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Immune Suppression by Neonicotinoid Insecticides at the Root of Global Wildlife Declines

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Abstract

Outbreaks of infectious diseases in honey bees, fish, amphibians, bats and birds in the past two decades have coincided with the increasing use of systemic insecticides, notably the neonicotinoids and fipronil. A link between insecticides and such diseases is hypothesised. Firstly, the disease outbreaks started in countries and regions where systemic insecticides were used for the first time, and later they spread to other countries. Secondly, recent evidence of immune suppression in bees and fish caused by neonicotinoids has provided an important clue to understand the sub-lethal impact of these insecticides not only on these organisms, but probably on other wildlife affected by emerging infectious diseases. While this is occurring, environmental authorities in developed countries ignore the calls of apiarists (who are most affected) and do not target neonicotinoids in their regular monitoring schedules. Equally, scientists looking for answers to the problem are unaware of the new threat that systemic insecticides have introduced in terrestrial and aquatic ecosystems.

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Key words

systemic insecticides; imidacloprid; infectious diseases; honeybees; bats; birds; fish; frogs; pollinators

Introduction

In 1991, Bayer CropScience introduced a new type of insecticide into the US; imidacloprid, the first member of a group now known as the neonicotinoids. Bayer Scientist Abbink[1] certified that: "imidacloprid is the first highly effective insecticide whose mode of action has been found to derive from almost complete and virtually irreversible blockage of post-synaptic nicotinic acetylcholine receptors (nAChRs) in the central nervous system (CNS) of insects." Imidacloprid differed from conventional spray pesticides in that it could be used as seed dressings or soil treatments. When used as a seed dressing the insecticide will migrate from the stem to the leaf tips, and eventually into the flowers and pollen. Any insect that feeds on the crop dies; but bees, bumblebees, hoverflies and butterflies that collect contaminated pollen or nectar from the crop are also poisoned.

In 1994 imidacloprid was licensed for use in Europe. In July 1994 beekeepers in France noticed something unexpected.[2] Over the course of a few days, just after the sunflowers had bloomed, a substantial number of their hives would collapse, as the worker bees flew off and never returned, leaving the queen and immature workers to die. The French beekeepers soon believed they knew the reason; a brand-new insecticide called Gaucho® with imidacloprid as active ingredient was being applied to sunflowers for the first time.

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In 2001, Belzunces and co-workers[3] reported an acute (48-hour) lethal dose of imidacloprid of only 40 ng per bee; a dose far smaller than most other insecticides. However, their important discovery was that the lethal dose from chronic exposure to imidacloprid was 4000 times less: ingesting 1 pg a day was enough to kill a bee within 10 days. Moreover, they showed that imidacloprid is degraded into six metabolites, some of which were even more toxic than the parent compound. Belzunces realised that the very small traces of imidacloprid in the range of µg kg^{-1} (ppb) of pollen constituted a significant risk for bees, particularly upon chronic exposure in the bee hive. Tennekes[4] explained Belzunces' findings in 2010 by showing that neonicotinoids can produce effects at any concentration level provided the exposure time in sufficiently long. In a more recent paper,[5] Tennekes and Sánchez-Bayo demonstrated that chemicals that bind irreversibly to specific receptors (neonicotinoids, genotoxic carcinogens and some metaloids) will produce toxic effects in a time-dependent manner, no matter how low the level of exposure.

In 2003, in a 108-page document, the Comité Scientifique and Technique commissioned by the French Ministry of Agriculture[6] concluded that: "the treatment of sunflowers with imidacloprid is a significant risk to bees in several stages of life." In 2004, in France, Colin et al. demonstrated that sub-lethal doses of 6 ppb imidacloprid or 2 ppb fipronil were enough to disrupt feeding on bees,[7] precisely what the manufacturer had advertised for the use of imidacloprid in termite control.[8] In 2008 Yang et al. confirmed the French scientists' findings of disruption of foraging.[9] Acute exposure to sub-lethal doses of imidacloprid (as low as 50 ppb) delayed the return visit of a bee, and the time delay was dose-dependent.

In 2006, deaths and disappearances amongst managed bee colonies in the US had reached such epidemic proportions that the term 'Colony Collapse Disorder' (CCD) came into use (**Fig. 1**). In fact high bee losses in the US had begun in 1995, when *Varroa* mites were first identified by beekeepers as a lethal threat to honeybee colonies.[10] Although treatment for the mites was instituted, colony losses had continued to escalate.[11] In January 2012, Steve Ellis, secretary of the US National Honey Bee Advisory Board and a beekeeper for 35 years said: "We are inching our way towards a critical tipping point." In 2011, he had so many abnormal bee die-offs that he will qualify for disaster relief from the US Department of Agriculture (USDA).[12]

In 2010, the Pesticides Industry and the US Environmental Protection Agency (US EPA) recognized that:[13] "Many who are familiar with pesticide risk assessment recognize that the methodology and testing scheme for foliar application products (where exposure may be primarily through surface contact) is not adapted to assess potential hazard and risk from systemic pesticides".

Suppression of immune response to infection in honey bees

Belzunces' observations have been recently substantiated by Pettis et al.[14] They demonstrated increased susceptibility of newly emerged worker bees to the gut pathogen *Nosema ceranae* following exposure of honey bee colonies during three brood generations to imidacloprid dosages of 5 ppb and 20 ppb (which are exposures below the levels demonstrated to cause effects on longevity or foraging in adult honey bees). The microsporidian pathogen *Nosema ceranae* targets the honeybee midgut and deprives infected bees of nutrients. Thus, they have a much greater chance of dying prematurely. Although residues of imidacloprid were found in bee bread and bees from exposed colonies, and increased in direct and expected proportion to the concentrations in the treated protein patties, newly emerged bees that were subsequently shown to be more susceptible to *Nosema ceranae* tested negative for imidacloprid. Therefore, the test bees could only have received pesticide exposure during larval development, and pesticide exposure to test bees could only have been indirectly from brood food from nurse bees. The evidence from these experiments shows that immune suppression by imidacloprid can occur in the absence of detectable imidacloprid residues.

Similar laboratory results with imidacloprid had been published by Alaux et al. two years before.[15] In 2011, Vidau et al.[16] also proved that exposure to sub-lethal doses of fipronil and thiacloprid highly increased the mortality of honeybees previously infected by *Nosema ceranae*. In fact, the manufacturer's own leaflet states that imidacloprid makes pathogenic soil fungi 10000 times more dangerous to termites.[8]

The microsporidian, *Nosema ceranae*, was first detected in the Asiatic honey bee, *Apis cerana* in 1994 in China, where imidacloprid had been used for rice production since 1991. Pettis et al. detected *N. ceranae* infections in US honey bee samples collected during the period 1995 to 2007,[17] significantly earlier than in Europe, where *N. ceranae* was found in Spain in 2004 and France, Germany and Switzerland in 2005. Klee et al.,[18] using genetic sequencing methods to screen microsporidian isolates across the world concluded that this emergent pathogen "had most likely jumped species, probably within the last decade."

Bumble bee declines in the US and Europe linked to infections

Massive declines in wild bumble bees in the US and Canada were reported in the late 1990s. In Ontario, previously a 'hot spot' for bumble bees, Colla and Packer sampled sites for three consecutive summers (2004-06).[19] Out of the 14 species that had been present in the same areas in 1971-73, three had disappeared completely and five of the remaining 11 were in steep decline. Of the species that had been fourth most common in the 1970s (14% of all samples), they found only one male. In 2008, the Xerces Society for Invertebrate Conservation reported that "at least four species of formerly common North American wild species have experienced catastrophic declines over the past decade - two of them may be on the brink of extinction". In a review by the Xerces Society, Robbin Thorp (UC Davis) and colleagues[20] stated that three formerly common species of bumble bee: "went from being widespread and commonly found to rare or absent within a relatively short period of time (about 7-10 years)." They were found to be infected with a series of unusual pathogens. In 2008, Otterstatter and Thomson[21] estimated 'spillover' of the pathogen *Crithidia bombi* from greenhouses where imported bumble bees were being used for pollination in Canada. Many bumble bee scientists became convinced that infections in imported bumble bees were at the heart of the declines in the wild population. By February 2010, the situation was so bad that a broad coalition of 67 scientists (many of them bumble bee experts) sent a letter to The Hon Tom Vilsack, Secretary USDA in Washington and the Administrator of the USDA's Animal and Plant Health Inspection Service (APHIS)[22] to regulate the movement and health of commercial bumble bees to safeguard wild, native bumble bee pollinators. However, Cameron et al. were unconvinced by this 'spillover' theory.[23] They showed that those species that had declined had significantly higher infection levels of the pathogen *Nosema bombi* and had low genetic diversity compared with those that had not. They concluded that "these observations are reminiscent of reports of other introduced fungal pathogens that pose widespread threats to some taxa," including frogs and bats.

Mommaerts et al.[24] in 2010 demonstrated negative effects of sub-lethal doses of imidacloprid on *Bombus terrestris* worker foraging behaviour. In 2012, UK researchers confirmed that when colonies of *B. terrestris* were exposed to field-realistic levels of imidacloprid, there was a significantly reduced growth rate and an 85% reduction in new queens when compared with control colonies.[25]

Global amphibian declines in relation to infection

In 1999, two novel pathogens were described in amphibians: the chytrid fungus (*Batratrachochytrium dendrobatidis* or *Bd*.) and the *ranavirus*.[26,27] Soon after, two species of once common frogs that had inhabited the thousands of lakes and ponds in California's Sierra Nevada were being wiped out by *chytridiomycosis*, a disease

Figure 1. a. Dead queen and workers. A typical dead colony from an area dominated by intensive arable crops - wheat, oilseed rape and barley, where imidacloprid and clothianidin are used all the time. About 50% of the colonies are lost every winter to what is called 'Fall Dwindling' - the bees stop rearing larvae in Autumn, and the colony is just too small to survive the winter. b. Workers fill the hive cells ('honeypots') with nectar. c. Worker bee returning to the hive with baskets full of oilseed rape pollen, which may be contaminated with imidacloprid, as it is commonly used on rape crops.

caused by the chytrid pathogen *Bd*. Vredenburg et al.[28] described the progress of the infection in a study area that comprised three lake basins separated by 20-50 km. *Bd.* was first detected in the smallest basin in June 2004 and in the two larger basins in August 2004 and July 2005 respectively. It took only one year to spread to virtually all the frog populations in the small basin and 3-5 years in the other two. The authors concluded that *Bd.* was a novel pathogen spreading through naïve host populations. For the decade after they were first reported, these two pathogens, chytrid fungus and *ranavirus* had between them caused mass deaths across the US in a wide variety of amphibian populations. By 2007 they had been detected in six continents.[28] In 2010, it was reported that there was still "no cure yet for the chytrid fungus which is devastating frog populations."[29] Maps of imidacloprid[30] and thiamethoxam[31] use from the US Geological Survey for 2002 show that the densest rates of application of imidacloprid and thiamethoxam were in the Central Valley running parallel to California's Sierra Nevada (**Fig. 2** & **Fig. 3**). Davidson et al.[32] reported in 2002 spatial patterns of decline for four California ranid frogs and matched the declines with the distribution of agricultural lands (also based on USGS land use maps and key predominant wind directions based on California Air Resources streamline wind maps). The authors stated that "In California, the transport and deposition of pesticides from the agriculturally intensive Central Valley to the adjacent Sierra Nevada is well documented, and pesticides have been found in the bodies of Sierra frogs". They raised the possibility that exposure to pesticides might weaken their immune systems increasing their susceptibility to disease. However, since the pesticides they targeted did not include neonicotinoids, they were unable to pinpoint the exact link between specific chemicals and amphibian losses.

The arrival of the chytrid fungus and *ranavirus* in Europe was later than in the US. However, at the Zoological Society of London in 2008, it was predicted that more than half of Europe's amphibians faced extinction by 2050.[33] In October 2010, the devastation of amphibian populations had hit the UK as well. In *Animal Conservation*, researchers reported that the rapidly spreading *ranavirus* "is killing common frogs in the UK in areas where it has never been seen before".[34] Joseph Mendelson wrote recently:[35] "The reality of amphibian declines and extinctions has shifted the ecological baseline in so many ecosystems, that an entire generation of biologists is conducting their research in a framework that has been very recently remodelled."

Evidence of deviation from normal pathogen/host relationships

Amphibians, particularly tadpoles, are considered to be environmental indicators of indirect ecosystem effects because of their unique niche at the boundary of the aquatic-terrestrial ecosystems as well as their sensitivity to pollutants. While tadpoles feed on periphyton, adult amphibians are strictly insectivorous animals. They were the first group of vertebrates to be affected by the epidemics of disease caused by uncommon pathogens.

In 2011, a global study published in Nature[36] noted that amphibian population declines from 1980s far exceeded those of other vertebrate groups. By the late 1990s emerging pathogens in wildlife had become an increasing cause for alarm.[26,27,37,38] Dobson and Foufopoulos[37] classified epidemics causing large-scale declines in wildlife species into three types. The first involves parasitic organisms that have recently invaded a wildlife population where there is high host susceptibility to a novel pathogen. The second is an emerging pathogen native to a specific host that is spreading because of an environmental factor such as pollution. The third involved extinctions caused by "pathogens that have recently invaded a naïve host population that is distressed or immune-compromised because of existing environmental conditions." Dobson and Foufopoulos[37] referred only to increased ultraviolet radiation and climate change. Hof et al. added land-use change,[36] and Davidson et al.[32] added pesticides into the equation. A link was suggested between pesticides and frog deformities. Kiesecker,[39] possibly unaware that the use of imidacloprid, clothianidin and thiamethoxam was by now widespread in the US,[30,31] found that atrazine (herbicide) and malathion (organophosphate insecticide) made frogs more susceptible to a parasite, a burrowing trematode worm, which affected tadpoles. Our hypothesis is, as had been proved with honey bees,[14-16] that exposure to small doses of the three neonicotinoid insecticides is likely to have occurred and may have weakened the amphibian immune systems, such that they became more susceptible to pathogens.

Environmental pollution; neonicotinoids have slipped through the safety water quality checks

Imidacloprid is stable in water, not easily biodegradable and can accumulate in soil and sediments, where it persists for a few

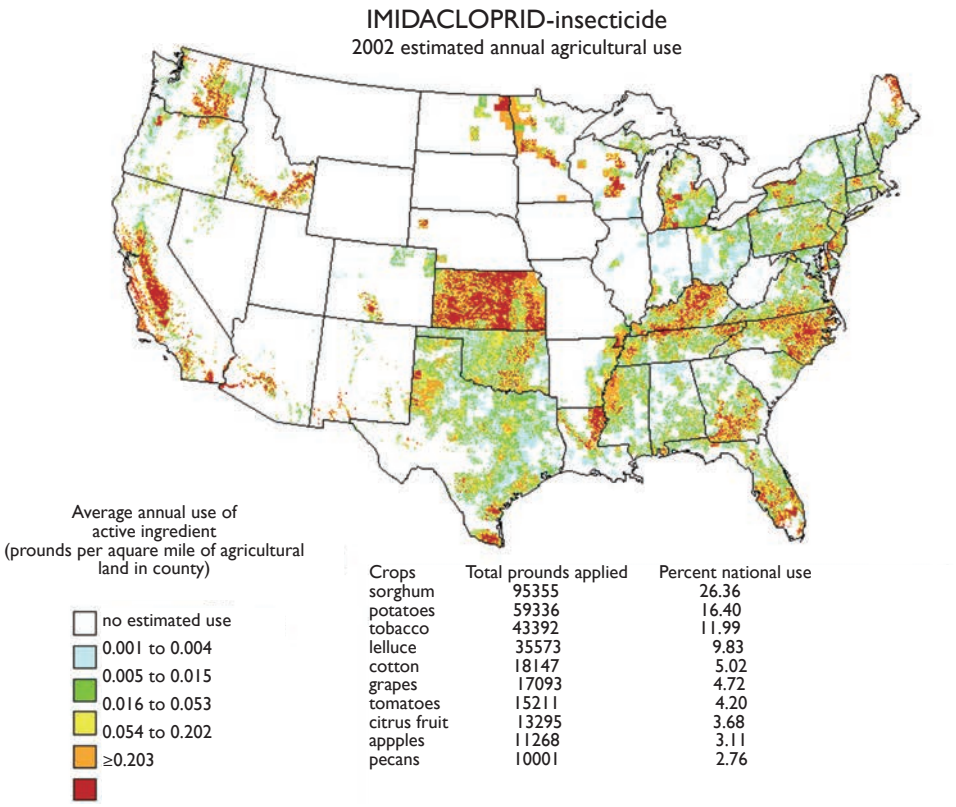

Figure 2. US Geological Survey National Water-Quality (NAWQA) Program. Pesticide National Synthesis Project. Pesticide Use Map. Imidacloprid insecticide. 2002 estimated annual agricultural use.

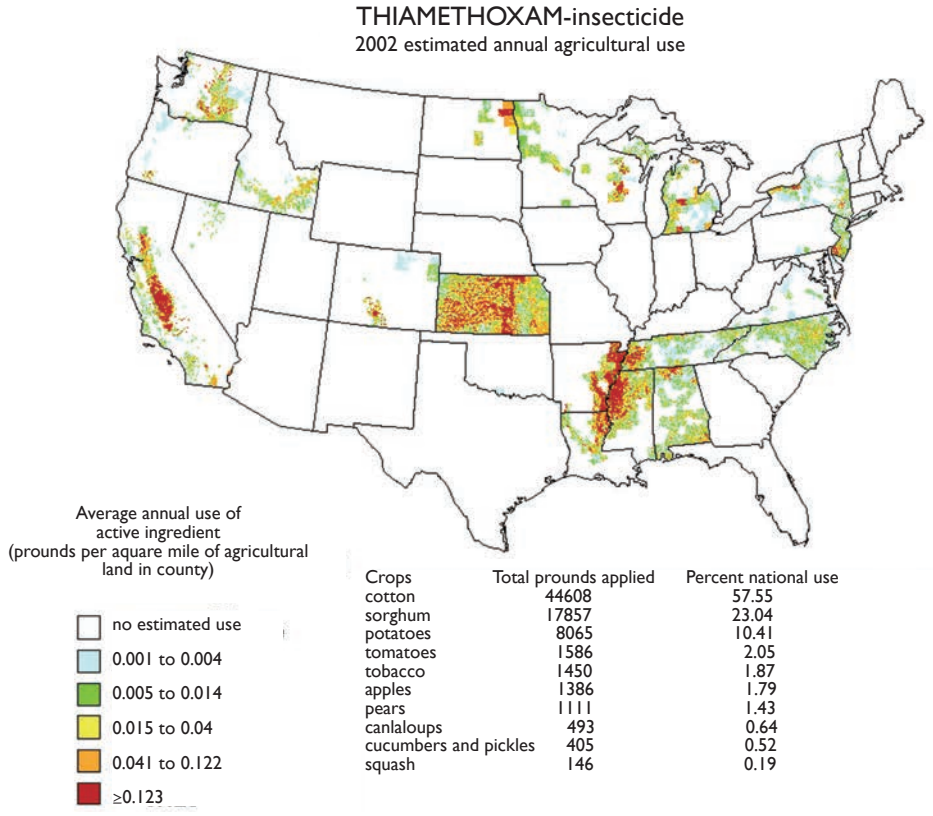

Figure 3. US Geological Survey National Water-Quality (NAWQA) Program. Pesticide National Synthesis Project. Pesticide Use Map. Thiamethoxam insecticide. 2002 estimated annual agricultural use.

Table 1. Persistence of neonicotinoid insecticides as expressed by their half-lives (days) in environmental matrices (Source: Footprint Database[93])

Compound	Water		Water-sediment	Soil**
	Photolysis	Hydrolysis*		
Acetamiprid	34	420	NA	3 (2-20)
Clothianidin	0.1	14	56	545 (13-1386)
Dinotefuran	0.2	stable	NA	82 (50-100)
Imidacloprid	0.2	~365	129	191 (104-228)
Nitenpyram	NA	2.9	NA	8
Thiacloprid	stable	stable	28	16 (9-27)
Thiamethoxam	2.7	11.5	40	50 (7-72)

NA=not available;

*median degradation time (DT50) in alkaline media (pH 9);

**typical value (range) for aerobic degradation.

months (half-life 191 days, **Table 1**).[40] Data on clothianidin, based on laboratory and field studies, are similar and show that this compound is more persistent (half-life over 500 days) and mobile, stable to hydrolysis, and has potential to leach into ground water and be transported via runoff to surface water bodies.[41] Imidacloprid and clothianidin are highly toxic to aquatic invertebrates.[40,41] Thiamethoxam is metabolised to clothianidin, so presumably the chemicals must have many features in common and are probably additive in their effects.

The systemic neonicotinoid insecticides did not feature in any of four recent reports on US national studies on the presence of pesticides in ground-water.[42-45] However, New York State has not registered clothianidin and has severely restricted the use of imidacloprid and thiamethoxam because of contamination of their water sources.[46,47]

In Europe, under the current statutory requirements of the EU Dangerous Substances Directive and the EU Water Framework, the five neonicotinoid pesticides licensed for use in the UK are not included in the list of chemical substances that are required to be monitored, but in the Netherlands a geographically low-lying country, with about 25% of its area below sea level (http://en.wikipedia.org/wiki/Netherlands#cite_note-milrek-8) and 50% of its land lying less than one metre above sea level, the Water Boards have been measuring imidacloprid levels in surface water for more than a decade.[4,48] Levels have increased from 2003-2008 and the increases correlated with decreasing abundance of flying insects of the order *Diptera*.[48] The Maximum Tolerable Risk (MTR) value, i.e. the environmental concentration of that substance at which species in an ecosystem are considered by Dutch regulators to be safe from effects caused by the substance, was exceeded, in some areas by more than five times and this was most noticeable in the regions where horticulture and agriculture were concentrated.[48] Pesticide Fact sheets show that imidacloprid, clothianidin and fipronil are just as toxic to non-target and beneficial invertebrates as they are to target ones.[40,41,49] The WWF Living Planet Report 2010 found that biodiversity was declining faster in freshwater than in any other biome, including coral reefs and tropical forests.[50] Neonicotinoids are also known to be toxic to aquatic invertebrates, and instructions for use state that they should not be applied where they can contaminate water. Based on laboratory and field studies, the available data on clothianidin show that the compound is persistent and mobile, stable to hydrolysis, and has potential to leach to ground water and be transported via runoff to surface water bodies.[41] According to US EPA ecologists, clothianidin is an acute risk to freshwater invertebrates. Acute lethal toxicity to benthic invertebrates also suggests this conclusion. These organisms are an integral part of the freshwater trophic systems and serve as both decomposers/predators that are important for nutrient cycles and a food source for larger predators (e.g. fish). Yearly application of neonicotinoids without monitoring surface or groundwater, in combination with flooding incidents (many of which have occurred in recent years), will increase insecticide runoff and decrease freshwater biodiversity.

Suppression of immune response to parasitic infections in fish

While studying Japanese medaka fish in experimental paddy fields, Sánchez-Bayo and Goka observed physiological stress in juvenile medaka and massive infections of the weaker fish by a *Trichodina* ectoparasite where rice was treated with imidacloprid (**Fig. 4**), compared with medaka in control rice fields.[51] In 1994 there was an epidemic of parasitic salmon lice *Lepeophtheirus salmonis* in salmon farms sited on the Atlantic coast of Canada. However, the first epidemic of *L. salmonis* involving the wild pink salmon (*Oncorhynchos gorbuscha*) populations on the Pacific coast of British Columbia occurred suddenly in spring 2001.[52] Louse-induced mortality in wild salmon is often more than 80%. If the outbreak were to continue, Krkošeck et al. predicted a 99% collapse within four salmon generations.[52] It became apparent that in areas without salmon farms, the prevalence of infestation was low whereas there was collapse in the wild salmon populations in the proximity of farms.[53]

Infections in bats in the US and Europe

In 2006, the same year that beekeepers realised the full implications of the disaster of CCD, White Nose Syndrome (WNS), a virulent and fatal fungus disease of hibernating bats, came to the attention of ecologists.[54] It was first found in a cave in New York State in the winter of 2005-6 and rapidly spread

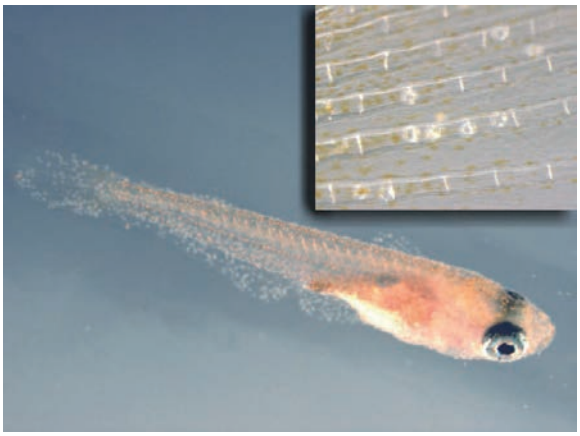

Figure 4. Medaka fish (*Oryzias latipes*) fry infested with the ectoparasite *Cyclochaeta* (=*Trichodina*) *domerguei* (white circles in insert). Fish reared in a rice experimental paddy treated with imidacloprid were significantly more infested with these parasites compared to fish reared in non-treated paddies (after Sánchez-Bayo and Goka, 2005).

through the north-eastern states. A powdery white nose tip was pathognomonic of the disease and when the powder was cultured a fungus, *Geomyces destructans* was grown. This pathogen infected the skin and wing membranes of bats and was associated with unprecedented numbers of deaths, affecting six different species of bat.

The mortality in a colony could be up to 95% and it was reported that 1 million bats had died since 2006. In August 2010 Frick et al., using a combination of existing field data on hibernacula counts, rate of spread of the disease and mathematical models, predicted that regional extinction of the little brown bat in the north-eastern US was likely to occur.[55] A map showed the rapid extension of WNS, year by year, starting in New York State and spreading throughout the north-eastern and Mid-Atlantic regions and into Ontario and Quebec in Canada. In March 2012, the US Fish & Wildlife Service Biologists estimated that at least 5.7 million to 6.7 million bats have died from WNS and they expect the disease to continue to spread.[56] Mortality rates at many sites in Eastern North America have reached up to 100%. The Fish & Wildlife Service is producing monthly maps showing the relentless progress of the disease.[57] Dan Ashe, the Director,[58] said: "Bats provide tremendous value to the US economy as natural pest control for American farms and forests every year, while playing an essential role in helping to control insects that can spread disease to people."

In Europe, in a German-led multicentre study published in August 2010, hibernating bats with obvious fungal growth were sampled in Germany, Switzerland and Hungary.[59] Despite laboratory confirmation that these bats were colonised by *G. destructans*, there was no evidence that they were sick. However, by November 2010, a report from the Czech Republic and Slovakia said that the numbers of hibernation cave sites in which the fungus was found were increasing rapidly, from 33 at the beginning of winter to 76 (out of 98 surveyed) at the end, and they said that sickness was starting to occur in some of the bats. The majority of these sites had been negative in the 2008/9 survey.[60] The authors concluded that "*G. destructans* was found to be widespread in the Czech Republic and Slovakia, with an epizootic incidence in bats during the most recent years. Further development of the situation urgently requires a detailed pan-Europe monitoring scheme."

Imidacloprid, thiamethoxam, clothianidin and other neonicotinoids applied to the seed on arable crops are non-selective and are toxic to non-target and beneficial insects as well.[40,41] Bats are insectivorous species, so their survival has been compromised since the numbers of insects have been drastically reduced.[48] Again, our hypothesis is that the thousands of invertebrates consumed in their diet will inevitably have exposed bats to small cumulative doses of these toxins.[4,5,14] In fact the abnormal neurological behaviour[55] which is also pathognomonic of bats affected by WNS, is very similar to the disorientation described in CCD honeybees that causes delay in foraging or eventual abandonment of the hive.[7,9]

Declines due to pathogens in birds in the US and Europe

A mycoplasmal conjunctivitis was first reported in wild house finches (*Carpodacus mexicanus*) in February 1994 in suburban Washington, DC. It was identified as *Mycoplasma gallisepticum*, a pathogen of poultry that had not previously been associated with wild songbirds.[61] In the first three years it killed an estimated 225 million finches and by 1995 it had spread to the American goldfinch (*Carduelis tristis*). There was a dramatic spread of disease to house finches in the mid-West and South East. Genome evolution in the bacterial pathogen was measured over 13 years and was found to be extremely rapid.[62] Degradation of phage-derived sequences in affected house finches had resulted in functional loss of bacterial immunity resulting in rapidly spreading deadly disease.

In Europe epidemics caused by a variety of novel pathogens in wild birds began in early 2000. Greenfinch (*Carduelis chloris*) numbers in Europe have been devastated by infections with *Trichomonas gallinae*, a protozoal organism which invades the bird's crop and mucosal lining of the beak. Deaths started in the UK around 2005.[63] At the same time, chaffinches (*Fringilla coelebs*) appeared in gardens with white, crusty growths on their legs and feet caused by a papilloma virus. The mortality is said to be about 20%, so the disease kills more slowly than with the Greenfinch *Trichomonas* infections.[64] In 2005, acute necrotising pneumonitis with *Suttonella ornithocola spp*. in the Paridae family of birds (tits) was first reported by researchers in Inverness and the Institute of Zoology, London; they found that a gram negative bacterium present in the diseased birds represented a novel species.[65] In 2006 there were further reports of mortality from this novel bacterium. The first case of avian pox was reported in the UK in 2006, but prior to that it had appeared in Austria, Czech Republic, Slovakia and Scandinavia.[66] Great Tits (*Parus major*) seem to be affected more severely than other species, with warty tumour-like growths around the eyes and beak, which sometimes interfered with feeding. According to Ben Sheldon, Director of the Edward Grey Institute near Oxford where records had been kept since 1947, the disease was detected for the first time in Wytham Woods in 2010. In September 2011, mass deaths

of Blackbirds (*Turdus merula*) were reported in the Rhine-Neckar area of Germany.[67] The Bernhard-Noct Institute for Tropical Diseases and the Friedrich-Loeffler Institute examined four birds and confirmed that it was the tropical *Usutu virus* from Africa. It was first seen in Austria in 2001, followed by reports from Italy, Hungary and Switzerland. In birds it first causes apathy, then signs of a central nervous system disorder, with unnatural movements of the head.

At the same time these diseases in birds originated and spread, the percentage of UK cropland treated with neonicotinoids has gone from 0.65% in 1994, to 30% in 2010. But the biggest increases have occurred in the last 10 years, from 1 million acres in 2000, to more than 3 million acres in 2010.[68] While the acreage of this kind of insecticides increases, the decline of more than 30 species of birds in the UK has been linked to the agricultural practices, including pesticides,[69] and the Pan-European Common Bird Monitoring Scheme recently reported devastating declines in farmland bird numbers: overall populations dropped from 600 million to 300 million between 1980 and 2009, with Britain being one of the nations worst affected by these losses.[70] Note that all species affected are insectivorous at least during the time of nesting and rearing their young. As with amphibians and bats, currently there is no evidence that any of these infections are caused by neonicotinoid insecticides alone. However, based on the timing of their appearance in the wild, we hypothesise here the obvious connection between all these pathogen outbreaks and the use of neonicotinoids.

Evidence of persistence of neonicotinoids in the environment

In March 2009 California's Department of Pesticide Regulation demanded re-evaluation of other uses of imidacloprid.[71] Their data noted two critical findings. One, high levels of imidacloprid in leaves and blossoms of treated plants (residues in some plants measured higher than 4 mg kg^{-1}) and two, increases in residue levels over time so that significant residues from the previous season are available to the treated plants. California's DPR issued a further notice of demands for re-evaluation.[72] In January 2011, all imidacloprid registrants voluntarily amended their labels removing their applications to almonds.

Clothianidin degradation in soil is slow, with half-lives ranging from 1 to over 2 years (**Table 1**), and in one case, no dissipation was recorded.[73] Persistence in the environment of clothianidin was confirmed by Krupke et al..[74] Field studies from Indiana showed widespread clothianidin contamination of bees and the environment close to maize fields, resulting in multiple routes of exposure throughout the foraging period. Residues were also found in dandelions (*Taraxacum spp*.) foraged by bees; in dead bees collected near hive entrances and in pollen collected by bees and stored in the hive. Maize pollen with clothianidin and other pesticides were fed by bees to the new queens. The levels of clothianidin in bee-collected pollen was 10-fold higher than those reported from experiments conducted on canola grown from clothianidin-treated seed in Canada and submitted by the manufacturer as a field study for registration, but subsequently rejected as inadequate by the US EPA.[75] Maize planting reached 35.7 million hectares in North America in 2010, and is expected to increase. Most of the maize is coated with neonicotinoid insecticides.[74]

Neonicotinoid insecticides can cause immunosuppression in mammals

There is now considerable evidence from basic neuroscience research that the neonicotinoids have effects on mammalian neurons as well as on invertebrate ones, and that the effects on the nicotinic acetylcholine receptors (nAChRs) are more significant in vertebrates than was initially stated by the pesticide companies.[76-78] Studies of the effects of clothianidin and imidacloprid on human neuronal-type α4β2 nAChRs showed that both chemicals had effects on human receptors.[79] In view of the current global usage figures and their persistence in the environment, several authors suggested that the neonicotinoids could have adverse effects on human health and the developing foetus. In fact, gestational exposure in rats to a single large nonlethal dose of imidacloprid produced significant neurobehavioural deficits and pathological alterations in their offspring.[77] Acetamiprid suppressed the immune system in female Wistar rats[80] and clothianidin has evidence of effects on the rat immune system, with juveniles being particularly susceptible.[41] Human clinical studies in 2010 demonstrated a connection between the nAChRs and the immune system.[81]

Consequences of the use and abuse of systemic insecticides

Over the last 20 years or so, the shift in pest management has moved away from reactive to prophylactic. Now many fungicides, pesticides and herbicides are applied to the seeds before sowing. Application of the chemical before pest damage has occurred often involves routine (calendar-based) spraying and pre-emptive treatments.[82]

The phenomena of insect and herbicide resistance have locked US farmers into a pesticide treadmill. Target pests and weeds are capable of becoming resistant to the repeated use of a single insecticide or herbicide such that successively larger doses have to be applied. Farmers and weed scientists across the heartland and cotton belt are now struggling to devise affordable and effective strategies to deal with the resistant weeds emerging in the wake of herbicide-tolerant crops.[83] Similar resistance to insecticides has developed in pests as a result of which new insecticides had to be developed.[84,85]

The neonicotinoids and fipronil appeared to be the ideal candidates for replacement of old generation insecticides; they are very effective in controlling insects, particularly sucking pests such as aphids, and at the same time quite innocuous to fish and vertebrates in general.[86] Thus, in England in 2010, about one third of arable land was treated with neonicotinoid insecticides out of a total cropped acreage of about 9.9 million acres. Some fields had up to four applications of various pesticides.[68] In the US in 2010, 88 million acres of maize, 77 million acres of soya and 53 million acres of wheat were treated with neonicotinoid insecticides. However, the neonicotinoids and fipronil are

systemic so they permeate all plant tissues and effectively reach the target and non-target insects that feed on the contaminated plants.[87] The consequences of this novel mode of insecticidal action are that insects die in droves, not only at the time of application, but also weeks later due to chronic toxicity. Among sub-lethal effects is a reduction in their immune systems, which have been demonstrated in bees and fish, thus exposing them to the lethal scourge of pathogenic diseases. This paper has reviewed the evidence to date on the latter account. While there is clear evidence implicating the role of neonicotinoids in suppressing the immunity in bees and fish, there is only circumstantial evidence that the new class of insecticides is responsible for most of the other pathogen epidemics that are affecting amphibians, bats and insectivorous birds around the world. The appearance of such epidemics in places where systemic insecticides had been used in large quantities in previous years is, however, far from coincidental. At the root of the problem lies a lack of data on the widespread and insidious contamination of these new chemicals.

On April 17th 2012 the EU Ombudsman[88] opened an investigation into bee mortality and neonicotinoid insecticides following a complaint from the Austrian Ombudsman Board, alleging that the European Commission (EC) had failed to take into account new scientific evidence arguing in favour of restricting the use of these insecticides as plant protection products. According to the Austrian Ombudsman Board, observations from beekeepers, as well as new scientific evidence, suggest that certain neonicotinoids have led to increased bee mortality in recent years. The EC asked the European Food Standards Authority (EFSA) Panel on Plant Protection Products to give a Scientific Opinion. The EFSA conclusions were that for the research on bees and bumblebees, the concentrations used by the authors were too high to be "field realistic" and that the experiments should be repeated.[89] However, the EFSA scientists, in their calculations, had failed to take into account evidence of pesticide residues in soil and water.[71,74]

Henry et al.[90] showed that nonlethal exposure of honey bees to thiamethoxam causes high mortality due to homing failure at levels that could put a colony at risk of collapse. Simulated exposure events on free-ranging foragers labelled with a radio-frequency identification tag suggest that homing is impaired by thiamethoxam intoxication. On 1st June 2012, the French Minister of Agriculture banned thiamethoxam.

Whitehorn et al.[25] exposed colonies of the bumble bee *Bombus terrestris* in the laboratory to field-realistic levels of the neonicotinoid imidacloprid, and allowed them to develop naturally under field conditions. Treated colonies had a significantly reduced growth rate and suffered an 85% reduction in production of new queens compared with control colonies. Given the scale of use of neonicotinoids, the authors suggested that neonicotinoid insecticides may be having a considerable negative impact on wild bumble bee populations across the developed world. When exposed to these insecticides, only 15% of the mated queens survive the winter to start new colonies. This is already happening around the world. A reduction of 85% in the production of new queens accounts for the massive declines (and some extinctions) reported in the US and Canada from the late 1990s onwards.[19,20,23]

In Regulation (EC) no 1107/2009 of the European Parliament[91] concerning the placing of plant protection products on the market, Annex II, Criteria for approval, page 43, it states that a plant protection product should not be persistent in the environment. The persistence criterion is fulfilled where the half-life in soil is higher than 120 days. "Assessment of persistency in the environment shall be based on available half-life data collected under appropriate conditions, which shall be described by the applicant." Registration documents confirm that the neonicotinoid insecticides are persistent in the environment (**Table 1)** and, according to EC laws, imidacloprid, clothianidin and thiamethoxam (which is metabolised to clothianidin) should never have been registered.

However, it would seem that the Registration Authorities let economics prevail when they register pesticides. On page 2, the Summary of the April 2012 Report by the EFSA Panel on Plant Protection Products and their Residues (PPR)[92] states: "The final decision on protection goals needs to be taken by risk managers. There is a trade-off between plant protection and the protection of bees. The effects on pollinators need to be weighted against increase in crop yields due to better protection of crops against pests." This suggests that crucially important pollinators may have to take second place to economics.

Sadly, most government departments responsible for ensuring the quality of our waters have failed to acknowledge the threats posed by systemic insecticides. Their widespread global use and contamination of the environment is unrecognized. As a result, researchers concerned about the emerging infectious diseases in the past two decades causing animal declines and local extinctions and the effects on biodiversity, ecosystem and human health, are unaware of the existence of the neonicotinoids. Even scientists who suspected the possibility of chemical pollution are working on pesticide data (and water quality measurements) that are 20 years out of date. Thus they target the wrong pesticides in their ecotoxicological studies (e.g. organophosphates, endosulfan, atrazine etc. instead of the neonicotinoids). This article is a wake-up call to the world authorities, environmental protection agencies and scientists to monitor thoroughly the waters and study the overall implications of systemic insecticides from a new perspective; that of human health and global biodiversity.

Contributors

Rosemary Mason, Henk Tennekes, Francisco Sánchez-Bayo and Palle Uhd Jepsen researched and wrote the paper. Henk Tennekes discovered toxicological proof of the actions of the neonicotinoid insecticides. Francisco Sánchez-Bayo provided further ecotoxicological information.

Conflict of interests

The authors declared no conflicts of interest.

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