pollen and nectar. Studies showed that bioavailability to pollinators at sublethal concentrations for most of the year 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 and larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene etc. Also, neonicotinoids exhibit a toxicity that can be amplified by various other agrochemicals and they synergistically reinforce infectious agents such as *Nosema ceranae* which together can produce colony collapse.\textsuperscript{56}

**Pollinators decline in the last decade and causes**

Historically, managed honey bee populations in the USA and Europe have been monitored due to their vital role in providing pollination services in agricultural systems. It was always difficult to determine whether pollinator species are declining, and no less challenging is determining the causes. Many explanations have been invoked by scientists to account for declines in pollinator populations including, among others, exposure to pathogens, parasites, and pesticides; habitat fragmentation and loss; climate change; market forces; intra- and inter-specific competition with native and invasive species; and genetic alterations.

Several studies indicate that American and European beekeepers were suffering large annual losses. It was estimated that in the USA, beekeepers have lost \textasciitilde30\% of their colonies every year since 2006, with total annual losses sometimes reaching as high as 42\%. Population changes in other insect pollinator species, such as other bee species, flies, butterflies and beetles have not been as closely tracked. Indeed, there are several hundred thousand species of pollinators and tracking all of them is not possible. However, surveys have documented disturbing population declines and even local extinctions of select pollinator species across Europe and the US. Initial studies for the causes of this decline were confusing. In the last
decades agricultural crops and technologies changed, wild and managed pollinators faced emerging stressors. Honey bees, other managed pollinator species such as bumble bees and orchard bees, and wild bees suffer from exposure to parasites and pesticides, and loss of floral abundance and diversity due to increased land-use. In addition, habitat destruction limits nesting sites for wild pollinators. Climate changes and harsh winters in some areas also play a role. Unfortunately, these stressors may interact synergistically to produce more detrimental effects on pollinator health.\textsuperscript{57}

Despite numerous physiological and behavioural defenses, honey bees are plagued by viral, fungal and bacterial infection. Furthermore, colonies are frequently infested with destructive parasitic mites or other insect pests. Some of the most damaging hive parasites were recently introduced to European honey bee (\textit{Apis mellifera}) populations from the related Asian honey bee (\textit{Apis cerana}) species.\textsuperscript{58} At present, 23 different viruses are known to infect honey bees. Notably, some of these viruses can cause asymptomatic infections, and depending virus levels and/or the presence of other stressors, infections may go undetected.\textsuperscript{59}

Honey bees are routinely exposed to natural and human applied chemicals. Pollen and nectar, the components of honey bee diet, contain diverse plant-produced chemicals. Honey bee digestive systems efficiently extract nutrients from nectar and pollen, while bee fat body tissue (analogous to human livers) can detoxify some plant-based food components that are harmful to bees. However, beekeepers may enlist numerous chemicals to control hive parasites and pests. Meanwhile, agricultural systems are treated with pesticides, fungicides and herbicides. Exposure to beekeeper applied treatments or crop chemicals can have negative effects on honey bee health, productivity and survival as bee detoxification systems may not be able to fully or efficiently eliminate human applied chemicals.\textsuperscript{60,61}
Figure 6. There is accumulating evidence of the last decade that in Europe and North America many species of pollinators are in decline, both in abundance and distribution. Most studies identified a long list of potential causes of pollinators decline, including, virus, microbial pathogens, parasites, climate change and very prolonged cold winters. Also, numerous studies link Neonicotinoid insecticide exposure and collapse of honey bee colonies in many countries.

Some of the most frequently found chemicals in pollen and/or wax samples were the miticides coumaphos and fluvalinate (applied by beekeepers to control Varroa infestation) and a fungicide, chlorothalonil. Each of these 3 commonly found chemicals has been shown to sublethal effects on larval bees and certain combinations result in synergistic toxicity. The fact that an average of 6 different chemicals are found in wax samples is of concern since these chemicals may interact to with negative, synergistic toxicity consequences.  

Scientists in the European Union (EU) estimated that €5 billion of annual EU agricultural output is directly attributed to pollinators, but nearly one in 10 bee and butterfly species is facing extinction, according to the European Red List. To tackle the decline, the European Commission is looking to develop a European initiative on pollinators and is calling on scientists, farmers, businesses, environmental organisations, public authorities and citizens to contribute.  

The accumulating evidence of the last decade that in Europe and North America many species of pollinators are in decline, both in abundance and distribution caused alarm in scientific circles. There was from the past a number of studies with a long list of potential causes of pollinators decline, including climate
change and harsh winters in some countries. The results from numerous agricultural and entomological projects added a new concern that neonicotinoid insecticides, in particular through their use as seed treatments are, play an important role. A paper (2014) summarized the natural science evidence base relevant to neonicotinoid insecticides and insect pollinators in as policy-neutral terms as possible. A series of evidence statements were listed in the paper and categorized according to the nature of the underlying information for the impact of neonicotinoids on honey bees.64

The latest studies on the impact of neonicotinoids on pollinators

Despite the numerous studies indicating that the widespread use of neonicotinoid insecticides threatens bees, research on this topic has been surrounded by controversy. A group of scientists produced a synthesis of research approaches that have examined the effect of neonicotinoids on bees and identified knowledge gaps. They reviewed most of research papers available on the Web of Science and PubMed in June 2015. They found that most of the 216 primary research studies were conducted in Europe or North America. The studies focused by 78% to the neonicotinoid imidacloprid. The pollinator concerned was by 75% the western honey bee *Apis mellifera*. The group in their review emphasized that there is considerable variation in ecological traits among bee taxa. The crops in the studies were dominated by seed-treated maize, oilseed rape (canola) and sunflower, but less whereas less for insect pollinated fruit and vegetable crops, or on lawns and ornamental plants. Most studies in the review used field approaches for bee exposure to neonicotinoids. Most studies measured effects on individual bees. The reviewers suggested that effects on honey bees should be linked to both mechanisms at the sub-individual level and also to the decline for the colony and wider bee populations. The review found difference in research approaches and knowledge gaps.65

Most studies in the last years reported declines in bee distribution and diversity and other pollinators that play important roles in ecosystems. From 2013 the European Commission put a moratorium on 3 neonicotinoids as seed
coatings in flowering plants (clothianidin, imidacloprid and thiamethoxam) to protect honeybees (Regulation EU No. 485/2013). Scientists were concerned also for the wild bees. A study in 2015 replicated and matched landscapes, with seed coating Elado, (insecticide containing a combination of the neonicotinoid clothianidin and the non-systemic pyrethroid β-cyfluthrin) and measured the effects on wild bees. It was applied to oilseed rape seeds, and the results showed reduced wild bee density, solitary bee nesting, and bumblebee colony growth and reproduction under field conditions. The scientists concluded that these insecticides posed a substantial risk to wild bees in agricultural landscapes.66

The decline of honey bees prompted some scientists to investigate the possible microbial pathogens as a profound impact on insect populations. Honey bees are suffering from elevated colony losses in the northern hemisphere possibly because of a variety of emergent microbial pathogens, with which pesticides may interact to exacerbate their impacts. To reveal such potential interactions, a scientific group (Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany) administered at sublethal and field realistic doses one neonicotinoid pesticide (thiacloprid) and two common microbial pathogens, the invasive microsporidian Nosema ceranae and black queen cell virus (BQCV), individually to larval and adult honey bees in the laboratory. Through fully crossed experiments in which treatments were administered singly or in combination, they found an additive interaction between BQCV and thiacloprid on host larval survival likely because the pesticide significantly elevated viral loads. In adult bees, two synergistic interactions increased individual mortality. The combination of two pathogens had a more profound effect on elevating adult mortality than N. ceranae plus thiacloprid. The scientists concluded that common microbial pathogens appear to be major threats to honey bees, while sublethal doses of pesticide may enhance their deleterious effects on honey bee larvae and adults.67

Another group of scientists investigated the losses of wild species (bees and bumblebees) in England (NERC Centre for Ecology and Hydrology, Wallingford, Oxfordshire OX10 8BB, UK). The group related 18 years of UK national wild bee distribution data for 62 species to amounts of neonicotinoid use in oilseed rape.
Using a multi-species dynamic Bayesian occupancy analysis, they found evidence of increased population extinction rates in response to neonicotinoid seed treatment use on oilseed rape. Species foraging on oilseed rape benefit from the cover of this crop, but were on average three times more negatively affected by exposure to neonicotinoids than non-crop foragers. Our results of the study suggested that sub-lethal effects of neonicotinoids could scale up to cause losses of bee biodiversity.68

A scientific investigation with honey bees in Germany (Free University Berlin, Institute for Biology-Neurobiology, and Julius Kühn-Institut, Institute for Bee Protection, Berlin) was focused on thiacloprid (thought to be less toxic to honey bees). Honey bees (Apis mellifera carnica) were exposed chronically to thiacloprid in the field for several weeks at a sublethal concentration. The study showed that foraging behaviour, homing success, navigation performance, and social communication were impaired. Thiacloprid residue levels increased both in the foragers and the nest mates over time. The effects observed in the field were not due to a repellent taste of the substance. The results were the first data for the risk evaluation of thiacloprid taken up chronically by honey bees in field conditions.69

A brief review by scientists from different countries (Australia, Italy, England, Japan and France) presented results for the negative impacts of insecticides on bees and other pollinators that have never been disputed. The review presented results on sub-lethal level (<LD50) of neurotoxic insecticide molecules that are known to influence the cognitive abilities of bees, impairing their performance and ultimately impacting on the viability of the colonies. Also, widespread systemic insecticides appear have introduced indirect side effects on both honey bees and wild bumblebees, by deeply affecting their health. Immune suppression of the natural defences by neonicotinoid and phenyl-pyrazole (fipronil) insecticides opens the way to parasite infections and viral diseases, fostering their spread among individuals and among bee colonies at higher rates than under conditions of no exposure to such insecticides. The reviewers argued that causal link between diseases and/or parasites in bees and neonicotinoids and other pesticides has eluded researchers for years because both factors are concurrent: while the former are the immediate
cause of colony collapses and bee declines, the latter are a key factor contributing to the increasing negative impact of parasitic infections observed in bees in recent decades.\textsuperscript{70}

Another study in the USA (University of Nebraska-Lincoln, Entomology, Lincoln, USA), investigated the potential effects of neonicotinoids on queen bees which may be exposed indirectly through trophallaxis (Trophallaxis is the transfer of food or other fluids among members of a community through mouth-to-mouth), or food sharing. The researchers in order to assess effects on queen productivity, small colonies of different sizes (1500, 3000, and 7000 bees) were fed imidacloprid (0, 10, 20, 50, and 100 ppb) in syrup for three weeks. The study found adverse effects of imidacloprid on queens (egg-laying and locomotor activity), worker bees (foraging and hygienic activities), and colony development (brood production and pollen stores) in all treated colonies. Some effects were less evident as colony size increased, suggesting that larger colony populations may act as a buffer to pesticide exposure. This study was the first to show adverse effects of imidacloprid on queen bee fecundity and behaviour and improved our understanding of how neonicotinoids may impair short-term colony functioning.\textsuperscript{71}

A study (2016) in the Bee Institute in Germany (LLH Bee Institute, Kirchhain, Germany) examined the immunosupression effects of neonicotinoids on bees. A strong immune defense is vital for honey bee health and colony survival. This defense can be weakened by environmental factors that may render honey bees more vulnerable to parasites and pathogens. The research group investigated the sublethal effects of the neonicotinoids thiacloprid, imidacloprid, and clothianidin on individual immunity, by studying three major aspects of immunocompetence in worker bees: total hemocyte number, encapsulation response, and antimicrobial activity of the hemolymph. The results from the laboratory experiments found a strong impact of all three neonicotinoids. Thiacloprid (24h oral exposure, 200 μg/l or 2000 μg/l) and imidacloprid (1 μg/l or 10 μg/l) reduced hemocyte density, encapsulation response, and antimicrobial activity even at field realistic concentrations. Clothianidin had an effect on these immune parameters only at
higher than field realistic concentrations (50–200 μg/l). These results suggest that neonicotinoids affect the individual immunocompetence of honey bees.72

In Germany a research project was supported by funds from the Federal Ministry of Food and Agriculture (BMEL), based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme. Germany, like many other European countries, witness substantial honey bee colony (periodically) in the last decades. Scientific evidence showed that the influence of pests and pathogens are beyond dispute, but in addition, chronic exposure to sublethal concentrations of pesticides has been suggested to affect the performance of honey bee colonies. The study aimed to elucidate the potential effects of a chronic exposure to sublethal concentrations (one realistic worst-case concentration) of the neonicotinoid thiacloprid to honey bee colonies in a three year replicated colony feeding study. Thiacloprid did not significantly affected the colony strength. No differences between treatment and control were observed for the mortality of bees, the infestation with the parasitic mite Varroa destructor and the infection levels of viruses. No colony losses occurred during the overwintering seasons. Furthermore, thiacloprid did not influence the constitutive expression of the immunity-related hymenoptaecin gene. However, upregulation of hymenoptaecin expression as a response to bacterial challenge was less pronounced in exposed bees than in control bees. Scientists concluded that under field conditions, bee colonies were not adversely affected by a long-lasting exposure to sublethal concentrations of thiacloprid. No indications were found that field-realistic and higher doses exerted a biologically significant effect on colony performance.73

Researchers from the Centre for Ecology & Hydrology (Oxfordshire, UK) publish results of a large-scale, field-realistic experiment to assess neonicotinoid impacts on honeybees and wild bees across Europe, in the peer-review journal Science (June 2017). The experiment – undertaken in the UK, Germany and Hungary – exposed three bee species to winter oilseed rape crops treated with seed coatings containing neonicotinoid clothianidin, from Bayer CropScience, or Syngenta’s thiamethoxam. Neonicotinoid seed coatings are designed to kill pests such as the
cabbage stem flea beetle, but were effectively banned in the EU in 2013 due to concerns regarding their impact on bee health. The researchers found that exposure to treated crops reduced overwintering success of honeybee colonies – a key measure of year-to-year viability – in two of the three countries. In Hungary, colony number fell by 24% in the following spring. In the UK, honeybee colony survival was generally very low, but lowest where bees fed on clothianidin-treated oilseed rape in the previous year. No harmful effects on overwintering honeybees were found in Germany.⁷⁴

Although most studies investigated the ecological and behavioural adverse effects of neonicotinoid insecticides on the health of bee pollinators, the molecular determinants were not explored. Scientists from the Bayer AG, Crop Science Division, Monheim Germany, the Dpt of Biointeractions and Crop Protection, Rothamsted Research, Harpenden, UK and College of Life and Environmental Eciences, Exeter University, UK), unraveled the molecular determinants of the toxicological impact of neonicotinoids in honey bees. In their study (2018), the researches noticed that although previous work has suggested that toxicity was due to rapid metabolism of neonicotinoids the specific gene(s) or enzyme(s) involved remained unknown. The study showed that the sensitivity of the two most economically important bee species to neonicotinoids is determined by cytochrome P450s of the CYP9Q subfamily. Radioligand binding and inhibitor assays showed that variation in honeybee sensitivity to \( N \)-nitroguanidine and \( N \)-cyanoamidine neonicotinoids does not reside in differences in their affinity for the receptor but rather in divergent metabolism by P450s. Functional expression of the entire CYP3 clade of P450s from honey bees identified a single P450, CYP9Q3, that metabolizes thiacloprid with high efficiency but has little activity against imidacloprid. Researchers demonstrated that bumble bees also exhibit profound differences in their sensitivity to different neonicotinoids, and they identify CYP9Q4 as a functional ortholog of honeybee CYP9Q3 and a key metabolic determinant of neonicotinoid sensitivity in this species.⁷⁵
Decisions of EU on neonicotinoids

In 2013, the European Commission severely restricted the use of plant protection products and treated seeds containing three of these neonicotinoids (clothianidin, imidacloprid and thiamethoxam) to protect honeybees (Regulation (EU) No 485/2013). The measure was based on a risk assessment of the European Food Safety Authority (EFSA) in 2012.

On the 27 of April 2018, the Standing Committee on Plants, Animals, Food and Feed of the European Union, after struggling for 7 months to achieve a majority vote, expanded a controversial ban on 3 neonocotinoids (clothianidin, imidacloprid and thiamethoxam) on the ground that they pose a threat to pollinators. In the voting 16 countries (Germany, France, UK, Spain, Italy, The Netherlands, Sweden, Greece, Cyprus, Austria, Portugal, Ireland, etc), voted in favour. Romania, Denmark, Czech Republic, Hungary) opposed the ban, and 13 countries abstained (Poland, Belgium, Finland, etc). Although Neonicotinoids are banned for outdoor crops, can be used in indoor permanent greenhouses. The decision was taken after an assessment by the European Food Safety Authority (EFSA) in a report that confirmed in February 2018, the dangers they posed to bees.

The EU has one of the strictest regulatory systems in the world concerning the approval of pesticides for agricultural crops. All pesticides on the EU market have been subject to a thorough assessment to ensure a high level of protection of both human and animal health and the environment. Insecticides are, by their nature, toxic to bees. However, their use should still be possible if exposure does not occur or is minimised to levels which do not generate harmful effects. In 2012, new scientific findings indicated that some insecticides showed high risks for bees, namely: Neonicotinoids, Fibronil (reviewed by EFSA and subjected to restrictions). Many years ago the European Commission requested for proper action on neonicotinoids. The EFSA has developed a guidance document on the risk assessment of plant protection products on bees and provided an opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (Apis mellifera, Bombus spp and solitary bees).
Scientific advice played a major part in persuading EU member states to support the ban. In 2013, the EU prohibited use of the three chemicals on flowering crops that are attractive to bees, including oilseed rape, sunflower and maize (corn), acting on advice from the European Food Safety Authority (EFSA) in Parma, Italy, an EU-funded independent scientific advisory body. Scientists have since better established the risks to bees, which led the European Commission to propose the outdoor-use ban last year.

What is happening in the USA and other countries with neonicotinoids

Each year the USA Department of Agriculture releases the **Bee Informed Partnership** (BIP), in cooperation with University of Maryland entomologist Dennis van Engelsdorp. The annual USA reports on ‘bee hive loss estimate’ presents whether there is ‘bee crisis’ or improvement. In recent years the popular narrative among USA journalists and on the Internet has been that honey bees and wild bees face impending doom—the subject has been dubbed a “beepocalypse” or “beemageddon”. The majority of reports put the blame on pesticides, especially neonicotinoids. The entomologists in the U.S. Department of Agriculture put the emphasis for honey bees decline on Varroa destructor mites that have been infesting bee hives at an accelerating rate over the past few decades, and present a serious and on-going threat. Pesticides rank low as a likely cause of bee health. Anti-pesticide campaigners have long rejected the conclusions of government agencies and scientists, deciding that bee health issues could not be driven by something as prosaic as a well-known parasite, and have focused instead on neonicotinoids and other insecticides. Although some laboratory studies point to potential serious problems due to neonicotinoids, field research, meta-studies and the hard numbers worldwide showed that bee hives are at record numbers globally. In the last years the BIP numbers showed winter losses mainly due to cold weather, than pesticide use, but the results are changing every year causing confusion.  

But in some U.S. States the ban on neonicotinoids has started. The first ban on sales begin in 1.1.2018 in Maryland. Connecticut will follow suit about a week
later with a similar ban. Also, there are pressures by various environmental societies on California (which has one of the environmental friendly legislation among U.S. states) to ban neonicotinoids. The American Bird Conservancy, following the ban by the EU, has voted to ban almost all outdoor uses of three neonicotinoids due to their environmental impact, harm to bees and pollution of waterways. Steve Holmer, Vice President of Policy for American Bird Conservancy, called on the U.S. Environmental Protection Agency (EPA) to follow suit and restrict the use of the most dangerous neonicotinoid pesticides to protect birds and bees, and to ensure that our environment will not continue accumulating these dangerous chemicals.\(^{78,79}\)

The EPA in the USA in 2018 has scheduled to review neonicotinoids’ safety and environmental impact. [EPA, Pollinator Protection. Schedule for review of neonicotinoid pesticides, 2018, https://www.epa.gov/pollinator-protection/schedule-review-neonicotinoid-pesticides ].


The Australian environmental authorities do not show any sign to ban the use of neonicotinoids. Neonicotinoids are widely used in Australia for crops. The Australian Pesticides and Veterinary Authority (APVMA) argued that there is no evidence of declining bee populations in Australia. [ABC News. EU ban on neonicotinoids triggers call for a similar ban in Australia to protect bees. 30.4.2018. [http://www.abc.net.au/news/rural/2018-04-30/beekeepers-call-for-neonicotinoids-australia-ban/9710252 ].
Conclusion

For many years, research by entomologists indicated that the widespread use of neonicotinoid insecticides affected honey bees. But the results of most of the research on this topic have been surrounded by controversy. Already, from 2015 there were more than 200 primary research studies, conducted in Europe or North America countries. Most studies focused on neonicotinoid imidacloprid and were concerned for the impact on western honey bee *Apis mellifera*. The problem in other countries of the globe seemed to be unknown. Another big challenge for scientists was the fact that there was considerable variation in ecological traits among bee taxa and studies on honey bees were not likely to fully predict impacts of neonicotinoids on other pollinator species. The majority of studies focused on seed-treated maize, oilseed rape (canola) and sunflower, and less on insect pollinated fruit and vegetable crops. Most studies were contacted in the laboratory and less in field-studies with realistic exposures to neonicotinoids.

The latest studies recognized beyond doubt that neonicotinoid insecticides pose a major threat to bees. Results showed that was strong evidence that neonicotinoids can affect bee behaviour and colony growth. But despite the evidence, major knowledge gaps remained in understanding how these pesticides affect pollinator health, especially the specific mechanisms by which neonicotinoid exposure affect bee colonies. Also, it is unclear how pesticide exposure interacts with other environmental stressors, which could either amplify or attenuate the effects of neonicotinoids.

Finally, In April 2018, the European Commission through the EFSA, based on strong scientific evidence, banned the use of neonicotinoids in outdoor crops. The ban was approved by member nations on the EU and will mean they can only be used in closed greenhouses. The decision was hailed by environmental organizations because honey bees and other insect pollinators are vital for global food production as they pollinate 75% of all crops. In many countries the plummeting numbers of pollinators in recent years has been blamed, in part, on the widespread use of neonicotinoids.
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