

# **Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and** Queen Production

Penelope R. Whitehorn, Stephanie O'Connor, Felix L. Wackers, Dave Goulson 1+

Growing evidence for declines in bee populations has caused great concern because of the valuable ecosystem services they provide. Neonicotinoid insecticides have been implicated in these declines because they occur at trace levels in the nectar and pollen of crop plants. We exposed colonies of the bumble bee Bombus terrestris in the laboratory to field-realistic levels of the neonicotinoid imidacloprid, then 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, we suggest that they may be having a considerable negative impact on wild bumble bee populations across the developed world.

now routinely treated with neonicotinoid in- crops (6).

ees in agroecosystems survive by feed- spreads to the nectar and pollen of flower-Bes in agroecosystems survive by feeding on wildflowers growing in field margins and patches of seminatural habiting from 0.7 to 10 µg kg <sup>-1</sup>(4, 5). Thus bee itat, supplemented by the brief gluts of flowers colonies in agroecosystems will be exposed provided by mass flowering crops such as oil- to 2- to 4-week pulses of exposure to neoseed rape and sunflower (1, 2). Many crops are nicotinoids during the flowering period of

have shown some evidence that neonicotinoids reduced forager success under field conditions, no studies have examined their impacts on colonies foraging naturally in the field. Here, we present an experiment, using 75 Bombus terrestris colonies, designed to simulate the likely effect of exposure of a wild bumble bee colony to neonicotinoids present on the flowers of a nearby crop. The colonies were randomly allocated to one of three treatments. Control colonies received ad libitum (ad lib) pollen and sugar water over a period of 14 days in the laboratory. Over the same period, colonies in the "low" treatment were fed pollen and sugar water containing 6 μg kg<sup>-1</sup> and 0.7 μg kg imidacloprid, respectively, representing the levels found in seed-treated rape (13). The "high"treatment colonies received double these doses, still close to the field-realistic range. After 2 weeks, all colonies were then placed in the field, where they were left to forage independently for a period of 6 weeks while their performance was monitored.

All colonies experienced initial weight gain followed by a decline as they switched from their growth phase to producing new reproductives. Colonies in both low and high treatments gained less weight over the course of



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## Report

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# A Common Pesticide Decreases Foraging Success and Survival in **Honey Bees**

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Non-lethal exposure of honey bees to thiamethoxam (neonicotinoid systemic pesticide) 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 labeled with an RFID tag suggest that homing is impaired by thiamethoxam intoxication. These experiments offer new insights into the consequences of common neonicotinoid pesticides used worldwide.

authorization procedures now require running mortality surveys to ensure doses encountered in the field remain below lethal levels for honey bees.

However, a growing body of evidence shows that sublethal doses, i.e. doses that do not entail direct mortality still have the potential to induce a variety of behavioral difficulties in foraging honey bees, such as memory and learning dysfunctions and alteration of navigational skills (9). Neonicotinoid pesticides used to protect crops against aphids and other sap-sucking insects are especially liable to provoke such behavioral troubles. They are highly potent and selective agonists of nicotinic acetylcholine receptors, which are important excitatory neurotransmitter receptors in insects (10, 11). Effects of sublethal neonicotinoid exposures in honey bees may include abnormal foraging activity (12-14), reduced olfactory memory and learning performance (15-17) and possibly impaired orienta-

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# Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees

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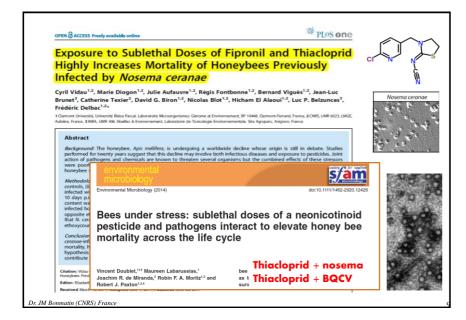
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Large-scale losses of honey bee colonies represent a poorly understood problem of global importance. Both biotic and abiotic factors are involved in this phenomenon that is often associated with high loads of parasites and pathogens. A stronger impact of pathogens in honey bees exposed to neonicotinoid insecticides has been reported, but the causal link between insecticide exposure and the possible immune alteration of honey bees remains elusive. Here, we demonstrate that the neonicotinoid insecticide clothianidin negatively modulates NF-kB immune signaling in insects and adversely affects honey bee antiviral defenses controlled by this transcription factor. We have identified in insects a negative modulator of NF-kB activation, which is a leucine-rich repeat protein. Exposure to clothianidin, by enhancing the transcription of the gene encoding this inhibitor, reduces immune defenses and promotes the replication of the deformed wing virus in honey bees bearing covert infections. This honey bee immunosuppression is similarly induced by a different neonicotinoid, imidacloprid, but not by the organophosphate chlorpyriphos, which does not affect NF-kB signaling. The occurrence at sublethal doses of this insecticide-induced viral proliferation suggests that the



nicotinoid insecticides are curpate (13). Over the last few years, I their use in agriculture, and they scrutiny of the European Food ently, three of them have been ropean Commission (17), based ice regarding the negative effects a shown that sublethal doses of homing capacity of honey bees n colony stability (18). Concurave provided further confirmaconicotinoids can have a wider (19, 20). Importantly, exposure ciated with a higher pathogenic gh the merely descriptive results ing and do not support any clear , due to significant gaps in our e insecticides act on honey bee ress this issue, focusing on the mechanism underlying the presumed immunosuppressive ac-



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# Sub-lethal exposure to neonicotinoids impaired honey bees winterization before proceeding to colony collapse disorder

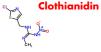
Chensheng Lu<sup>1</sup>, Kenneth M. WARCHOL<sup>2</sup>, Richard A. CALLAHAN<sup>3</sup>

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Imidaclopri

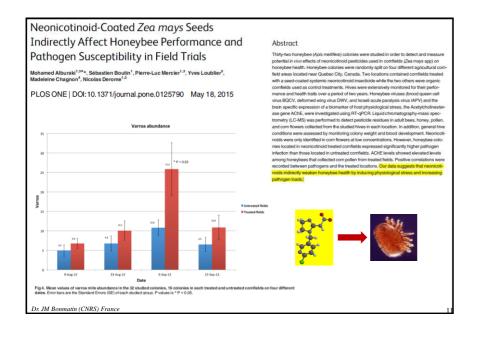


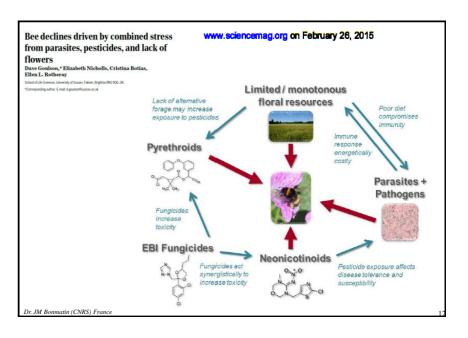


### **Abstract**

Honey bee (Apis mellifera L.) colony collapse disorder (CCD) that appeared in 2005/2006 still lingers in many parts of the world. Here we show that sub-lethal exposure of neonicotinoids, inidacloprial or clothiandidin, affected the winterization of healthy colonies that sub-sequently leads to CCD. We found honey bees in both control and neonicotinoid-treated groups progressed almost identically through the summer and fall seasons and observed no acute morbidity or mortality in either group until the end of winter. Bees from six of the twelve neonicotinoid-treated colonies had abandoned their hives, and were eventually dead with symptoms resembling CCD. However, we observed a complete opposite phenomenon in the control colonies in which instead of abandonment, they were re-populated quickly with new emerging bees. Only one of the six control colonies was lost due to Nosema-like infection. The observations from this study may help to elucidate the mechanisms by which sub-lethal neonicotinoids exposure caused honey bees to vanish from their hives.

Key words: colony collapse disorder, CCD, honey bee, neonicotinoids, imidacloprid, clothianidin.

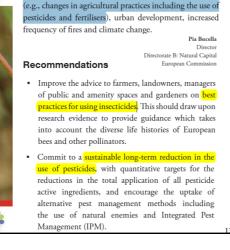


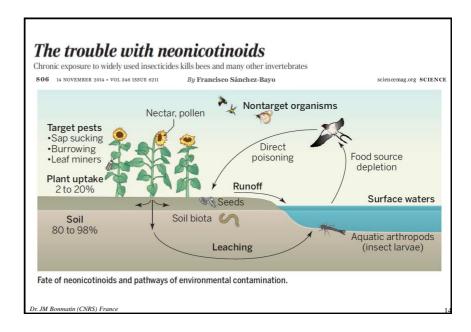


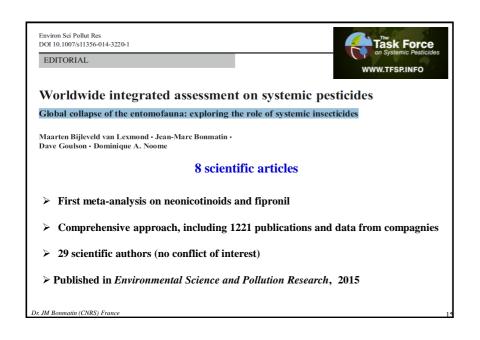
# European Red List of Bees Ana Neto, Staart DM. Roberts, James Keep, Pierre Basmont, Michael Kahhmann, Mariana Garcta Crisdo, Jacobeur C. Biermeijer, Pierr Bogusch, Holger H. Dathe, Pilar De la Rüs, Thibaut De Meulemeenster, Manuel Delhon, Alexandre Dewolf, Francisco Javier Ortis Sanchez, Patrick Lhomma, Malin Pauly, Simon G. Detts, Christopher Par, Marino Quantant, Vladimir G. Radchesko, Erwin Scheuchl, Jan Smit, Jakub Straka, Michael Fizra, Bogdan Tomozii, Jemma Window and Denis Michae

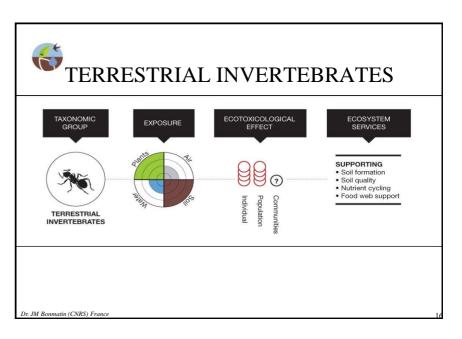
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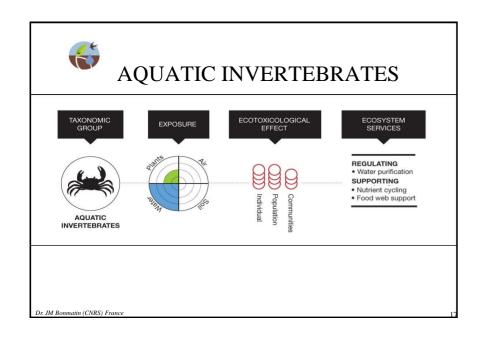
The European Red List of Bees provides, for the first time, factual information on the status of all bees in Europe, nearly 2,000 species. This new assessment shows us that 9% of bees are threatened with extinction in Europe mainly due to habitat loss as a result of agriculture intensification (e.g., changes in agricultural practices including the use of pesticides and fertilises), urban development, increased frequency of fires and climate change.

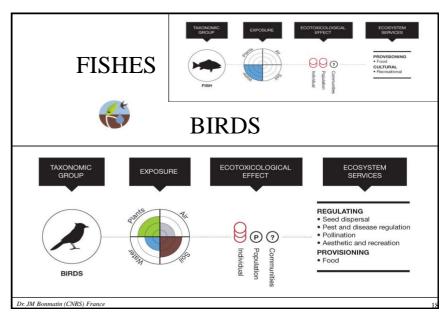












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# Declines in insectivorous birds are associated with high neonicotinoid concentrations

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Recent studies have shown that neonicotinoid insecticides have adverse effects on non-target invertebrate species1-6. Invertebrates constitute a substantial part of the diet of many bird species during the breeding season and are indispensable for raising offspring7. We investigated the hypothesis that the most widely used neonicotinoid insecticide, imidacloprid, has a negative impact on insectivorous bird populations. Here we show that, in the Netherlands, local population trends were significantly more negative in areas with higher surface-water concentrations of imidacloprid. At imidacloprid concentrations of more than 20 nanograms per litre, bird populations tended to decline by 3.5 per cent on average annually. Additional analyses revealed that this spatial pattern of decline appeared only after the introduction of imidacloprid to the Netherlands, in the mid-1990s. We further show that the recent negative relationship remains after correcting for spatial differences in land-use changes that are known to affect bird populations in farmland. Our results suggest that the impact of neonicotinoids on the natural environment is even more substantial than has recently been reported and is reminiscent of the effects of persistent insecticides in the past. Future legislation should take into account the potential cascading effects of neonicotinoids on ecosystems.

The present study takes advantage of two standardized, long-term, country-wide monitoring schemes in the Netherlands (see Methods)—the Dutch Common Breeding Bird Monitoring Scheme\*) and surface-water quality measurements\*—to investigate the extent to which average concentrations of imidacloprid residues in the period 2003–2009 spatially correlate with bird population trends in the period 2003–2010. We selected 15 passerine species that are common in farmlands and depend on invertebrates during the breeding season (Extended Data Table 1 and Supplementary Methods). We interpolated concentrations of imidacloprid in surface water to bird monitoring plots (Extended Data Figs 1–3, Supplementary Data and Supplementary Methods) and examined how local bird trends correlate with these concentrations (Fig. 1).

The average intrinsic rate of increase in local farmland bird populations was negatively affected by the concentration of imidacloprid (Fig. 1b, linear mixed effects regression (LMER): d.f. = 1,443, t = -5.64, P < 0.0001). At the separately tested individual species level, 14 out of 15 of the tested species had a negative response to interpolated imidacloprid concentrations, and 6 out of 15 had a significant negative response at the 95% confidence level after Bonferroni correction (Table 1 and Extended Data Fig. 4). Thus, higher concentrations of imidacloprid in surface water in

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# **Ecosystem services, agriculture and neonicotinoids**



Critical to assessing the effects of neonicotinoids on ecosystem services is their impact on non-target organisms: both invertebrates and vertebrates, and whether located in the field or margins, or in soils or the aquatic environment. Here, the Expert Group finds the following.

- There is an increasing body of evidence that the widespread prophylactic use of neonicotinoids has severe negative effects on non-target organisms that provide ecosystem services including pollination and natural pest control.
- There is clear scientific evidence for sublethal effects of very low levels of neonicotinoids over extended periods on non-target beneficial organisms. These should be addressed in EU approval procedures.
- Current practice of prophylactic usage of neonicotinoids is inconsistent with the basic principles of integrated pest management as expressed in the EU's Sustainable Pesticides Directive.
- Widespread use of neonicotinoids (as well as other pesticides) constrains the potential for restoring biodiversity in farmland under the EU's Agrienvironment Regulation.



### RESEARCH COMMUNICATIONS RESEARCH COMMUNICATIONS.

# Large-scale trade-off between agricultural intensification and crop pollination services

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Unprecedented growth in human populations has required the intensification of agriculture to enhance crop productivity, but this was achieved at a major cost to biodiversity. There is abundant local-scale evidence that both pollinator diversity and pollination services decrease with increasing agricultural intensification. This raises concerns regarding food security, as two-thirds of the world's major food crops are pollinator-dependent. Whether such local findings scale up and affect crop production over larger scales is still being debated. Here, we analyzed a country-wide dataset of the 54 major crops in France produced over the past two decades and found that benefits of agricultural intensification decrease with increasing pollinator dependence, to the extent that intensification failed to increase the yield of pollinator-dependent crops and decreased the stability of their yield over time. This indicates that benefits from agricultural intensification may be offset by reductions in pollination services, and supports the need for an ecological intensification of agriculture through optimization of ecosystem services.

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### ORIGINAL PAPER

### Imidacloprid-mediated effects on survival and fertility of the Neotropical brown stink bug Euschistus heros

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Abstract Enhanced reproductive output after sublethal insecticide exposure, including neonicotinoid exposure, has been reported in a diversity of arthropods. Suspicions of such a phenomenon in the Neotropical brown stink bug, Euschistus heros (Hemiptera: Pentatomidae), were sparked by the increasing densities of naturally occurring populations of this insect pest species in Brazilian soybean fields. Here, we tested whether the sublethal exposure to imidacloprid would induce changes in the survival and reproductive performances of E. heros adult females. The imidacloprid estimated LC50 was 0.83 (0.60-1.25) µg a.i./ cm2, and the dose recommended for field applications (4.2 μg a.i./cm2) was within the concentration range of the imidacloprid estimated LCso [2.66 (1.65-5.49) ug a.i./ cm2]. Newly emerged (<24 h) adult females were exposed for 48 h to dry imidacloprid residues (0.042 µg/cm<sup>2</sup>, equivalent to 1 % of the field rate dose) and exhibited higher levels of cell damage, greater ovariole length, and a • Females of E. heros increased their reproductive output larger area of the most developed follicle in their ovaries up to the 6th day of adulthood. Furthermore, these females exhibited reduced rates of survival but higher fecundity and

fertility rates compared with untreated females. Our results thus suggest that females of E. heros increased their reproductive output in response to the imidacloprid sublethal exposure. These findings suggest a potential involvement of sublethal exposure to neonicotinoids in the recent outbreaks of the Neotropical brown stink bug E. heros observed in Brazilian soybean-producing regions.

Keywords Reproductive responses · Hormesis · Insect ovaries · Damaged cells · Stink bugs

- · Insecticide-induced changes in Euschistus heros reproduction capacity has been sparked by the increasing densities of this pest in Brazilian soybean fields.
- (fecundity and fertility rates) to overcome imidacloprid-induced sublethal stress (higher number of damaged ovarian cells and reduction on female's survival).
- These findings suggest a potential link between imidacloprid sublethal exposure and the recent outbreaks of E. heros observed in the Brazilian soybean fields.

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# **Quantitative Analysis of Neonicotinoid Insecticide Residues in Foods: Implication for Dietary Exposures**

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ABSTRACT: This study quantitatively measured neonicotinoids in various foods that are common to human consumption. All fruit and vegetable samples (except nectarine and tomato) and 90% of honey samples were detected positive for at least one neonicotinoid; 72% of fruits, 45% of vegetables, and 50% of honey samples contained at least two different neonicotinoids in one sample, with imidacloprid having the highest detection rate among all samples. All pollen samples from New Zealand contained multiple neonicotinoids, and five of seven pollens from Massachusetts detected positive for imidacloprid. These results show the prevalence of low-level neonicotinoid residues in fruits, vegetables, and honey that are readily available in the market for human consumption and in the environment where honeybees forage. In light of new reports of toxicological effects in mammals, the results strengthen the importance of assessing dietary neonicotinoid intakes and the potential human health effects.

KEYWORDS: neonicotinoid insecticides, dietary exposure, pollen, honey

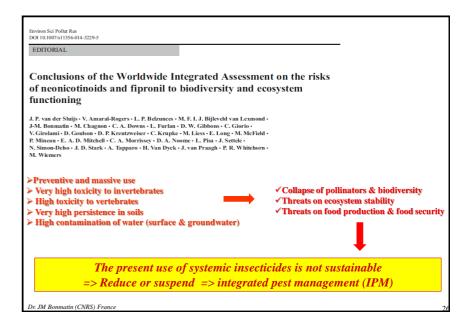
# Neonicotinoids and public health

- Neonics are less toxic to humans (less nicotinic receptors for our CNS)
- Only few studies (despite that neonics represent 1/3 of the global insecticide market):
   (non-target species > 1000 publications; humans < 20 publications)</li>

### BUT

- EPA 2002 then UE then ANSES (2013): Thiacloprid carcinogen
- -ARLA 2001, 2004, 2007: Three neonics are potential endocrine disruptors
- 2012: Genotoxicity and cytotoxicity of neonics
- 2012: Neonics have similar effects than nicotine
- EFSA 2013: Neuro-developmental risk for humans
- 2014: Effects on hepatic enzymes (toxic accumulation of delta-ALA)
- 2014: Cytotoxic effects of formulations >> active ingredients, on human cells
- 2014: Effects on thyroid and testicles (endocrine disruptor)
- 2014: Synergies with other pesticides (pyrethroid and carbamate)
- Japan 2014: description of sub-acute effects on poisoned people (hospital)
- Japan 2014 & 2015: human urine contains neonics (90% of tested people)
- 2015: Another new toxic pathway of neonics on the CNS (glutamate receptors)...

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