THOMAS SPENDER/GETTY IMAGES

A protest against the use of neonicotinoid pesticides takes place outside the Houses of Parliament in London in April 2013.

PESTICIDES

Seeking answers amid a toxic debate

Some see the European Union's ban on neonicotinoid pesticides as a victory for pollinators, but the data suggest that limiting these compounds may do little to stave off honeybee losses.

BY MICHAEL EISENSTEIN

A camera crew followed Dennis vanEngelsdorp's every move as he returned to his laboratory with an unfortunate cargo — packages of dead honeybees. The year was 2006, and the film-makers were chronicling colony collapse disorder (CCD), a newly coined phenomenon in which entire hives die from the catastrophic loss of adult bees. Scientists were still grappling with CCD, but the media had already found a culprit. "As we were opening the first packages, the crew were asking me, 'This was neonics, wasn't it?'" recalls vanEngelsdorp, then the acting state apiarist in Pennsylvania. "And this was before we had even done anything!"

Neonics — short for neonicotinoids — are insecticides that were introduced in the early 1990s as a more environmentally benign approach to agricultural pest management. Rather than being sprayed directly onto crops — the common method of applying insecticides — neonicotinoids are typically coated onto seeds, ostensibly limiting opportunities for exposure by bees and other non-target organisms. But traces can be found throughout treated plants, including in the pollen and nectar that bees subsist on. Barely a year after neonicotinoids were first used in agriculture, French bee-keepers were connecting the chemicals with hive losses. The evidence was circumstantial, but a series of apparent bee-poisoning cases in Europe and the United States fuelled the fire.

The resulting debate has seen environmental groups and their supporters rallying around scientists who believe that this class of chemicals contributes to CCD; other scientists think that there is only a minor risk and accuse the press and activists of inflaming fears of 'killer nerve agents'. "In Britain, it brought out all kinds of campaign organizations and slogans," says Francis Ratnieks, a bee behaviourist at the University of Sussex in Brighton, UK, who sees little evidence linking neonics with honeybee losses. In the European Union (EU), the furore culminated in a two-year moratorium on the three most widely used neonics, enacted in December 2013. Still, the extent of harm caused by neonics to bee colonies remains an open question, and two decades of research have yielded as much controversy as clarity.

LIFE ON WHITE/ALAMY

ALIVE, BUT UNWELL

Ratnieks notes that in past decades, the evidence for pesticide poisoning was unambiguous. "I lived in the US 30 years ago, when there was heavy spraying of insecticides like carbaryl to control insects in sweetcorn," he says. "We'd see heaps of dead and dying bees

in front of hives." Other, older pesticides were also harmful to humans — most notably, organophosphates, nerve agents that were historically used as chemical weapons as well as to control pests.

By contrast, neonicotinoids have relatively low toxicity in mammals. Furthermore, seed distributors typically apply these treatments before sale, limiting opportunities for overuse. In North America — and until recently, most European nations — neonic seed treatments are widely used on several crops (see 'No clear pattern'). "In the US, almost no corn is planted without them," says Christian Krupke, an entomologist at Purdue University in West Lafayette, Indiana. For honeybees, the most important neonic-treated crop is probably oilseed rape (also known as rapeseed or canola), which blooms with bee-attractive yellow flowers. As a source for both vegetable oil and biodiesel, oilseed rape is a highly valuable cash crop in Europe and Canada.

There is no question that all three neonicotinoids banned by the EU — imidacloprid, thiamethoxam and clothianidin — are highly toxic to bees. Treated seeds carry doses that could kill hundreds of thousands of bees. But by the time the crop blooms, only small amounts of the active ingredients are present in the nectar and pollen. Still, laboratory studies have shown that, even at low doses, neonics can have a serious impact on a bee's brain function. "Neonics affect parts of the brain where sensory information is integrated, including information related to orientation," says Mickaël Henry, a behavioural ecologist at the French National Institute for Agricultural Research in Avignon. The fear is that these effects essentially confuse bees, making it harder for them to find good sources of nutrition or return safely home with sustenance for their hive-mates.

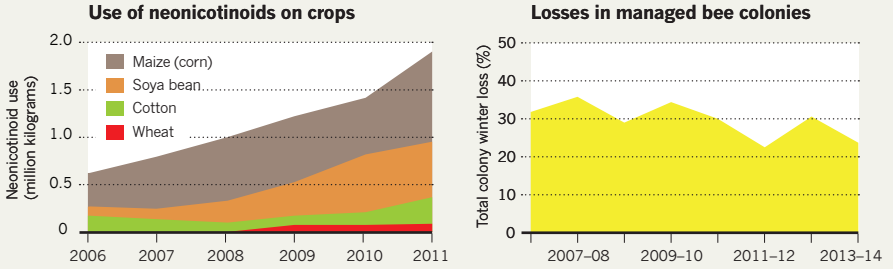
Henry is among the scientists concerned that prolonged exposure to low doses of neonicotinoids may degrade colony robustness by depleting hives of both bees and food. In a trial conducted in France in 2012, he and his colleagues used radio-frequency identification tags to monitor the homing capabilities of 653 forager bees that were released up to 1 kilometre away from their colony[1]. They found that bees treated with sublethal doses of thiamethoxam in sugar water before being released were considerably less likely to return to their hive, with results worse for those foraging in unfamiliar surroundings. Subsequent computer modelling indicated that this steady loss of foragers could jeopardize the hive. "For the first time, we showed that the effects of sublethal doses can lead to indirect mortality, because of bee disorientation, at levels that can put a colony at risk of collapse," says Henry.

A second study[2] in 2012, by entomologist Dave Goulson, then at the University of Stirling, UK, reached a similar conclusion regarding the bumblebee species *Bombus terrestris*. Bumblebees differ from honeybees in that queens live for only one year rather than several, so annual queen production is crucial to the survival of a colony. For two weeks, Goulson's team fed bees in 75 colonies with either plain pollen and sugar water or the same foodstuffs containing imidacloprid; for the next six weeks, they observed the colonies foraging freely. The treated colonies showed an 85% reduction in the production of queen bees, and subsequent findings from Goulson[3] have implicated impaired foraging. These results highlighted potential real-world consequences for low-level pesticide exposure in bumblebees. "Most studies up to that point had been done with bees in a greenhouse or a cage or even a plastic container, where the bee doesn't have to be very good at navigating," says Goulson. Indeed, several other studies have since suggested that non-honeybee pollinator species may be particularly vulnerable to neonicotinoids' effects (see 'Plight of the bumblebee').

"Neonics affect parts of the brain where sensory information is integrated."

NO CLEAR PATTERN

The application of neonicotinoids on maize (corn), soya bean and other crops in the United States continues to climb each year. By contrast, nationwide surveys by bee researchers, in collaboration with the Apiary Inspectors of America and the US Department of Agriculture, show highly variable winter losses for honeybee colonies. Equivalent multi-year data are not publicly available for many European countries, although the few data that there are hint at a similar lack of correlation.



SOURCES: LEFT: DOUGLAS, M. R. & TOOKER, J. F. *ENVIRON. SCI. TECHNOL.* **49**, 5088–5097 (2015); RIGHT: THE BEE-INFORMED PARTNERSHIP

REALITY FIELD

By examining bees in real-world environments, the studies by Henry and Goulson invigorated the neonic debate — indeed, France moved to ban thiamethoxam within months of the publications. But these studies still relied on forced dosing of bees, based on an experimentally determined range of neonicotinoid concentrations — and some experts are wary of their validity. Ratnieks and his University of Sussex colleague Norman Carreck have looked at results from forced-dose trials and found that the studies that showed the greatest risk to bees used doses that were either based on unrealistic or at least worst-case assumptions, and therefore may be of little relevance in field conditions[4]. Ratnieks adds that there could also be considerable variability in the effects of neonics on bees depending on the manner of the dosing. A worker honeybee going about its daily business of picking up multiple, small doses of neonics in the nectar it collects has a chance to metabolize the insecticide and prevent it from building up as opposed to a bee that has received the same dose applied by a researcher at one time. "Just like if you drink a whole bottle of whiskey in a single session versus having a glass or two per day for a week," says Ratnieks.

Without clear agreement on how much pesticide bees actually encounter, conclusions drawn from forced-dose studies remain controversial. "There should be increased efforts to do sound studies with real exposure, not just 'realistic' laboratory exposure," says Jens Pistorius, head of bee risk assessment at the Julius Kühn Institute in Berlin, and a bee-keeper himself.

Only a handful of peer-reviewed studies have examined foraging in actual treated crops, and these generally offer little evidence for ill effects in honeybees. One was published[5] in 2013 by scientists at Syngenta, the Swiss agrochemical company based in Basel that developed thiamethoxam. The study ran for four years in France and examined several indicators of hive robustness for honeybees foraging in either seed-treated fields of maize (corn) or oilseed rape or untreated control fields. "We saw absolutely no effect on the honeybee colonies in those trials, including overwintering success," says Peter Campbell, Syngenta's senior environmental specialist, referring to the hive's capacity to rebound from winter population losses.

A second field study[6], performed in Canada, reached a similar conclusion in 2014 after comparing bee deaths, honey production and other measures of health in 40 colonies that foraged in fields of untreated or clothianidin-treated oilseed rape. "We are not seeing any impact on honeybees as a result of exposure to canola grown from neonic-treated seeds," says Cynthia Scott-Dupree, a pest management specialist and toxicologist at the University of Guelph in Ontario, Canada, who co-authored the study.

Both studies have been criticized for conflict of interest. The study that took place in

BAYER CROPSCIENCE

Maize (corn) seeds in their natural form (yellow) and treated with neonicotinoids (purple and red).

France was conducted by a neonicotinoid manufacturer, and the Canadian study was financed by one: Bayer CropScience, the German company based in Monheim that developed clothianidin and imidacloprid. "Any research into the safety of agrochemicals should be funded by a government or completely independent entity," says Goulson, now at the University of Sussex. But Scott-Dupree notes that field trials of pesticides at the scale necessary to yield meaningful results are complicated and expensive, and she bristles at the notion that the funding affected her findings. "It cost us close to a million dollars for a one-year study — where are we going to get that if not from private funds?" she says. "I didn't get any paybacks to generate data that would support my funders."

The two studies have also been criticized for inadequate test fields. Both used plots of 2 hectares (0.02 square kilometres) — less than the honeybee's typical springtime foraging area of 3–12 km^2, and much smaller than real-world fields. This raises the possibility that bees supplemented their diet with untreated outlying plants, and may have ingested lower doses of the chemical than was assumed.

However, these trials are at least partly supported by field data from Swedish researchers at Lund University — a study funded entirely by government and non-profit foundation resources[7]. The scientists examined the well-being of multiple bee species after the bees had been foraging in untreated or clothianidin-treated fields of oilseed rape with an average size of around 9 hectares. The data showed evidence for adverse effects on the health of bumblebees and other wild bees, whereas honeybee colonies remained largely unscathed. "This doesn't mean that there aren't any negative effects on honeybees, but so far I don't see any evidence from field studies supporting that," says lead author Maj Rundlöf.

"We saw absolutely no effect on the honeybee colonies in those trials."

Numerous real-world factors could be mitigating the known toxicity of neonics. Honeybees do not simply binge on their favourite flower. By analysing pollen samples and the waggle dance that honeybees use to communicate the location of nearby food sources to other hive members, Ratnieks learned that bees that live near highly desirable oilseed rape spend barely half their time foraging in the crop[8]. In Britain, he explains, oilseed rape mostly blooms in the spring, when plenty of other flowers offer the bee a variety of food choices. What is more, honeybee colonies can often shake off moderate losses, with enthusiastic springtime reproduction making up for individual deaths — particularly when nutrient-rich crops such as oilseed rape are available. "If you have a steep increase in colony strength, it's questionable whether you would still find very small effects caused by neonicotinoids," says Pistorius.

Scientists have also analysed additional data from national bee-health surveys and observations from bee-keepers. Although many keepers continue to report bee die-offs, no clear thread directly links these to neonicotinoids. "In the last decade of having many colonies in treated oilseed rape in real agricultural settings, I have never had a single incident," says Pistorius. He acknowledges that this observation is anecdotal, but notes that his team's national bee-monitoring system reports similar findings across Germany, with no incidents apparently associated with neonicotinoid exposure to nectar, pollen or dust drift during sowing from seed-treated oilseed rape since 2005. Carreck, also a lifelong bee-keeper, describes similar observations from the UK government's Wildife Incident Investigation Scheme. "There hasn't been a confirmed incident of honeybees being killed by the approved use of an agricultural pesticide since 2003," he says.

WHAT BEES SEE

There is one scenario in which the danger of neonicotinoids to bees is unambiguous. In spring 2008, there was an abrupt rise in bee deaths in southern Germany that affected more than 11,000 colonies. The cause turned out to be clothianidin-contaminated dust, abraded from the surface of treated seeds, that became airborne during machine-assisted planting of maize. Similar incidents throughout Europe and North America have also revolved around maize, which is planted in spring when other crops and wildflowers are in bloom — elevating the risk to bees. Krupke, who works in the heart of the US corn belt, began investigating similar reports in 2010. "In all cases, we found that the dead bees had neonicotinoids on them," he says. His group systematically analysed[9] the extent of contamination and obtained striking data regarding the seed dust. "It was so incredibly toxic — a bee flying behind a corn planter would just die on the spot," he says.

After the 2008 incidents, Germany banned neonicotinoid seed treatments for maize. Other European nations required farmers to use deflectors that minimize dust release. In 2013, Bayer CropScience released a new lubricant for pneumatic planters that limits seed abrasion and hence dust, and the Canadian government mandated its use for treated maize and soya bean. "In last year's report from Canada's Pest Management Regulatory Agency, loss of bees due to corn planting had dropped by 70%," says Scott-Dupree. Unfortunately, neither measure is commonplace in the vast cornfields of the United States; until planting practices change, Krupke is seeking other means to protect bee colonies. "We're trying to figure out how far from cornfields they have to be before there is no risk of contacting toxic levels of planter dust," he says.

BANS AND CONSEQUENCES

The central question of whether neonicotinoid seed treatments, when properly applied, are harming honeybees remains murky. Some experts rule out serious danger from the doses found in nectar or pollen: "I just don't see the exposure being there, and I don't see the evidence of colony-level effects for honeybees," says vanEngelsdorp, who is now at the University of Maryland in College Park.

Nevertheless, a cautionary scientific report produced by the European Food Safety Authority (EFSA) in May 2012 — alongside considerable political pressure that included an online petition signed by two and a half million people — moved the European Commission (EC) to action. In spring 2013, following a close vote, the EC enacted a two-year moratorium on imidacloprid, thiamethoxam and clothianidin treatment for bee-attractive crops (including oilseed rape and maize).

Both the initial scientific report and the subsequent EFSA guidance document on assessing pesticide risk have come under fire as politically motivated rush jobs. Unsurprisingly, Syngenta is among the most vocal critics, claiming that the guidance requires pesticide manufacturers to demonstrate safety at a level that is statistically unfeasible. "It requires the ability to detect a 7% effect on honeybee colonies, which is below the natural variability you would see," says Campbell, adding that the guidance remains the subject of ongoing debate two years after its release. "The EC was using a very controversial, very conservative approach that has not yet been agreed upon within the EU," he says.

Manufacturers are not the only critics, however; some scientists are calling for a more nuanced approach to pesticide evaluation. "The scientific discussion is often very emotional, and there is also a lot of political pressure," says Pistorius. "The risk from neonics certainly varies greatly for different routes of exposure, different crops and applications, and this issue requires a substantially more differentiated evaluation that considers these various uses and conditions."

Conversely, pesticide manufacturers were quick to offer dire predictions of agricultural

THINKSTOCK

disaster that, for now, bear little resemblance to reality. A report funded by Bayer CropScience and Syngenta suggested that, over five years, continued suspension of neonicotinoids could cost the EU between €17 billion (US$19 billion) and €23 billion. But the data thus far indicate few negative effects — indeed, the EC's crop-monitoring report from December 2014 described a highly productive year for crops such as maize and sunflowers. However, oilseed rape is not included: because of the timing of the planting season, the first harvest of untreated crops will not happen until later this year. Early data from Britain's Home Grown Cereals Authority suggest that only 5% of seedlings in England and Scotland were lost to the flea beetles normally thwarted by neonics. But the report also notes that certain regions were hit especially hard, experiencing losses of 40% or more. "The problem is that it was almost impossible to predict where flea beetle attacks would occur," says Campbell, "and once a farmer knew they had a problem it was too late and the damage was done."

NO QUICK FIX

The EU moratorium may prove to be a missed opportunity for science. Its short duration makes tracking trends a challenge — even if there were a way to detect them. "It's kind of daft," says Goulson. "I don't understand why the EU didn't introduce some measures to at least try to monitor the effects." Furthermore, use of alternative pesticides may be masking any benefits of the moratorium. Several European nations have pursued 'derogations' that allow temporary use of seed treatments — essentially sidestepping the moratorium — and the UK government has authorized the spraying of neonicotinoids. Many farmers are also increasing their use of pyrethroid pesticides, spraying crops up to five times a year rather than just the normal one or two. In addition to also being highly toxic to bees, the route of application for pyrethroids could mean more accidental exposure for non-target organisms such as bees.

Perhaps a greater concern is the illusion that politicians are 'doing something' about bee deaths, but ignoring other important threats. "There's a consensus among bee scientists that long-term declines are primarily due to changes in land use that leave less forage and fewer places to nest," says Carreck. And although vanEngelsdorp's early investigations of CCD-ravaged hives uncovered no straightforward answers, they did reveal high levels of the parasitic mite *Varroa destructor* and widespread viral and fungal infections. Over the ensuing nine years, vanEngelsdorp and many other bee researchers have become increasingly convinced that this mite, which carries diseases and also weakens immunity against other infections, is public enemy number one for bee-keepers. "We've been dealing with *Varroa* mites for a long time," he says. "But today's mite is different from the *Varroa* of 30 years ago, and the viruses have also changed." He adds that neonics are not the only chemicals that bees encounter — he and his colleagues found measurable amounts of more than 120 agricultural chemicals or derivatives in honeybee colonies[10], any of which might affect bee health.

For now, no national ban is under consideration in the United States or Canada, but the issue remains bitterly politicized anywhere there are farms and apiaries. Neonicotinoid manufacturers and advocates claim that environmental organizations are colluding to promote biased research. Environmental (and some bee-keeping) groups accuse researchers who find no clear evidence for neonic harm of being in the pocket of big agribusiness. "We're not funded by these companies," says Ratnieks, "but you have the feeling that if you're not careful, being objective may easily be perceived as being 'pro-pesticide' — which we're not." And in general, the media leave little opportunity for scientists to address these issues with nuance. Carreck recalls speaking to a British journalist the day the EU ban went into effect. "He wanted a debate, and said he wanted to get 'both sides'. I told him: 'I'm not on anyone's side — I'm trying to be objective here!'" says Carreck. "He immediately lost interest." ■

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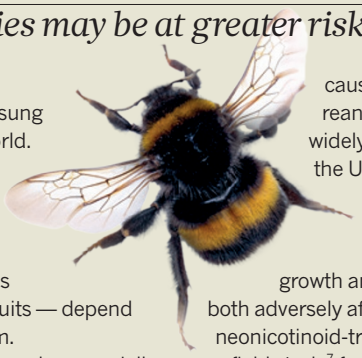
PLIGHT OF THE BUMBLEBEE

Other bee species may be at greater risk from pesticides



The plump bumblebee, *Bombus terrestris,* is an unsung hero of the agricultural world. Many experts believe that the majority of pollination is conducted by these insects, and that some crops — such as tomatoes and most soft fruits — depend almost exclusively on them.

However, bumblebees may be especially susceptible to the effects of neonicotinoid pesticides. A team led by Dave Goulson, an entomologist at the University of Sussex in Brighton, UK, has found that bumblebees experiencing the neurological effects of these chemicals provide poor support for their hives[3]. "You get a big drop in the number of bees that come back with pollen, and that's the hive's only source of protein," he says, "so they can't rear enough queens."

Even scientists who are sceptical of the risk to honeybees recognize the importance of assessing potential impact on other pollinators. "Bumblebee colonies are clearly different from honeybee colonies," says Peter Campbell, a senior environmental specialist at Swiss agrochemical firm Syngenta, who is based in Bracknell, UK. "But Syngenta has conducted and submitted for publication a field study that clearly showed no effects of the neonicotinoid thiamethoxam on bumblebees."

Nevertheless, a steady trickle of data over the past few years has given increased cause for concern. After reanalysing data from a widely criticized 2013 study by the UK Food & Environment Research Association, Goulson's team found[11] that bumblebee-colony growth and queen production were both adversely affected by exposure to neonicotinoid-treated crops. In parallel, a field study[7] from the University of Lund in Sweden showed that when foraging in oilseed-rape plants grown from clothianidin-treated seeds, both colony growth and queen production were stunted for the bumblebee, and that solitary bees from the species *Osmia bicornis* failed to build nests. "I don't think any of us expected to see what we saw," says lead author Maj Rundlöf.

Unfortunately, the tests used to assess pesticide toxicity may prove irrelevant to bumblebees — to say nothing of the thousands of wild solitary bee species (see page S62). Given that many of these species are endangered, the problem is all the more pressing. "A honeybee colony has got a huge degree of resilience, but a solitary bee is just that — if a female doesn't lay eggs that hatch, that's the end of her," says Norman Carreck, a bee researcher at the University of Sussex. "We have to look at as many species as we can, because there is quite good evidence that not all of them are as good at detoxifying these substances as others." M.E.

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