

WORLDWIDE INTEGRATED ASSESSMENT

OF THE IMPACTS OF SYSTEMIC PESTICIDES
ON BIODIVERSITY AND ECOSYSTEMS





http://www.tfsp.info/assets/WIA_2015.pdf

Report in brief

The Task Force on Systemic Pesticides is an independent group of scientists from all over the globe, who came together to work on the Worldwide Integrated Assessment of the Impact of Systemic Pesticides on Biodiversity and Ecosystems.

The mandate of the Task Force on Systemic Pesticides (TFSP) has been *“to carry out a comprehensive, objective, scientific review and assessment of the impact of systemic pesticides on biodiversity, and on the basis of the results of this review to make any recommendations that might be needed with regard to risk management procedures, governmental approval of new pesticides, and any other relevant issues that should be brought to the attention of decision makers, policy developers and society in general”* (see appendix 2).

The Task Force has adopted a science-based approach and aims to promote better informed, evidence-based, decision-making. The method followed is Integrated Assessment (IA) which aims to provide policy-relevant but not policy-prescriptive information on key aspects of the issue at hand. To this end a highly multidisciplinary team of 30 scientists from all over the globe jointly made a synthesis of 1,121 published peer-reviewed studies spanning the last five years, including industry-sponsored ones. All publications of the TFSP have been subject to the standard scientific peer review procedures of the journal (<http://www.springer.com/environment/journal/11356>).

Key findings of the Task Force have been presented in a special issue of the peer reviewed Springer journal “Environmental Science and Pollution Research” entitled “Worldwide Integrated Assessment of the Impacts of Systemic Pesticides on Biodiversity and Ecosystems” and consists of eight scientific papers, reproduced here with permission of Springer.

In summary the TFSP’s scientific assessment indicates that the current large-scale prophylactic use of systemic insecticides is having significant unintended negative ecological consequences. The evidence indicates that levels of systemic pesticides that have been documented in the environment are sufficient to cause adverse impacts on a wide range of non-target organisms in terrestrial, aquatic, wetland, marine and benthic habitats. There is also a growing body of evidence that these effects pose risks to ecosystem functioning, resilience and services such as for example pollination and nutrient cycling.

Notre Dame de Londres, 9 January 2015

www.tfsp.info

Table of contents

1. Worldwide integrated assessment on systemic pesticides: Global collapse of the entomofauna: exploring the role of systemic insecticides	1
2. Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites	5
3. Environmental fate and exposure; neonicotinoids and fipronil	35
4. Effects of neonicotinoids and fipronil on non-target invertebrates	68
5. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife	103
6. Risks of large-scale use of systemic insecticides to ecosystem functioning and services	119
7. Alternatives to neonicotinoid insecticides for pest control: case studies in agriculture and forestry	135
8. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning	148

Appendix 1

Neonicotinoids, bee disorders and the sustainability of pollinator services

Appendix 2

IUCN resolution WCC-2012-Res-137-EN: Support for a comprehensive scientific review of the impact on global biodiversity of systemic pesticides by the joint task force of the IUCN Species Survival Commission (SSC) and the IUCN Commission on Ecosystem Management (CEM)

Worldwide integrated assessment on systemic pesticides

Global collapse of the entomofauna: exploring the role of systemic insecticides

Maarten Bijleveld van Lexmond · Jean-Marc Bonmatin ·
Dave Goulson · Dominique A. Noome

Received: 13 June 2014 / Accepted: 17 June 2014 / Published online: 23 August 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Keywords Neonicotinoids · Fipronil · Insecticides ·
Ecosystem services · Biodiversity · Non-target organisms

The appeal of Notre Dame de Londres

In July 2009, a group of entomologists and ornithologists met at Notre Dame de Londres, a small village in the French department of Hérault, as a result of an international enquiry amongst entomologists on the catastrophic decline of insects (and arthropods in general) all over Europe.

They noted that a perceptible and gradual decline of insects, as part of the general impoverishment of the natural environment, had set in from the 1950s onwards. Amongst many others, they recognized as root causes of this decline the intensification of agriculture with its accompanying loss of natural habitats and

massive use of pesticides and herbicides, the manifold increase in roads and motorized traffic as well as a continent-wide nocturnal light pollution and nitrogen deposition.

They equally agreed that a further degradation of the situation, a steeper decline in insect populations, had started in the decade 1990–2000. This first began in western Europe, followed by eastern and southern Europe, is nowadays apparent in the scarcity of insects splattered on windscreens of motorcars and squashed against their radiators and is best documented in the decline of butterflies and the global disorders amongst honey bees. They concluded that these phenomena reflected the now general collapse of Europe's entomofauna.

They also noted that the massive collapse of different species, genera and families of arthropods coincided with the severe decline of populations of different insectivorous bird species up to now considered as “common” such as swallows and starlings.

On the basis of existing studies and numerous observations in the field as well as overwhelming circumstantial evidence, they came to the hypothesis that the new generation of pesticides, the persistent, systemic and neurotoxic neonicotinoids and fipronil, introduced in the early 1990s, are likely to be responsible at least in part for these declines.

They, therefore, issued the Appeal of Notre Dame de Londres under the heading “No Silent Spring again” referring to Rachel Carson's book “Silent Spring” then published almost half a century ago:

The disappearance of honey bees is only the most visible part of a phenomenon now generalized in all of Western Europe. The brutal and recent collapse of insect populations is the prelude of a massive loss in biodiversity with foreseeable dramatic consequences for natural ecosystems, the human environment and public health. The systematic use of persistent neurotoxic insecticides in intensive agriculture and horticulture (neonicotinoids such as imidacloprid and thiamethoxam, and fipronil as

Responsible editor: Philippe Garrigues

M. B. van Lexmond · D. A. Noome
Task Force on Systemic Pesticides, Pertuis-du-Sault,
2000 Neuchâtel, Switzerland

M. B. van Lexmond
e-mail: mbvl@club-internet.fr

D. A. Noome
e-mail: dominiquenoome@gmail.com

J.-M. Bonmatin (✉)
Centre National de la Recherche Scientifique, Centre de Biophysique
Moléculaire, rue Charles Sadron, 45071 Orléans Cedex 02, France
e-mail: bonmatin@cnr-orleans.fr

D. Goulson
School of Life Sciences, University of Sussex, Brighton BN1 9QG,
UK
e-mail: d.goulson@sussex.ac.uk

D. A. Noome
Kasungu National Park, c/o Lifupa Conservation Lodge, Private Bag
151, Lilongwe, Malawi

a phenylpyrazole), which now form an invisible, widespread, toxic haze on land, in water and in the air, is regarded as a principal cause of this collapse observed by entomologists beginning in the middle of the 1990's and followed by the decline of insectivorous and other bird species by the ornithologists.

For this reason the undersigned raise an alarm and demand a much stricter adherence to the « Precautionary Principle » as enshrined in the E.U. Commission's Directive 91/414, and defined by UNESCO in 2005 as « When human activities may lead to morally unacceptable harm that is scientifically plausible but uncertain, actions shall be taken to avoid or diminish that harm ».

The international scientific Task Force on Systemic Pesticides (TFSP)

In response, an international scientific Task Force on Systemic Pesticides of independent scientists was set up shortly afterwards by a Steering Committee of which Maarten Bijleveld van Lexmond (Switzerland), Pierre Goeldlin de Tiefenau (Switzerland), François Ramade (France) and Jeroen van der Sluijs (The Netherlands) were the first members. Over the years, membership grew and today counts 15 nationalities in four continents. The Task Force on Systemic Pesticides (TFSP) advises as a specialist group two IUCN Commissions, the *Commission on Ecosystem Management* and the *Species Survival Commission*. Its work has been noted by the *Subsidiary Body on Scientific, Technical and Technological Advice* under the Convention on Biodiversity (CBD) and was brought to the attention of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) in the context of the fast-track thematic assessment of pollinators, pollination and food production.

In undertaking the Worldwide Integrated Assessment (WIA), over the course of the last 4 years, the TFSP has examined over 800 scientific peer-reviewed papers published over the past two decades. The TFSP areas of expertise span diverse disciplines, including chemistry, physics, biology, entomology, agronomy, zoology, risk assessment and (eco) toxicology, and this has enabled a truly interdisciplinary evaluation of the evidence, necessary to understand the diverse ramifications of the global use of systemic pesticides on individual organisms, on ecosystems and on ecosystem processes and services.

The findings of the TFSP-WIA

Neonicotinoids were introduced in the early 1990s and are now the most widely used insecticides in the world. They are

neurotoxins, binding to nicotinic acetylcholine receptors (nAChRs) in the central nervous system and causing nervous stimulation at low concentrations but receptor blockage, paralysis and death at higher concentrations. Fipronil is another widely used systemic insecticide that shares many of the properties of neonicotinoids and was introduced around the same time; hence, this compound is also included here. Both neonicotinoids and fipronil exhibit extremely high toxicity to most arthropods and a lower toxicity to vertebrates (although fipronil exhibits high acute toxicity to fish and some bird species). They are relatively water soluble and are readily taken up by plant roots or leaves, so they can be applied in a variety of ways (e.g. foliar spray, soil drench and seed dressing). The predominant use of these chemicals, in terms of the area of land over which they are used, is as a seed dressing, whereby the active ingredient is applied prophylactically to seeds before sowing and is then absorbed by the growing plant and spreads throughout the plant tissues, hence protecting all parts of the crop (Simon-Delso et al. 2014).

A range of concerns have emerged as to the impacts of neonicotinoids and fipronil on the environment (Bonmatin et al. 2014; Pisa et al. 2014; Gibbons et al. 2014; Chagnon et al. 2014; Furlan and Kreutzweiser 2014):

- It has become apparent that neonicotinoids can persist for years in soils and so cause environmental concentrations to build up if regularly used. This is likely to be impacting substantially on soil invertebrates, which as a group perform a vital service in maintaining soil structure and in cycling nutrients. Being water soluble, neonicotinoids leach into ponds, ditches and streams and contaminate groundwater. Contamination of marine environments has been observed but as yet has not been monitored systematically. Concentrations exceeding the LC_{50} for aquatic insects frequently occur in waterways, and much higher concentrations have been found in surface water in arable fields and in adjacent ditches. Waterways with higher neonicotinoid concentrations have been found to have depleted insect abundance and diversity.
- Dust created during drilling of treated seeds is lethal to flying insects and has caused large-scale acute losses of honeybee colonies. When applied as foliar sprays, drift is likely to be highly toxic to non-target insects. Non-crop plants, such as those growing in field margins, hedgerows and near contaminated waterways can become contaminated with neonicotinoids either via dust created during drilling, spray drift or contaminated water. This provides the potential for major impacts on a broad range of non-target herbivorous invertebrates living in farmland.
- Neonicotinoids and fipronil are found in nectar and pollen of treated crops such as maize, oilseed rape and sunflower and also in flowers of wild plants growing in farmland. They have also been detected at much higher concentrations in

guttation drops exuded by many crops. In bees, consumption of such contaminated food leads to impaired learning and navigation, raised mortality, increased susceptibility to disease via impaired immune system function and reduced fecundity, and in bumblebees, there is clear evidence for colony-level effects. Studies of other pollinators are lacking. Bees in farmland are simultaneously exposed to some dozens of different agrochemicals, and some act synergistically. The impact of chronic exposure of non-target insects to these chemical cocktails is not addressed by regulatory tests and is very poorly understood.

- Although vertebrates are less susceptible than arthropods, consumption of small numbers of dressed seeds offers a potential route for direct mortality in granivorous birds and mammals, for such birds need to eat only a few spilt seeds to receive a lethal dose. Lower doses lead to a range of symptoms including lethargy, reduced fecundity and impaired immune function. In addition, depletion of invertebrate food supplies is likely to indirectly impact on a broad range of predatory organisms, from arthropods to vertebrates.
- The prophylactic use of broad-spectrum pesticides (as seed dressings) goes against the long-established principles of Integrated Pest Management (IPM) and against new EU directives which make adoption of IPM compulsory. Continual exposure of pests to low concentrations of neonicotinoids is very likely to lead to the evolution of resistance, as has already occurred in several important pest species. Although systemic pesticides can be highly effective at killing pests, there is clear evidence from some farming systems that current neonicotinoid use is unnecessary, providing little or no yield benefit. Agrochemical companies are at present the main source of agronomic advice available for farmers, a situation likely to lead to overuse and inappropriate use of pesticides.

Overall, a compelling body of evidence has accumulated that clearly demonstrates that the wide-scale use of these persistent, water-soluble chemicals is having widespread, chronic impacts upon global biodiversity and is likely to be having major negative effects on ecosystem services such as pollination that are vital to food security and sustainable development. There is an urgent need to reduce the use of these chemicals and to switch to sustainable methods of food production and pest control that do not further reduce global biodiversity and that do not undermine the ecosystem services upon which we all depend (van der Sluijs et al. 2014).

The systemic insecticides, neonicotinoids and fipronil, represent a new chapter in the apparent shortcomings of the regulatory pesticide review and approval process that do not fully consider the risks posed by large-scale applications of broad-spectrum insecticides to ecosystem functioning and services. Our inability to learn from past mistakes is remarkable.

Acknowledgments This manuscript benefited from the discussions in the International Task Force on Systemic Pesticides during its plenary meetings in Paris (2010), Bath (2011), Cambridge (2012), Montegrotto-Padova (2012), Louvain-la-Neuve (2013) and Legnaro-Padova (2013). The work has been funded by the Triodos Foundation's Support Fund for Independent Research on Bee Decline and Systemic Pesticides. This Support Fund has been created from donations by Adessium Foundation (The Netherlands), Act Beyond Trust (Japan), Utrecht University (Netherlands), Stichting Triodos Foundation (The Netherlands), Gesellschaft fuer Schmetterlingsschutz (Germany), M.A.O.C. Gravin van Bylandt Stichting (The Netherlands), Zukunft Stiftung Landwirtschaft (Germany), Study Association Storm (Student Association Environmental Sciences Utrecht University) and citizens. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

We very much acknowledge ESPR Editor-in-Chief Philippe Garrigues and Editorial Assistant Emmanuelle Pignard-Péguet for their help during the preparation of this Special Issue.

The review process was coordinated by ESPR Editor-in-Chief, according to the strict ethical guidelines of Springer, with independent reviewers chosen by ESPR Editors.

The authors are listed in alphabetic order.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser D, Krupke C, Liess M, Long E, Marzaro M, Mitchell EAD, Noome DA, Simon-Delso N, Tapparo A (2014) Environmental fate and exposure; neonicotinoids and fipronil. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3332-7
- Chagnon M, Kreutzweiser DP, Mitchell EAD, Morrissey CA, Noome DA, van der Sluijs JP (2014) Risks of large scale use of systemic insecticides to ecosystem functioning and services. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3277-x
- Furlan L, Kreutzweiser DP (2014) Alternatives to Neonicotinoid insecticides for pest control: case studies in agriculture and forestry. *Environ Sci Pollut Res* (this issue)
- Gibbons D, Morrissey C, Mineau P (2014) A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3180-5
- Pisa L, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs C, Goulson D, Kreutzweiser D, Krupke C, Liess M, McField M, Morrissey C, Noome DA, Settele J, Simon-Delso N, Stark J, van der Sluijs JP, van Dyck H, Wiemers M (2014) Effects of neonicotinoids and fipronil on non-target invertebrates. *Environ Sci Pollut Res* (this issue). doi:10.1007/s11356-014-3471-x
- Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, van Dyck H, van Praagh J, van der Sluijs JP, Whitehorn PR and Wiemers M (2014) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environ SciPollut Res* (this issue). doi:10.1007/s11356-014-3470-y
- Van der Sluijs JP, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Simon-Delso N, Stark JD, Tapparo A, van Dyck H, van Praagh J, Whitehorn

PR and Wiemers M (2014) Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3229-5



Dr Maarten Bijleveld van Lexmond is a biologist and conservationist by training. He studied at Leiden and Amsterdam Universities obtaining his PhD. in 1974 with the publication of his first book: *Birds of Prey in Europe*. As one of the founders of the World Wildlife Fund in the Netherlands he joined the WWF international secretariat in Switzerland and later led the Commission on Ecology of the International Union for the Conservation of Nature (IUCN).

In the mid-eighties he founded the Swiss Tropical Gardens in Neuchâtel, now in Kerzers (Switzerland), in parallel with the Shipstem Nature Reserve in Belize, Central America. For many years he also served as President of the Foundation for the Conservation of the Bearded Vulture which succeeded in reintroducing the species into the Alps and other parts of Europe. At present, dividing his time between Switzerland and the south of France most of it since 2009 is taken up by his function as Chairman of the international Task Force on Systemic Pesticides (TFSP) which now looks into the worldwide impact of these chemicals on biodiversity and ecosystems, and in particular on pollinators such as honey bees, bumble bees, butterflies, but also at suspected consequences for public health.



Dr Jean-Marc Bonmatin is researcher for the Centre National de la Recherche Scientifique (CNRS, France). He completed his thesis in 1987 at the University of Bordeaux (Chemistry and Physics) by studying interaction mechanisms between biological membranes and peptides by various spectroscopic techniques. This was his first scientific contact with the fascinating world of bees because these peptides have included bee venom. Just after, he worked for the National Research

Council of Canada (Ottawa, Canada) until 1989. Here he was interested in dynamics of cholesterol in membranes by solid state NMR. He joined the Centre de Biophysique Moléculaire late 1989 (CBM, CNRS, Orléans, France) where he started his researches on structure-activity relationships of various biomolecules by high resolution NMR. These biomolecules have in common to be toxic to their target (antibacterial, antifungal, neurotoxins from arthropods, etc.). He shares the idea that 'knowing how it kills, gives clues on biological mechanisms and may allow saving'. From 2008 he was involved during twelve years in European programs on what is called the Colony Collapse Disorder (CCD), especially concerning analytics of pesticides in soil, water, pollen and honey, as well as concerning the finding of a virus of bee mites (*Varroa destructor*).

He joined the Task Force on Systemic Pesticides very early and he is a member of its Steering Committee. He is also involved in risk assessments for pollinators for several public organisms, at national and international levels, such as ITSAP (French Institute of Bee and Pollination), ANSES (French Agency of Environmental and Food Safety) and the Organisation for Economic Co-operation and Development (OECD).



Dave Goulson is Professor of Biology at the University of Sussex. He received his bachelor's degree in biology from Oxford University, followed by a doctorate on butterfly ecology at Oxford Brookes University. Subsequently, he lectured in biology for 11 years at the University of Southampton, before moving to Stirling in 2006, and then to Sussex in 2013. Goulson works mainly on the ecology and conservation of bumble bees. He has published more than 200 scientific articles on the ecology and conservation

of insects, with a particular focus on bumblebees. He is the author of *Bumblebees; Their Behaviour, Ecology and Conservation*, published in 2010 by Oxford University Press, and of *A Sting in the Tale*, a popular science book about bumble bees, published in 2013 by Jonathan Cape. Goulson founded the Bumblebee Conservation Trust in 2006, a UK-based charity which has grown to 8,000 members. For his work on bumblebee conservation he was made BBSRC Social Innovator of the year in 2010, and received the Zoological Society of London's Marsh Award for Conservation Biology in 2013. He was also elected a Fellow of the Royal Society of Edinburgh in 2013.



Dominique Noome MSc is currently project coordinator for the Task Force on Systemic Pesticides and conservation manager in Kasungu National Park, Malawi. Originally a veterinary epidemiologist, she studied the hematology of Kenyan cattle and economic impacts of emerging infectious diseases on livestock in the Netherlands during her MSc. After graduating as an animal health specialist at Wageningen University, she continued as an independent conservation scientist, being involved with

the IUCN Commission on Ecosystem Management, and Foundation Chimbo. During this period she first got acquainted with the Task Force on Systemic Pesticides, starting with field work in 2011 which evolved into project coordinator over the years. In Malawi, where she has just concluded writing the general management plan for Kasungu National Park, she is now focused on coordination of research projects identified in the management plan. Her main areas of interest are protected areas management, more specifically wildlife health, law enforcement and strategies for ecosystem restoration. This also extends to systemic pesticide use in African countries, such as Malawi, where many knowledge gaps about the scale of use and associated impact of these substances still exist.

Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites

N. Simon-Delso • V. Amaral-Rogers • L. P. Belzunces • J. M. Bonmatin • M. Chagnon • C. Downs • L. Furlan • D. W. Gibbons • C. Giorio • V. Girolami • D. Goulson • D. P. Kreutzweiser • C. H. Krupke • M. Liess • E. Long • M. McField • P. Mineau • E. A. D. Mitchell • C. A. Morrissey • D. A. Noome • L. Pisa • J. Settele • J. D. Stark • A. Tapparo • H. Van Dyck • J. Van Praagh • J. P. Van der Sluijs • P. R. Whitehorn • M. Wiemers

Received: 4 May 2014 / Accepted: 15 August 2014 / Published online: 19 September 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract Since their discovery in the late 1980s, neonicotinoid pesticides have become the most widely used class of insecticides worldwide, with large-scale applications ranging from plant protection (crops, vegetables, fruits),

veterinary products, and biocides to invertebrate pest control in fish farming. In this review, we address the phenyl-pyrazole fipronil together with neonicotinoids because of similarities in their toxicity, physicochemical profiles, and presence in the

Responsible editor: Philippe Garrigues

N. Simon-Delso • L. Pisa • J. P. Van der Sluijs
Environmental Sciences, Copernicus Institute, Utrecht University,
Heidelberglaan 2, 3584 CS Utrecht, The Netherlands

N. Simon-Delso (✉)
Beekeeping Research and Information Centre (CARI), Place Croix
du Sud 4, 1348 Louvain-la-Neuve, Belgium
e-mail: noa.simondelso@student.uclouvain.be

V. Amaral-Rogers
Buglife, Bug House, Ham Lane, Orton Waterville, PE2
5UU Peterborough, UK

L. P. Belzunces
INRA, UR 406 Abeilles & Environnement, Laboratoire de
Toxicologie Environnementale, Site Agroparc, 84000 Avignon,
France

J. M. Bonmatin
Centre National de la Recherche Scientifique, Centre de Biophysique
Moléculaire, rue Charles Sadron, 45071 Orléans Cedex 02, France

M. Chagnon
Université du Québec À Montréal, Département des sciences
biologiques, Case Postale 8888, succursale Centre-ville, Montréal,
Québec, Canada H3C 3P8

C. Downs
Haereticus Environmental Laboratory, P.O. Box 92, Clifford,
VA 24533, USA

L. Furlan
Veneto Agricoltura, Legnaro, PD, Italy

D. W. Gibbons
Centre for Conservation Science (RSPB), The Lodge, Sandy,
Bedfordshire SG19 2DL, UK

C. Giorio
Department of Chemistry, University of Cambridge, Lensfield Road,
CB2 1EW Cambridge, UK

V. Girolami
Dipartimento di Agronomia Animali Alimenti Risorse Naturali e
Ambiente, Università degli Studi di Padova, Agripolis, viale
dell'Università 16, 35020 Legnaro, Padova, Italy

D. Goulson
School of Life Sciences, University of Sussex, Brighton BN1 9RH,
UK

D. P. Kreutzweiser
Canadian Forest Service, Natural Resources Canada, 1219 Queen
Street East, Sault Ste Marie, ON, Canada P6A 2E5

C. H. Krupke • E. Long
Department of Entomology, Purdue University, West Lafayette, IN,
USA

M. Liess
Department of System Ecotoxicology, Helmholtz Centre for
Environmental Research - UFZ, 04318 Leipzig, Germany

M. McField
Healthy Reefs for Healthy People Initiative, Smithsonian Institution,
Belize City, Belize

environment. Neonicotinoids and fipronil currently account for approximately one third of the world insecticide market; the annual world production of the archetype neonicotinoid, imidacloprid, was estimated to be ca. 20,000 tonnes active substance in 2010. There were several reasons for the initial success of neonicotinoids and fipronil: (1) there was no known pesticide resistance in target pests, mainly because of their recent development, (2) their physicochemical properties included many advantages over previous generations of insecticides (i.e., organophosphates, carbamates, pyrethroids, etc.), and (3) they shared an assumed reduced operator and consumer risk. Due to their systemic nature, they are taken up by the roots or leaves and translocated to all parts of the plant, which, in turn, makes them effectively toxic to herbivorous insects. The toxicity persists for a variable period of time—depending on the plant, its growth stage, and the amount of pesticide applied. A wide variety of applications are available, including the most common prophylactic non-Good Agricultural Practices (GAP) application by seed coating. As a result of their extensive use and physicochemical properties, these substances can be found in all environmental compartments including soil, water, and air. Neonicotinoids and fipronil operate by disrupting neural transmission in the central nervous system of invertebrates. Neonicotinoids mimic the action of neurotransmitters, while fipronil inhibits neuronal receptors. In doing so, they continuously stimulate neurons leading ultimately to death of target invertebrates. Like virtually all insecticides, they can also have lethal and sublethal impacts on non-target organisms, including insect predators and vertebrates. Furthermore, a range of synergistic

effects with other stressors have been documented. Here, we review extensively their metabolic pathways, showing how they form both compound-specific and common metabolites which can themselves be toxic. These may result in prolonged toxicity. Considering their wide commercial expansion, mode of action, the systemic properties in plants, persistence and environmental fate, coupled with limited information about the toxicity profiles of these compounds and their metabolites, neonicotinoids and fipronil may entail significant risks to the environment. A global evaluation of the potential collateral effects of their use is therefore timely. The present paper and subsequent chapters in this review of the global literature explore these risks and show a growing body of evidence that persistent, low concentrations of these insecticides pose serious risks of undesirable environmental impacts.

Keywords Neonicotinoid · Fipronil · Trends · Mechanism of action · Agriculture · Seed treatment · Systemic insecticides · Metabolites

Introduction

Neonicotinoids and the phenyl-pyrazole fipronil are insecticides with systemic properties. Their physicochemical characteristics, mainly assessed in terms of their octanol water partition coefficient (K_{ow}) and dissociation constant (pKa), enable their entrance into plant tissues and their translocation to all its parts (Bromilow and Chamberlain 1995; Bonmatin et al. 2014). Regardless of the manner of application and route

P. Mineau

Pierre Mineau Consulting, 124 Creekside Drive, Salt Spring Island V8K 2E4, Canada

E. A. D. Mitchell

Laboratory of Soil Biology, University of Neuchâtel, Rue Emile Argand 11, 2000 Neuchâtel, Switzerland

E. A. D. Mitchell

Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58, 2000 Neuchâtel, Switzerland

C. A. Morrissey

Department of Biology and School of Environment and Sustainability, University of Saskatchewan, 112 Science Place, Saskatoon, SK S7N 5E2, Canada

D. A. Noome

Kijani, Oud Blaricumweg 36b, 1411JT Naarden, The Netherlands

J. Settele · M. Wiemers

UFZ, Helmholtz Centre for Environmental Research, Department of Community Ecology, Theodor-Lieser-Str. 4, 06120 Halle, Germany

J. Settele

German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

J. D. Stark

Puyallup Research and Extension Centre, Washington State University, Puyallup, WA 98371, USA

A. Tapparo

Dipartimento di Scienze Chimiche, Università degli Studi di Padova, via Marzolo 1, 35131 Padova, Italy

H. Van Dyck

Behavioural Ecology and Conservation Group, Biodiversity Research Centre, Université Catholique de Louvain (UCL), Croix du Sud 4-5 bte L7.07.04, 1348 Louvain-la-Neuve, Belgium

J. Van Praagh

Scientific Advisor, Hassellstr. 23, 29223 Celle, Germany

J. P. Van der Sluijs

Centre for the Study of the Sciences and the Humanities, University of Bergen, Postboks 7805, 5020 Bergen, Norway

P. R. Whitehorn

School of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK

of entry to the plant, they translocate throughout all plant tissues making them toxic to any insects (and potentially other organisms) that feed upon the plant. This protects the plant from direct damage by herbivorous (mainly sap feeding) insects and indirectly from damage by plant viruses that are transmitted by insects. The discovery of imidacloprid by Shinzo Kagabu, and its subsequent market introduction in 1991, started the era of the neonicotinoid class of insecticides (Tomizawa and Casida 2011). Imidacloprid was followed in 1999 by thiamethoxam (Maienfisch et al. 2001a) and clothianidin, which is a metabolite of thiamethoxam (Meredith et al. 2002). Over the following two decades, neonicotinoids have become the most widely used insecticides of the five major chemical classes (the others being organophosphates, carbamates, phenyl-pyrazoles, and pyrethroids) on the global market (Jeschke and Nauen 2008; Jeschke et al. 2011; Casida and Durkin 2013).

The French company Rhône-Poulenc Agro (now Bayer CropScience) discovered and developed fipronil between 1985 and 1987 (Tingle et al. 2003), reaching the market in 1993 (Tomlin 2000). It is noteworthy that substances belonging to the phenyl-pyrazole family have in principal herbicidal effects, whereas fipronil is a potent insecticide.

By the 1980s, many pest insects had developed resistance to the organophosphates, carbamates, and pyrethroids then on the market (Georghiou and Mellon 1983; Denholm et al. 1998; Alyokhin et al. 2008). Set against this background of increased resistance to existing insecticides, the neonicotinoid and fipronil were presented as having several key attributes that led to their rapid adoption in both agricultural and urban environments. These included the following: lower binding efficiencies to vertebrate compared to invertebrate receptors, indicating selective toxicity to arthropods, high persistence, systemic nature, versatility in application (especially as seed treatments), high water solubility, and assumed lower impacts on fish and other vertebrates.

The binding sites of neonicotinoids to nicotinic acetylcholine receptors (nAChRs) and fipronil to γ -aminobutyric acid (GABA) receptors in the nervous systems of vertebrates are different from those in insects. In general, vertebrates have lower numbers of nicotinic receptors with high affinity to neonicotinoids, which is why neonicotinoids generally show a priori higher toxicity to invertebrates than vertebrates (including human, e.g., USEPA 2003a; Tomizawa and Casida 2003; Tomizawa and Casida 2005; Liu et al. 2010; Van der Sluijs et al. 2013). Similarly, the binding of fipronil to insect GABA receptors is tighter than that observed for vertebrate receptors (Cole et al. 1993; Grant et al. 1998; Hainzl et al. 1998; Ratra and Casida 2001; Ratra et al. 2001; Narahashi et al. 2010). This, combined with the frequent use on neonicotinoids and fipronil in seed/soil treatments rather than sprays, is supposed to make them comparatively safe for agricultural workers. This is in contrast to some of the

alternatives that they have replaced, such as organophosphates and carbamates (Marrs 1993). Neonicotinoids and fipronil are also relatively persistent, offering the potential for long-term crop protection activity. The half-lives of these compounds in aerobic soil conditions can vary widely, but are measured in months or longer (e.g., 148–6,931 days for clothianidin; USEPA 2003a; Gunasekara et al. 2007; Goulson 2013; Sánchez-Bayo and Hyne 2014). Extensive information about the physicochemical characteristics of neonicotinoids and fipronil can be found in Bonmatin et al. (2014), together with information about their environmental fate.

Arguably, however, it is the systemic nature of these insecticides that has made them so successful. Irrespective of their mode of application, neonicotinoids become distributed throughout the plant, including the apices of new vegetation growth, making them particularly effective against sucking pests, both above ground and below. Although it is not a neonicotinoid, fipronil also acts systemically mainly when it is co-formulated with polymers to increase its systemic activity (Dieckmann et al. 2010a; Dieckmann et al. 2010b; Dieckmann et al. 2010c). Neonicotinoids and fipronil belong to a wide family of substances jointly referred to as the “systemic insecticides” due to their systemic properties, some carbamate and organophosphorus substances, however, can also act systemically (Sanchez-Bayo et al. 2013). Neonicotinoid and fipronil should theoretically not target organisms lacking nervous systems, such as protists, prokaryotes, and plants. Very little research has been done on these non-target organisms and the ecosystem functions they are responsible for. Nevertheless, some studies have revealed negative effects: for example, a negative effect of fipronil on soil microorganisms was suggested as a possible cause for the slower (ca. four-fold) degradation of this pesticide at high vs. low application in Australian soils (Ying and Kookana 2006).

Seven separate neonicotinoid compounds are available commercially worldwide (Jeschke et al. 2011). These 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 eighth compound, sulfoxaflor (Zhu et al. 2010), has recently come onto the market in China (Shao et al. 2013b) and the USA (Dow Agro Sciences 2013; USEPA 2013) and has been reviewed by the European Food Safety Authority (EFSA) for approval in the European Union (EFSA 2014). In China, new neonicotinoid compounds are being developed and tested (e.g., guadipyr and huanyanglin), and are nearing market release (Shao et al. 2013b; Shao et al. 2013b). Some of these novel neonicotinoids are the *cis*-neonicotinoids, which are isomers of neonicotinoids in which the nitro or cyano group are in the *cis*, rather than *trans*, orientation. It is well known that *trans* and *cis* isomers can differ markedly in their toxicity. More than 600 *cis*-

neonicotinoid compounds have already been synthesized, two of which, paichongding and cycloxaprid (Shao et al. 2013a), might also soon be available on the Chinese market; both are highly effective against Homoptera and Lepidoptera. Through hydrolysis, cycloxaprid forms imidacloprid within the plant, thereby acting as a time-released imidacloprid source, prolonging the protection of the crop. The molecular structures of these systemic pesticides are reported in Fig. 1.

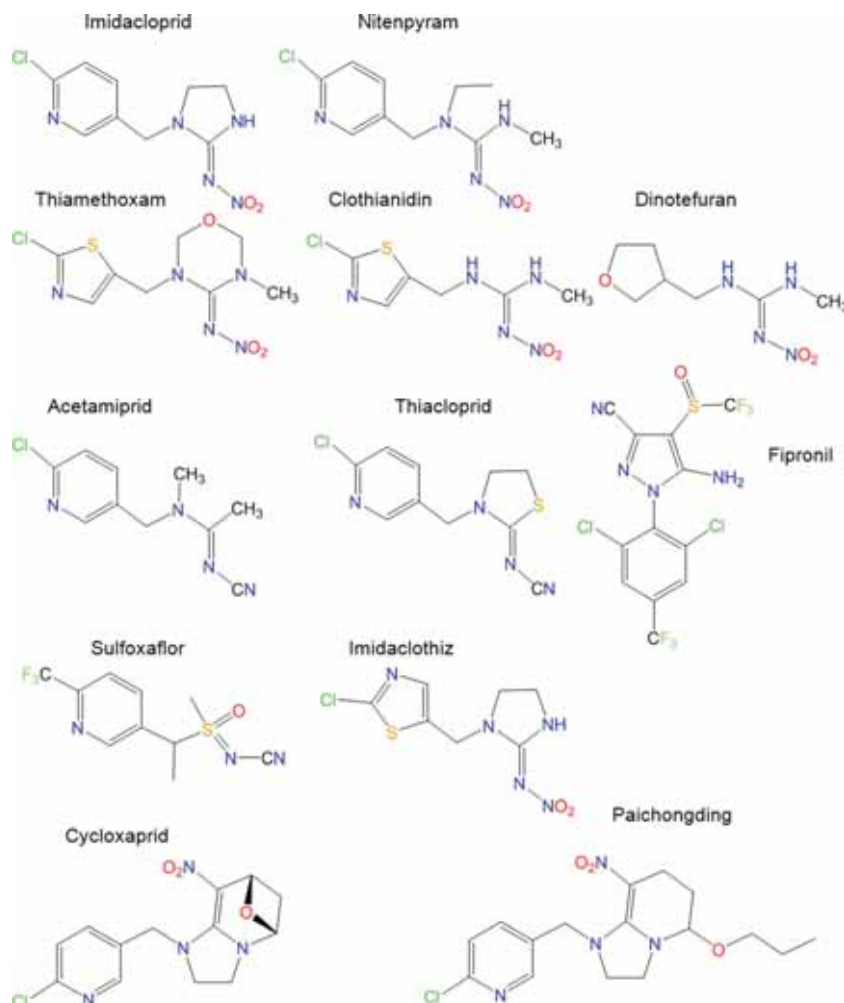
Neonicotinoids are active against a broad spectrum of economically important crop pests, including Aphidae (aphids), Aleyrodidae (whitefly), Cicadellidae (leafhoppers), Chrysomelidae (among others western corn rootworm), Elateridae (wireworms), Fulgoroidea (planthoppers), Pseudococcidae (mealybugs), and phytophagous mites (Elbert et al. 2008; Jeschke et al. 2011). Some of these groups (e.g., aphids) can also transmit viruses, so neonicotinoids can also contribute to the control of insect vectors of crop viral diseases. However, their broad spectrum leads to undesirable effects on non-target insects (Balança and de Visscher 1997; Sánchez-Bayo and Goka 2006; Maini et al. 2010; Lanzoni et al. 2012; Hayasaka et al. 2012a, b; Lu et al. 2012; Fogel

et al. 2013; Goulson 2013; Matsumoto 2013; Sanchez-Bayo et al. 2013; Van der Sluijs et al. 2013; Lu et al. 2014; Feltham et al. 2014; Bonmatin et al. 2014; Pisa et al. 2014). Pisa et al. (2014) focus specifically on the undesirable effects of neonicotinoids and fipronil on non-target invertebrates.

Global growth in the insecticide market

In 1990, the global insecticide market was dominated by carbamates, organophosphates, and pyrethroids. By 2008, one quarter of the insecticide market was neonicotinoid (rising to 27 % in 2010; Casida and Durkin 2013), and nearly 30 % was neonicotinoid and fipronil combined, with the other classes correspondingly reduced (Jeschke et al. 2011). In the same year, imidacloprid became the world's largest selling insecticide, and second largest selling pesticide (glyphosate was the largest; Pollack 2011) with registered uses for over 140 crops in 120 countries (Jeschke et al. 2011). Neonicotinoids are now in widespread use for a wide variety of crops worldwide.

Fig. 1 Common names and molecular structures of the systemic insecticides



By 2009, the global neonicotinoid market was worth US \$2.63 billion (Jeschke et al. 2011). Imidacloprid accounted for the greatest proportion (41.5 %) of this, and was worth US \$1.09 billion, with—in decreasing order of market share—thiamethoxam, clothianidin, acetamiprid, thiacloprid, dinotefuran, and nitenpyram worth US \$0.63, 0.44, 0.28, 0.11, 0.08, and 0.008 billion, respectively. Over the period 2003–2009, sales of individual neonicotinoid products (with the single exception of nitenpyram) rose by between 1.6- and 14.6-fold, with total sales across all products rising 2.45-fold (Table 1).

According to one estimate, ca. 5,450 tonnes of imidacloprid were sold worldwide in 2008 (Pollack 2011). A separate study estimated that ca. 20,000 tonnes of imidacloprid were produced globally in 2010 (CCM International 2011). This difference may reflect real growth, but may also be because imidacloprid became generic (off-patent) in 2006 (Jeschke et al. 2011), and/or because the estimates differ in the way they were measured, and what they include (e.g., agrochemicals and/or veterinary products, etc.; whether seed treatment is considered as insecticidal or not). Of the estimated 20,000 tonnes, 13,620 tonnes were produced in China (CCM International 2011). Shao et al. (2013b) similarly estimate that China currently produces 14,000 tonnes of imidacloprid annually, exporting 8,000 tonnes. Considering these figures, the estimation of CCM International 2011 seems realistic.

More recently, imidacloprid has been replaced by thiamethoxam and clothianidin in some parts of the world. Consequently, the worldwide sales of thiamethoxam reached US \$1 billion in 2011 (Syngenta 2012), and US \$1.1 billion in 2012 (Syngenta 2013). In the USA, clothianidin is now registered for use on 146 agricultural crops, and between 2009 and 2011 was applied to about 46 million acres (18.6 million ha) of these crops annually, of which 45 million (18.2 million ha) was corn (maize), *Zea mays* (Brassard 2012). In the USA, the use of clothianidin in 2011 is estimated to be 818 tonnes with corn accounting for 95 % of that use; imidacloprid 811 tonnes (2011) with soybeans and cotton accounting for

60 % of that use; and thiamethoxam 578 tonnes (2011) with soybeans, corn, and cotton accounting for 85 % of that use (US Geological Survey 2014).

Obtaining country or state-specific information on annual trends in quantities used of neonicotinoid insecticides and fipronil is challenging. Such information is rare in the peer-reviewed literature. Furthermore, in those countries/states in which information is available (e.g., Great Britain, Sweden, Japan, and California), quantities are measured in different ways (sold, used, shipped, etc.) and comparisons of absolute amounts are not straightforward, though trends can be determined. For each of these countries and states, the overall use of neonicotinoids and fipronil has risen markedly since their first introduction in the early 1990s (Figs. 2a–d). There is little suggestion that the quantities sold, used, or shipped are reaching an asymptote (Fig. 3), which concords with the growth in their annual global sales (Table 1).

The quantities of neonicotinoid insecticides produced, sold, and applied may well continue to grow. This will be aided by the increases in the acreage of crops where they are heavily used, development of combined formulations (e.g., neonicotinoids combined with pyrethroids or fungicides), formulation technologies (e.g., Bayer CropScience's Q-TEQ technology, which facilitates leaf penetration), the rise of generic (off-patent) products (Elbert et al. 2008; Jeschke et al. 2011), or possible development of molecules with properties of multiple pesticide classes (e.g., combinations of herbicidal and insecticidal properties).

Many insect pests have developed resistance to conventional insecticides such as organophosphates, carbamates, pyrethroids, chlorinated hydrocarbons, and insect growth regulators. Similarly, after nearly two decades of use, several target pests of neonicotinoids have begun to develop resistance (Jeschke et al. 2011). Examples are the greenhouse whitefly, *Trialeurodes vaporariorum* (Karatolos et al. 2010), the whitefly, *Bemisia tabaci* (Prabhakar et al. 1997; Cahill et al. 1996), and the Colorado potato beetle, *Leptinotarsa decemlineata* (Nauen and Denholm 2005; Szendrei et al. 2012; Alyokhin et al. 2007).

Table 1 Growth in global annual turnover (US \$ million) of neonicotinoid insecticides. Sales figures for 2003, 2005 & 2007 taken from [http://www.agropages.com/BuyersGuide/category/Neonicotinoid-](http://www.agropages.com/BuyersGuide/category/Neonicotinoid-Insecticide-Insight.html)

[Insecticide-Insight.html](http://www.agropages.com/BuyersGuide/category/Neonicotinoid-Insecticide-Insight.html). Sales figures for 2009, and number of crop uses taken from (Jeschke et al. 2011). Products sorted by rank of sales in 2009

Product	Crop uses	Company	2003	2005	2007	2009
imidacloprid	140	Bayer CropScience	665	830	840	1091
thiamethoxam	115	Syngenta	215	359	455	627
clothianidin	40	Sumitomo//Bayer CS	<30	162	365	439
acetamiprid	60	Nippon Soda	60	95	130	276
thiacloprid	50	Bayer CropScience	<30	55	80	112
dinotefuran	35	Mitsui Chemicals	<30	40	60	79
nitenpyram	12	Sumitomo	45	<10	<10	8

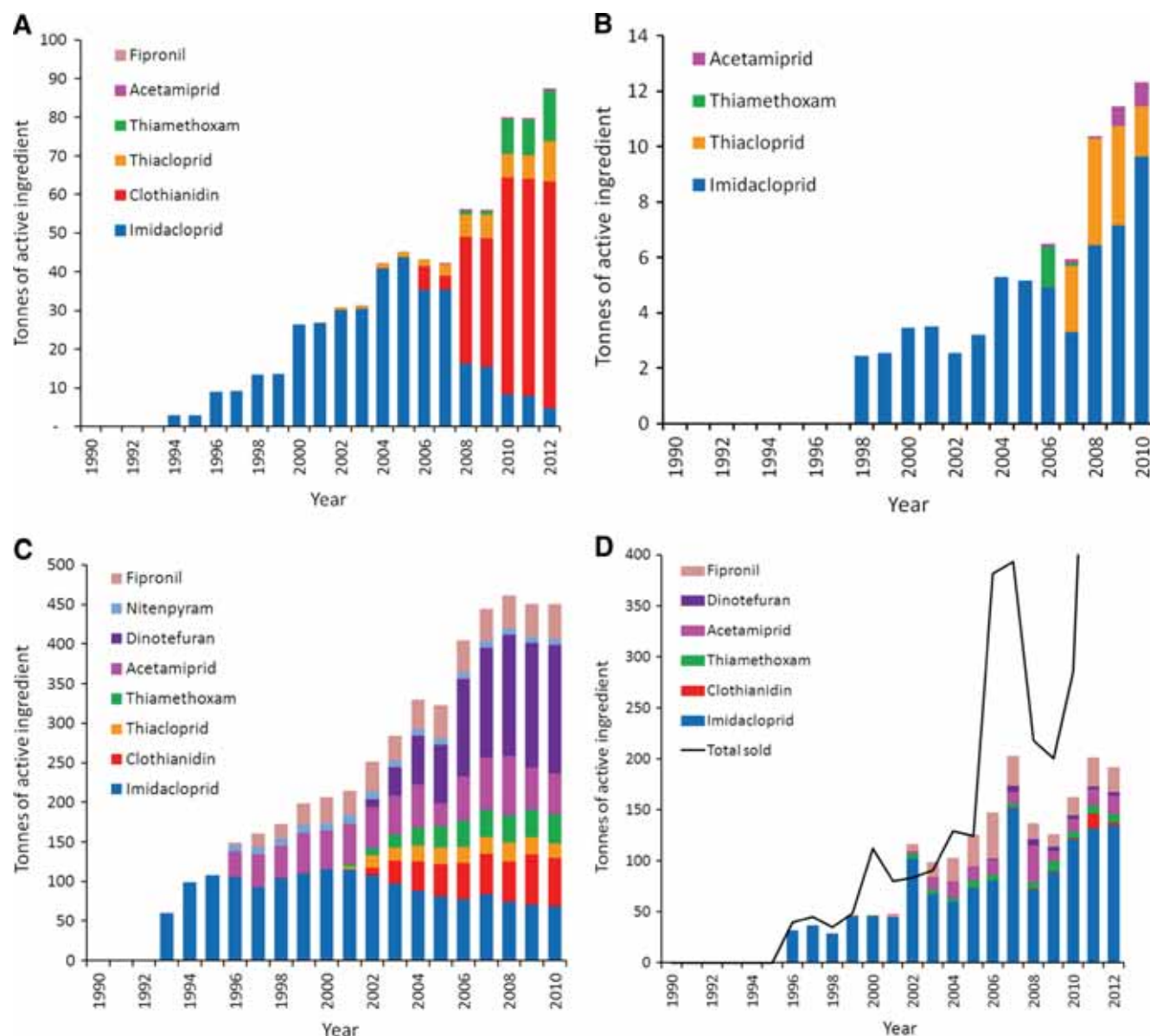


Fig. 2 **A** Trend in the agricultural use of neonicotinoid insecticides in Britain from 1990, measured in tonnes of active ingredient applied per year. Data from <http://pusstats.csl.gov.uk/index.cfm>. **B** Trend in the quantities of neonicotinoid insecticides sold in Sweden from 1998, measured in tonnes of active ingredient per year. Data from Swedish Chemicals Agency, KEMI, quoted in (Bergkvist 2011). **C** Trend in the domestic shipment of neonicotinoid insecticides and fipronil in Japan

from 1990, measured in tonnes of active ingredient per year. Data from Japan's National Institute for Environmental Studies database, provided by Mizuno, R. in litt., 2012. **D** Trend in the quantity of neonicotinoid insecticides and fipronil used in California from 1990, measured in tonnes of active ingredient applied per year. Data taken from <http://www.cdpr.ca.gov/docs/pur/purmain.htm>. Also shown are the total quantities sold, from <http://www.cdpr.ca.gov/docs/mill/nopdsold.htm>

Wang et al. (2007) demonstrated a relationship between imidacloprid and acetamiprid resistance in cotton aphids (*Aphis gossypii*). An increase in the frequency of resistance to three neonicotinoids (acetamiprid, clothianidin, and thiamethoxam) has also been reported for *A. gossypii* by Herron and Wilson (2011). Shi et al. (2011) noted no cross-resistance between imidacloprid and two other neonicotinoids (thiamethoxam and clothianidin), but did find a 3.68–5.79-fold cross-resistance for acetamiprid, nitenpyram, and thiacloprid. These researchers concluded that resistance to

acetamiprid and thiacloprid should be avoided on imidacloprid-resistant populations of *A. gossypii*.

Bioassays performed by Elbert and Nauen (2000) revealed a high degree of cross-resistance for the tobacco white fly (*B. tabaci*) to acetamiprid and thiamethoxam. Cross-resistance between imidacloprid and thiamethoxam was also confirmed under field conditions although Elbert and Nauen (2000) suggest that such problems are sometimes quite localized and that generalizations regarding resistance to imidacloprid or other neonicotinoids based on a few monitoring results

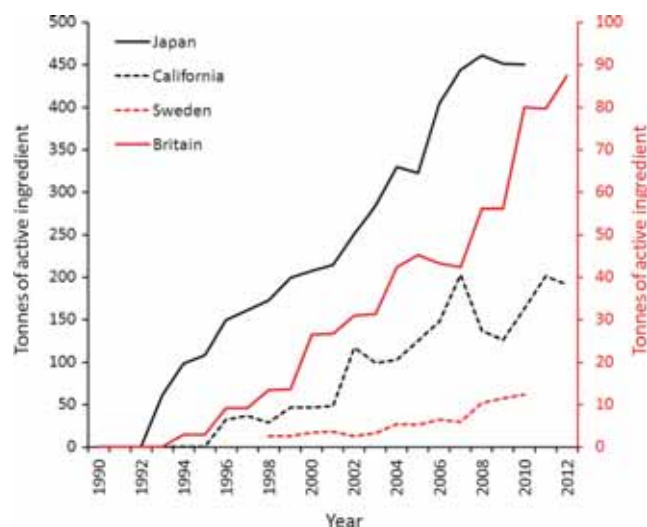


Fig. 3 Trend in the sales (Sweden), domestic shipment (Japan), use (California) and agricultural use (Britain) of all neonicotinoid insecticides and fipronil. See Figs. 2a–d for further details. All measured in tonnes of active ingredient per year. Note the separate vertical axes for California/Japan, and Britain/Sweden

should be avoided. Cross-resistance also appeared between imidacloprid, thiamethoxam, and clothianidin in the Colorado potato beetle, *L. decemlineata* (Alyokhin et al. 2007).

A recent study by Kavi et al. (2014) shows that resistance alleles to imidacloprid are present in the genetics of house flies (*Musca domestica*) in Florida. Imidacloprid selection resulted in a highly resistant strain of housefly, although the resistance was not stable and decreased over the course of several months. Incompletely dominant resistance of house flies to fipronil was found by Abbas et al. (2014).

The development of insecticide resistance against neonicotinoids in the brown planthopper (*Nilaparvata lugens*) was first observed in Thailand in 2003 and has since been found in other Asian countries such as Vietnam, China, and Japan. This problem has exacerbated yield losses in rice production in eastern China. Matsumura et al. (2008) found positive cross-resistance between imidacloprid and thiamethoxam in whitebacked planthopper, *Sogatella furcifera*, and also indicated that insecticide resistance of this crop pest against fipronil occurred widely in East and Southeast Asia. Planthopper resistance to imidacloprid has been reconfirmed following studies by Wang et al. (2008) and Azzam et al. (2011). According to Matsumura and Sanada-Morimura (2010) resistance to neonicotinoids is increasing. More recently, Zhang et al. (2014) studied nine field populations of the brown planthopper (*N. lugens*) from Central, East, and South China, and resistance to two neonicotinoids was monitored from 2009 to 2012. All nine field populations collected in 2012 had developed extremely high resistance to imidacloprid. Resistance to imidacloprid was much higher in 2012 than in 2009. Of the nine field

populations, six populations showed higher resistance to nitenpyram in 2012 than in 2011.

Neonicotinoids are of enormous economic importance globally, especially in the control of pests that have previously developed resistance to other classes of insecticides (Jeschke et al. 2011). However, as for many pest control products, resistance to neonicotinoids may become a barrier to market growth if not managed appropriately. The systemic properties of neonicotinoid pesticides and fipronil, combined with prophylactic applications, create strong selection pressure on pest populations, thus expediting evolution of resistance and causing control failure. There is clearly a need to be judicious in our patterns of neonicotinoid use, given that the emergence of insecticide resistance can pose threats to crop production and food security.

Uses

The use of neonicotinoids and fipronil covers four major domains: plant protection of crops and ornamentals against herbivorous insects and mites, urban pest control to target harmful organisms such as cockroaches, ants, termites, wasps, flies, etc., veterinary applications (against fleas, ticks, etc. on pets and cattle, and fleas in cattle stables) and fish farming (to control rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) infestations in rice-crayfish (*Procambarus clarkii*) rotation (Barbee and Stout 2009; Chagnon et al. 2014)). Figures on the relative economic importance of these four domains of application are scarce, but to give an indicative example, the 2010 imidacloprid sales of Bayer CropScience (covering plant protection and biocide uses) amounted to 597 million Euro (Bayer CropScience 2011), while the 2010 imidacloprid sales of Bayer Healthcare (veterinary applications) amounted to 408 million Euro (Bayer Healthcare 2011). Overall, the largest use seems to be protection of crops, ornamentals, and trees in agriculture, horticulture, tree nursery, and forestry.

In agriculture, horticulture, tree nursery and forestry, neonicotinoids and fipronil can be applied in many different ways such as (foliar) spraying, seed dressing, seed pilling, soil treatment, granular application, dipping of seedlings, chemigation, (soil) drenching, furrow application, trunk injections in trees, mixing with irrigation water, drenching of flower bulbs and application with a brush on the stems of fruit trees. Seed and soil applications represent approx. 60 % of their uses worldwide (Jeschke et al. 2011). In Europe for instance, more than 200 plant protection products containing imidacloprid, thiamethoxam, clothianidin, acetamiprid, or thiacloprid are on the market. In 2012, these products had more than 1,000 allowed uses for the treatments of a wide range of crops and ornamentals including potato, rice, maize, sugar beets, cereals (incl. maize), oilseed rape, sunflower, fruit, vegetables, soy, ornamental plants, tree nursery, seeds

for export, and cotton (EFSA 2012). In 2012, imidacloprid and thiamethoxam accounted for the largest share of authorized uses in Europe, with >30 and >25 %, respectively. Thiacloprid and acetamiprid accounted for >15 %, while clothianidin accounts for <5 %. These uses include field, greenhouse, and indoor applications. The largest share is field uses representing >60 % (EFSA 2012). Approximately 70 % of the number of allowed field uses in Europe were spray applications in 2012, while less than 20 % were seed treatment and less than 20 % were other methods of application such as drip irrigation, soil treatment. However, it is worthwhile noting here that “percentage of number of allowed uses” is not the same as “percentage of the total volume of active substance,” nor is it representative of the extent of treated area. Thiacloprid and acetamiprid are authorized in the EU as spray or soil treatments. In Europe, no uses as seed treatment were noted for acetamiprid, and a single use was noted for thiacloprid (maize) (EFSA 2012). In Asia, major large-scale applications of neonicotinoids include spraying of rice fields and other crops (Taniguchi et al. 2012), as well as granular applications (Thuyet et al. 2011, 2012) and seed coatings.

By far, the largest and most popular application in crop protection is the prophylactic seed coating. It is an a priori treatment against target pests that may decrease production yields. During germination and growing, the active substance in the seed coating is taken up by the roots and translocated to all parts of the crop, making the crop toxic to insects that attempt to feed upon it (Van der Sluijs et al. 2013). The global market for coating crop seeds with insecticides grew dramatically (more than six-fold) between 1990 and 2008, when its total value approached a billion Euros (Jeschke et al. 2011). This growth was almost entirely due to seeds being treated with neonicotinoids, which are well suited to this form of application (Elbert et al. 2008). In Britain, for example, of the 87.2 tonnes of neonicotinoid applied in 2012, 75.6 tonnes was as a seed treatment. In fact, 93 % by weight of all insecticidal seed treatment was with neonicotinoids (Fig. 4).

Similarly, the largest use of these compounds in North America is via application to seed in many annual row crop systems. Corn (maize) is the largest single use—in fact, production of corn for food, feed, and bioethanol production represents the largest single use of arable land in North America. Pest management of seed and seedling disease and insect pests in corn is achieved almost exclusively using prophylactic applications of pesticide “cocktails” that routinely include neonicotinoid seed treatments for insect control. One coated maize seed typically is coated with between 1,500 and 4,500 ppm of insecticide (or 0.5–1.5 mg per seed). Systemic and long-lasting high concentrations allow not only the protection of the seedling from soil-bound insects but also offer some suppression of western corn rootworm, *Diabrotica virgifera virgifera*, whose attacks usually start one or more weeks after the sowing (van Rozen and Ester 2010).

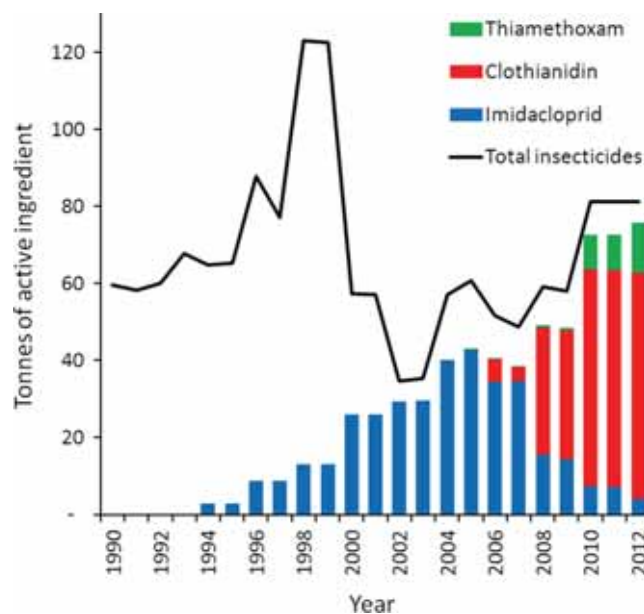


Fig. 4 Trend in the agricultural use of neonicotinoid insecticides as seed treatments in Britain from 1990, measured in tonnes of active ingredient per year (bars). The total usage of all insecticidal seed treatments (solid line) is also shown. Data from <http://pusstats.csl.gov.uk/index.cfm>

Maize planting reached unprecedented levels in the USA in 2013 at 96 million acres, or 38.8 million ha (USDA-NASS 2013). This level of production is expected to increase in 2014 and beyond. Virtually all of the seeds planted in North America (the lone exception being organic production= 0.2 % of total acreage, USDA –NASS 2013) are coated with neonicotinoid insecticides. The two major compounds used are clothianidin and thiamethoxam; the latter is metabolized to clothianidin in insects, other animals, plants, and soil (Nauen et al. 2003). Although maize is the largest single use, seed treatments in other large acreage crops, including soybeans (31.4 million ha), wheat (23 million ha), and cotton (4.2 million ha) combine to make this class of insecticides the most widely used in the USA in history, when measured by area of application (USDA-NASS 2013).

Neonicotinoid seed treatments are routinely applied to the vast majority of grain and oilseed crops in developed countries, regardless of pest pressures or field histories. Untreated seeds are often unavailable for purchase. In fact, in many of the most important crops grown in North America (notably maize), there are no non-neonicotinoid seed alternatives readily available to producers in the marketplace. Because any subsequent crop insurance claims by producers must document that accepted standard practices were used during planting, there is an inherent risk in requesting seed that is markedly different from the standard. This may present a disincentive for producers that would otherwise attempt growing untreated seeds in some fields. Several efficacy studies have demonstrated that applications of neonicotinoids can reduce pest population densities, defoliation, and crop damage (e.g.,

Maienfisch et al. 2001b; Kuhar et al. 2002; Nault et al. 2004; Koch et al. 2005). This can result in increased crop yields compared to crops with no pest management (see review by Jeschke et al. 2013).

However, because the pests targeted by neonicotinoids are generally occasional, sporadic, and secondary pests, these benefits are not routinely found: a review of literature by Stevens and Jenkins (2014) found inconsistent benefits in 11 of 19 peer-reviewed papers examined, and no benefit in the remaining 8 articles. Considering the nature of the pests targeted, this is not altogether surprising. By definition, these secondary pests are often not present or present in subeconomic levels. However, they do occur and it is crucial that crop producers have options for management. These resources do exist: there is a significant base of knowledge for managing these secondary pests, and agricultural practices such as crop rotation drastically reduce the need for control through neonicotinoids in many cases (Apenet 2009, 2010, 2011). Indeed, the cost-effectiveness of the prophylactic use of neonicotinoids has in the past and recently been questioned (Maini et al. 2010; Stevens and Jenkins 2014). Several studies have shown that the use of neonicotinoids does not necessarily result in increased yield or economic benefit, thereby bringing into question the advisability of a widespread and prophylactic use of neonicotinoid insecticides (Apenet 2011; Mole et al. 2013; Stokstad 2013). Macfadyen et al. (2014) showed that imidacloprid-treated seeds tended to increase yields of canola, but no such benefit was found for wheat. Similarly, Royer et al. (2005) found that imidacloprid-treated seeds sometimes increased yields of wheat but did not always result in a positive economic return. Neonicotinoid insecticidal seed treatments provided no yield benefits over a 2-year study in experimental soybean applications (Seagraves and Lundgren 2012). De Freitas Bueno et al. (2011) also found that the prophylactic use of neonicotinoids on soybeans did not significantly increase production in comparison to other pest management approaches. Johnson et al. (2009) found that although imidacloprid treatments increased the yield of soybeans, the economic return from imidacloprid-treated crops was not as high as those from crops under an integrated pest management program. In citrus orchards of California, imidacloprid treatments were ineffective or marginally effective at controlling damage from scales or mites and the insecticides suppressed natural enemies such that overall benefits to citrus crops were less than from other pest management options including growth regulators (Grafton-Cardwell et al. 2008). Taken as a whole, these data reflect that use levels for neonicotinoid seed treatments are dramatically out of step with the actual need; in most cases, pests are absent or present at such low numbers that seed treatments cannot demonstrate any benefit.

Alternatives to this prophylactic use of neonicotinoids including those presented by Furlan and Kreutzweiser (2014)

may help to minimize the risk of insect and other arthropod resistance (see above) to neonicotinoids and reduce overall operational costs.

Mode of action on invertebrates

Neonicotinoids can be considered substances acting as agonists on nAChRs opening cation channels (Casida and Durkin 2013). Voltage-gated calcium channels are also involved (Jepson et al. 2006) in their insecticidal activity (Liu et al. 1995; Orr et al. 1997; Nishimura et al. 1998; Tomizawa and Casida 2001, 2003, 2005). Differences in properties and structure of the subunits between insects and mammalian nAChRs explain in part the high selectivity of neonicotinoids to arthropods and the supposed relatively low toxicity to vertebrates (Nauen et al. 1999; Lansdell and Millar 2000; Matsuda et al. 2001; Tomizawa and Casida 2003, 2005). Electrophysiological studies have shown that the binding potency of neonicotinoids to brain membranes is well and positively correlated with their agonistic and insecticidal activity. This suggests that the channel opening of nAChRs induced by the binding of neonicotinoids to receptors leads to insecticidal activity (Nishimura et al. 1998; Nishiwaki et al. 2003). As a result, their agonistic action induces continuous excitation of the neuronal membranes, producing discharges leading to paralysis and cell energy exhaustion. This binding potency is conferred by a unique molecular conformation (Tomizawa and Casida 2011). However, the interaction of this conformation with the receptor may vary depending on their different chemical substituents and on the species considered (Honda et al. 2006). In addition, the sensitivity of insect nAChRs to neonicotinoids may be modulated by phosphorylation mechanisms, as shown for imidacloprid (Salgado and Saar 2004), leading to variation in the insecticidal activity. Thus, imidacloprid selectively inhibits desensitizing nicotinic currents, while displaying a selective desensitization toward certain nAChR subtypes (Oliveira et al. 2011). This indicates that selective desensitization of certain nAChR subtypes can account for the insecticidal actions of imidacloprid.

The characterization of the binding sites, the recognition subsites, and the toxicophores of neonicotinoids have been studied in depth (Hasegawa et al. 1999; Kagabu et al. 2002; Kanne et al. 2005; Matsuda et al. 2005; Kagabu 2008; Kagabu et al. 2008; Kagabu et al. 2009). Photoaffinity labelling has enabled identification of the amino acids involved in the molecular interaction between neonicotinoids and nAChRs or the acetylcholine binding protein (AChBP) (Tomizawa and Casida 1997; Kagabu et al. 2000; Tomizawa et al. 2001a; Tomizawa et al. 2001b; Zhang et al. 2002, 2003; Tomizawa et al. 2007; Tomizawa et al. 2008; Tomizawa and Casida 2009). It appears that, in the same binding pocket, two very different interactions drive the recognition of

neonicotinoids. The electronegative toxicophore of neonicotinoids and the cationic toxicophore of nicotinoids (nicotine, epibatidine, and desnitro-imidacloprid) lead to them docking in opposite directions at the binding sites (Tomizawa et al. 2003; Tomizawa and Casida 2009).

Neonicotinoids appear to bind to multiple sites on membranes of neural tissues in various insect species. The American cockroach, *Periplaneta americana*, expresses two types of receptors resistant to α -bungarotoxin (α -BgTx), an antagonist of nicotinic receptors: nAChR1, which is sensitive to imidacloprid, and nAChR2, which is not (Courjaret and Lapied 2001; Courjaret et al. 2003; Tan et al. 2007; Thany et al. 2008). As a result, while imidacloprid acts on nAChR1 and not on nAChR2, nicotine, acetamiprid, and clothianidin act as agonists of nAChR2 (Bordereau-Dubois et al. 2012; Calas-List et al. 2013).

The first generation of neonicotinoids included nitenpyram, imidacloprid, acetamiprid, and thiacloprid. Imidacloprid and its metabolites are highly toxic to bees (Suchail et al. 2000, 2001). It behaves like a partial agonist of the nicotinic nAChRs in Kenyon cells of the honey bee (*Apis mellifera*) mushroom body, which are involved in higher order neuronal processes in the brain such as olfactory learning (Déglise et al. 2002). However, the pharmacological properties and the molecular composition of nAChRs differ in Kenyon cells and in neurons from antennal lobes (Barbara et al. 2008; Dupuis et al. 2011). In antennal lobe neurons, the characterization of type I nAChR currents, which exhibit slow desensitization, and type II currents, which exhibit fast desensitization, strongly suggest the presence of at least two different types of nAChRs. The presence of two types of receptors displaying different affinities for imidacloprid and its metabolites was proposed on the basis of the complex toxicity profile after acute and chronic exposures in the honey bee (Suchail et al. 2001). Such complex profiles can be shown both on mortality rates and on sublethal effects on reproduction. This has been recently exemplified for common fruit fly, *Drosophila melanogaster*, after chronic exposure to imidacloprid, at concentrations far below the levels found in the field (Charpentier et al. 2014). A study designed to demonstrate the absence of different biological targets of imidacloprid and its metabolites (Nauen et al. 2001) proved inconclusive for several reasons: (1) a binding of [3 H]-imidacloprid occurs at nanomolar concentrations, whereas ionic currents are induced at micromolar concentrations (30 μ M here), (2) the pharmacology of the current induced by imidacloprid, 5-OH-imidacloprid and olefin (two important metabolites of imidacloprid, see metabolites section for details) has not been investigated, (3) no Scatchard analysis is presented, therefore no analysis for receptor binding interactions is provided, and (4) displacement experiments have been performed at nanomolar concentrations instead of micromolar concentrations, which prevent the dual characterization of

high and low-affinity targets. Studies on the effects of imidacloprid and two of its metabolites, 5-OH-imidacloprid and olefin-imidacloprid, on the habituation phenomenon have enabled the characterization of two receptors differentially expressed during honey bee development (Guez et al. 2001; Guez et al. 2003).

The occurrence of two types of imidacloprid targets, which could explain the differential toxicity of imidacloprid at low and very low doses observed in bees, has been demonstrated in the green peach aphid (*Myzus persicae*). Saturable binding of [3 H]-imidacloprid has revealed a high-affinity binding site, with a dissociation constant (K_d) of 0.14 nM, and a low-affinity binding site, with K_d of 12.6 nM, whose pharmacology resembles that of nAChR (Lind et al. 1998). Another study confirming these results presented similar dissociation constants of 0.6 and 7.2 nM (Wiesner and Kayser 2000). In addition, the pharmacology of the high-affinity binding site is similar to that of α -BgTx binding sites in the honey bee and the hawk moth (*Manduca sexta*) (Lind et al. 1999). The existence of two imidacloprid binding sites has been confirmed in the brown planthopper (*N. lugens*) (Li et al. 2010). Two [3 H]-imidacloprid binding sites have been identified with different affinities ($K_d=3.5$ pM and $K_d=1.5$ nM) and subunit co-assemblies ($\alpha 1$, $\alpha 2$, and $\beta 1$ for the low-affinity nAChR and $\alpha 3$, $\alpha 8$, and $\beta 1$ for the high-affinity nAChR). In fact, the existence of multiple binding sites in insects seems to appear as a relatively common feature of neonicotinoids, since it has also been observed in the aphid (*Aphis craccivora*) and in the locust (*Locusta migratoria*) (Wiesner and Kayser 2000).

Contrary to acetylcholine, acetylcholinesterase does not act on nicotine nor imidacloprid, and possibly on the other neonicotinoids, leading to their prolonged action on the nAChRs (Thany 2010). Furthermore, poor neuronal detoxification mechanisms may contribute to a prolonged action at this level (Casida and Durkin 2013). 6-chloronicotinic acid (6-CNA) is a metabolite common to chloropyridinyl neonicotinoids (Ford and Casida 2008; Casida 2011). Some of these metabolites have proved to be highly toxic to bees leading to significant mortalities by chronic exposure (Suchail et al. 2001). Thus, the risk posed by 6-CNA to the honey bee might be common to the use of imidacloprid, thiacloprid, acetamiprid, and nitenpyram. These features may contribute to the delayed and chronic lethality observed with some neonicotinoids, e.g., thiacloprid, imidacloprid (Suchail et al. 2001; Beketov and Liess 2008; Tennekes and Sánchez-Bayo 2011; Roessink et al. 2013).

Imidacloprid has been shown to stimulate plant growth of genetically modified stress tolerant plants, even in the absence of damaging pest species, leading to increase in crop yield. As a result, treated plants respond better to the effects of abiotic stressors such as drought (Thielert et al. 2006). The metabolite 6-CNA has been suggested to be responsible for the physiological plant changes as it is known to induce a plant's own

defenses against plant disease. Consequently, imidacloprid together with acetamiprid, thiacloprid, and nitenpyram are included within the so-called Stress Shield™ technology (Bayer 2006).

Thiamethoxam, a second-generation neonicotinoid (Maiefisch et al. 2001a), acts differently to first-generation neonicotinoids. Thiamethoxam is a poor agonist of insect nAChRs (Nauen et al. 2003; Tan et al. 2007; Benzidane et al. 2010). However, it is a full agonist at cercal afferent/giant interneuron synapses (Thany 2011) where it induces a strong depolarization that can be partially lowered by the muscarinic antagonist atropine. This suggests that thiamethoxam is able to bind to mixed nicotinic/muscarinic receptors (Lapied et al. 1990). Metabolic N-desmethylation of thiamethoxam (TMX-dm) results in an increase in the affinity to the [³H]-imidacloprid binding site (Wiesner and Kayser 2000). However, although it does not occur in lepidopteran larvae, TMX-dm can be produced in mammals and insects (Nauen et al. 2003; Ford and Casida 2006b). It can interact with insect nAChRs, but is about 25 times less potent than thiamethoxam as an insecticide (Nauen et al. 2003), but is nevertheless marketed in its own right. The thiamethoxam metabolite, clothianidin, presents insecticidal activity (Nauen et al. 2003). It can act on imidacloprid-sensitive nAChR1 and imidacloprid-insensitive nAChR2 subtypes (Thany 2009, 2011). Studies involving neurophysiology, behavioral experiments, and chemical analysis have revealed that the effect of thiamethoxam on cockroach locomotor activity is closely associated with the appearance of its metabolite clothianidin (Benzidane et al. 2010). These two molecules are often presented together in environmental matrices (Bonmatin et al. 2014), and their toxic action may therefore be enhanced.

The third-generation neonicotinoid dinotefuran (Wakita et al. 2003) can interact with insect nAChRs (Mori et al. 2002; Kiriya et al. 2003). A high-affinity binding site, exhibiting a dissociation constant of 13.7 nM, has been characterized in the nerve cord membranes of the American cockroach (*P. americana*) (Miyagi et al. 2006). However, Scatchard analysis suggests the occurrence of two binding sites. Dinotefuran can exhibit a nerve-excitatory activity, which is lower than that of imidacloprid and comparable to that of clothianidin, and a nerve-blocking activity, which is comparable to that of imidacloprid and slightly higher than that of clothianidin (Kiriya and Nishimura 2002). Such a nerve-blocking action has also been described in cockroaches with thiacloprid and its derivatives (Kagabu et al. 2008). The insecticidal activity of dinotefuran and its derivatives is better correlated to nerve-blocking activity than to nerve-excitatory activity, a characteristic also observed with other neonicotinoids (Kagabu et al. 2008). Both the nitroguanidine and the terahydro-3-furylmethyl parts of the molecule are

important for the insecticidal activity of dinotefuran (Wakita et al. 2004a; Wakita et al. 2004b; Wakita 2010). However, compared to imidacloprid and acetamiprid, dinotefuran appears more effective in inducing depolarizing currents in terms of current amplitude and concentration dependence (Le Questel et al. 2011).

Sulfoxaflor is a fourth-generation neonicotinoid that exhibits a high insecticidal activity against a broad range of sap-feeding insects (Babcock et al. 2011). It can also act on nAChRs and may be considered as a neonicotinoid. This needs to be taken into account when considering possibilities for insecticide rotation in order to manage resistance toward neonicotinoids (Cutler et al. 2013). The nature of the interactions with nAChRs differs between sulfoxaflor and the other neonicotinoids (Sparks et al. 2013). Sulfoxaflor induces currents of high amplitude when tested on nAChR hybrids of *D. melanogaster* $\alpha 2$ nAChR subunit and chicken $\beta 2$ subunit in the african clawed frog (*Xenopus laevis*) oocytes (Watson et al. 2011). The maximum intensity ($I_{\max}$) of sulfoxaflor-induced currents is much higher than those of imidacloprid, acetamiprid, thiacloprid, dinotefuran, and nitenpyram. Conversely, sulfoxaflor presents a weak affinity to displace [³H]-imidacloprid from green peach aphid (*M. persicae*) membranes. In stick insect (Phasmatodea) neurons, sulfoxaflor potently desensitizes fast-desensitizing currents, I_{ACh1H} , and both slowly desensitizing components, I_{ACh2H} and I_{ACh2L} (Oliveira et al. 2011). These studies clearly show that the action of sulfoxaflor and other sulfoximines, similar to that of imidacloprid, involves receptor desensitization, receptor selectivity, a differential action at low and high doses and, probably, receptor desensitization after a prolonged exposure. Additionally, the use of *D. melanogaster* strains presenting mutations at D $\alpha 1$ and D $\beta 2$ nAChR subunits, or resistant silverleaf whitefly (*B. tabaci*) strains revealed no cross-resistance between sulfoxaflor and imidacloprid or spinosyns (family of compounds with insecticidal activity produced from fermentation of two species of *Saccharopolyspora*, including active ingredients such as spinosad; Perry et al. 2012; Longhurst et al. 2013), despite the fact that sulfoxaflor shares nAChR as a common target with other neonicotinoids.

The pharmacology of cycloxaprid, a *cis*-neonicotinoid also belonging to the fourth generation, has been subjected to fewer investigations due to its recent discovery. In the housefly, [³H]-cycloxaprid binds to head membranes with a K_d of 28 nM (Shao et al. 2013b). Displacement studies show that the cycloxaprid metabolite, [³H]-nitromethylene imidazole (NMI), is 19, 15, and 41-fold more potent than cycloxaprid on housefly, honey bee, and mouse (*Mus musculus*) brain membranes, respectively.

Neonicotinoids induce depolarizing currents in insects by an agonist action on nAChRs. However, as seen above, they

also exert a nerve-blocking activity that contrasts with their agonist action and their nerve-excitatory activity, as shown for thiacloprid and its derivatives (Kagabu et al. 2008; Toshima et al. 2008). Studies carried out at chicken neuromuscular junction strongly suggest that imidacloprid is an antagonist at muscle cell nAChRs (Seifert and Stollberg 2005). In *N. lugens*, the Y151S mutation in $\text{Nl}\alpha 1$ subunit is associated with a resistance to imidacloprid, but has little effect on the action of acetylcholine (Liu et al. 2005; Liu et al. 2006). Replacement of tyrosine with methionine (Y151M mutation), as found in *Caenorhabditis elegans* in the site equivalent to Y151, instead of serine, results in $\text{Nl}\alpha 1/\beta 2$ nAChR on which imidacloprid acts as an antagonist (Zhang et al. 2008). This shows that very subtle differences in subunit sequence can lead to nAChRs resistant to neonicotinoids or to nAChRs on which neonicotinoids can act agonistically or antagonistically.

As with carbamates and organophosphates, fipronil exerts its insecticidal activity by acting on the inhibiting system of the nervous system. It binds to GABA receptors (Tingle et al. 2003) and to glutamate receptors coupled to chloride channels (Barbara et al. 2005). In doing so, fipronil blocks the inhibiting receptors leading to an excitation of the nervous system. It leads to neuronal hyperexcitation due to accumulation of the neurotransmitter (GABA) at the synaptic junctions. Its mode of action is, therefore, antagonistic, whereas that of neonicotinoids is agonistic. Glutamate receptors are insect specific, which is the reason why fipronil is more effective on invertebrates than on vertebrates (Narahashi et al. 2007). Furthermore, it seems to have low affinity to vertebrate receptors (Grant et al. 1998). Fipronil shows a higher selectivity for insects than for humans, with affinity constant ($K_I = \text{IC}_{50}/(1 + [L]/K_d)$) of 4 nM for the housefly GABA_A receptors and 2,169 nM for human GABA_A receptors (Ratra and Casida 2001). However, selectivity and sensitivity may vary with the subunit composition of the human GABA_A receptors. Competition with the binding of 4- $[\text{}^3\text{H}]$ -ethylnylbicycloorthobenzoate ($[\text{}^3\text{H}]$ -EBOB) to GABA receptors was performed to compare the relative affinity of fipronil to GABA receptors of different subunit compositions (Ratra et al. 2001). Fipronil is highly selective to the $\beta 3$ receptors (inhibitory concentration 50 % (IC_{50}) = 2.4 ± 0.3 nM; K_I = 1.8 nM), but presents a lower selectivity to native GABA_A receptors (IC_{50} = $2,470 \pm 370$ nM; K_I = 2,160 nM). The fact that native receptors show a lesser affinity to fipronil than $\beta 3$ receptors suggests that the other subunits of the human GABA_A receptors modulate the sensitivity of GABA receptors to fipronil (Casida and Quistad 2004). Fipronil derivatives show a higher affinity for native receptors than fipronil, with IC_{50} values ranging between 237 ± 45 and 343 ± 49 nM for the derivatives, and $2,470 \pm 370$ nM for fipronil (Ratra et al. 2001). Fipronil interacts with AChR receptors with lower affinity than neonicotinoids (Barbara et al. 2005).

Metabolites

Metabolism of the seven major commercial neonicotinoids can be divided into two phases. Phase I metabolism, largely dependent on cytochrome P450, includes reactions such as demethylation, nitro reduction, cyano hydrolysis, hydroxylation of imidazolidine and thiazolidine accompanied by olefin formation, hydroxylation of oxadiazine accompanied by ring opening, and chloropyridinyl and chlorothiazolyl dechlorination (Ford and Casida 2008; Casida 2011). For some neonicotinoids, cytosolic aldehyde oxidase together with cytochrome P450 is responsible for nitro reduction in mammals (Dick et al. 2005; Casida 2011). Phase I metabolites have been found in both small mammals and plants (Chen et al. 2005; Casida 2011). Phase II metabolism is mainly responsible for conjugate formation, which differ between plants and animals (Ford and Casida 2008; Casida 2011). Several metabolites are common to different neonicotinoids but others are compound specific (Schulz-Jander and Casida 2002; Ford and Casida 2006a, 2008; Shi et al. 2009; Casida 2011).

Neonicotinoids are subjected to intense metabolism in plants leading to the appearance of different metabolites during the plant life or, at least, up to the harvest of plants consumed by humans or breeding animals (Laurent and Rathahao 2003; Greatti et al. 2006; Ford and Casida 2008; Karmakar et al. 2009; Karmakar and Kulshrestha 2009). As a result, metabolites may induce a long-lasting action of neonicotinoids against pests, particularly plant-sucking pests such as aphids (Nauen et al. 1998). Tables 2 and 3 show the metabolites of neonicotinoids and fipronil, respectively.

Thiamethoxam, clothianidin, and dinotefuran

Animals

The metabolism of thiamethoxam (hereafter also TMX) is closely related to that of clothianidin (hereafter also CLO). As a result, thiamethoxam produces both metabolites in common with clothianidin as well as some specific metabolites (Ford and Casida 2006a). The main metabolic pathways of thiamethoxam involve hydroxylation at the oxadiazine part of the molecule, accompanied by ring opening, leading to the production of clothianidin, its principal intermediate in mammals, insects, and plants (Nauen et al. 2003; Ford and Casida 2006a; Karmakar et al. 2009; Casida 2011). Other metabolic pathways of both TMX and CLO are N-demethylation and/or nitro reduction reactions (Ford and Casida 2006a; Casida 2011; Kim et al. 2012), leading to TMX-dm and CLO-dm or their N-nitroso- or N-amino-guanidines derivatives. These are two metabolites with toxicity comparable to those of the parent compounds, maintaining almost unaltered binding affinity to the nAChR (Chen et al. 2005; Ford and Casida 2006a). In fact, N-desmethyl

Table 2 Metabolites of neonicotinoids in various media and organisms. Metabolites known to be active toward invertebrates or mammals are highlighted in bold

Parent compound	Metabolites	Formation medium	Reference
Thiamethoxam (TMX)	Clothianidin, CLO	Soil, mice, mammals, insects, plants	Ford and Casida 2006a; Nauen et al. 2003; PPDB 2013; FAO thiamethoxam
	Thiamethoxam-dm, TMX-dm, N-desmethyl thiamethoxam TMX-NNO	Mice	Ford and Casida 2006a
		Mice, soil bacteria (<i>Pseudomonas</i> sp.)	Ford and Casida 2006a Pandey et al. 2009
	TMX-NNH2	Mice	Ford and Casida 2006a
	TMX-NH	Mice, soil bacteria (<i>Pseudomonas</i> sp.), water (photodegradation), soil	Ford and Casida 2006a; Pandey et al. 2009; De Uderzo et al. 2007; FAO thiamethoxam
	TMX-Urea	Mice, soil bacteria (<i>Pseudomonas</i> sp.), water (photodegradation), soil	Ford and Casida 2006a; Pandey et al. 2009; Schwartz et al. 2000; FAO thiamethoxam
	TMX-dm-NNO	Mice	Ford and Casida 2006a
	TMX-dm-NH2	Mice	Ford and Casida 2006a
	TMX-dm-NH	Mice	Ford and Casida 2006a
	TMX-dm-Urea	Mice	Ford and Casida 2006a
	hydroxy thiazole urea derivative	Plants (tomato)	Karmakar et al. 2009
	6-hydroxy oxadiazinon	Plants (tomato)	Karmakar et al. 2009
	ether derivative	Plants (tomato)	Karmakar et al. 2009
	NG-A	Mammals	Ford and Casida 2006a
	NG-B	Mammals	Ford and Casida 2006a
	NG-C	Mammals	Ford and Casida 2006a
	NG-D	Mammals	Ford and Casida 2006a
	5-methyl-2(3H)-thiazolone	Water (photodegradation)	De Uderzo et al. 2007
	oxazine derivative	Water (photodegradation)	De Uderzo et al. 2007
	acrylonitrile derivative	Water (photodegradation)	De Uderzo et al. 2007
	carbonyl sulfide	Water (photodegradation)	De Uderzo et al. 2007; Schwartz et al. 2000
	isocyanic acid	Water (photodegradation)	De Uderzo et al. 2007; Schwartz et al. 2000
Clothianidin/ Thiamethoxam	TZNG, CLO-dm N-(2-chlorothiazol-5-ylmethyl)-N'-nitroguanidine	Soil, plants, mammals	PPDB 2013; Kim et al. 2012; Ford and Casida 2006a, 2008; FAO clothianidin ;
	CLO-NNO	Mice, insects, plants	Ford and Casida 2006a, 2008 Kanne et al. 2005; Karmakar et al. 2009
	CLO-dm-NNO	Mice, insects, plants	Ford and Casida 2006a, 2008; Kanne et al. 2005
	CLO-NNH2, ATMG	Mice, insects	Ford and Casida 2006a; Kanne et al. 2005
	CLO-dm-NNH2, ATG	Mice, insects	Ford and Casida 2006a; Kanne et al. 2005
	CLO-NH, TMG, N-(2-chlorothiazol-5-ylmethyl)-N'-methylguanidine	Soil, plants, sediment, mammals	Kim et al. 2012; Ford and Casida 2006a, 2008; FAO clothianidin
	CLO-dm-NH, TZG	Mammals, plants	Ford and Casida 2006a, 2008; FAO clothianidin
	CLO-Urea, TZMU, N-(2-chlorothiazol-5-ylmethyl)-N-methylurea	Soil, Plants, mammals, water	PPDB 2013; Kim et al. 2012; FAO clothianidin ; Ford and Casida 2008; Karmakar et al. 2009; Žabar et al. 2012; Schwartz et al. 2000
	CLO-dm-Urea, TZU, 2-chloro-1, 3-thiazole-5-ylmethylurea	Mammals, plants, soil	Kim et al. 2012; Ford and Casida 2006a, 2008; FAO clothianidin
	THMN, N-hydroxy clothianidin, N-2-Chlorothiazol-5-ylmethyl-N-hydroxy-N'-methyl-N''-nitroguanidine	Rat, apple	FAO clothianidin
	2-chloro-1,3-thiazole-5-methylamine	Tomato cell culture	Karmakar et al. 2009

Table 2 (continued)

Parent compound	Metabolites	Formation medium	Reference
Thiamethoxam/ Clothianidin/ Dinotefuran	2-chloro-1,3-thiazole-5-methyl isocyanate	Tomato cell culture	Karmakar et al. 2009
	TZA, CTM-a	Mammals	Ford and Casida 2006a
	TZOH, CTM-b*	Mammals	Ford and Casida 2006a
	CTM-c, CTA, CTCA, 2-chloro-1, 3-thiazole-5-carboxylic acid	Mammals, plants	Kim et al. 2012; Ford et al. 2010; Ford and Casida 2008, 2006a
	CTM-i, cACT, 2-chlorothiazol-5-ylmethylamine	Water	FAO clothianidin
	CTM-f	Mammals	Ford and Casida 2006a
	CTNU, N-(2-chlorothiazol-5-ylmethyl)-N'-nitrourea	Water	FAO clothianidin
	HMIO, 4-hydroxy-2-methylamino-2-imidazolin-5-one	Water	FAO clothianidin
	MIT, 7-methylamino-4H-imidazo [5,1-b][1,2,5]thiadiazin-4-one	Water	FAO clothianidin
	FA, Formamide	Water	FAO clothianidin
	MU, Methylurea	Water	FAO clothianidin
	MNG, NG-E, N-methyl-N-nitroguanidine	Soil, plants, mammals	PPDB 2013; Ford and Casida 2006a, b; FAO clothianidin
	MG, NG-F, Methylguanidine	Water, plants, mammals	Kim et al. 2012; Ford and Casida 2006a; FAO clothianidin
	NG-G, NTG, nitroguanidine	Mammals, soil, plants	Ford and Casida 2006a; FAO clothianidin
	DIN-dm, FNG, N-desmethyl dinotefuran, 2-nitro-1-(tetrahydro-3-furylmethyl) guanidine	Mammals, plants, soil (aerobic)	Ford and Casida 2006a; 2008; FAO dinotefuran
	DIN-NNO	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-dm-NNO	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-NNH2	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-dm-NNH2	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-NH, DN, 1-Methyl-3-(tetrahydro-3-furylmethyl)guanidine	Mammals, plants, water (photolysis), soil (anaerobic)	Ford and Casida 2006a, 2008; FAO dinotefuran; USEPA 2004b
Dinotefuran	DIN-dm-NH, 3-(tetrahydro-3-furylmethyl)guanidine	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-Urea, UF, 1-Methyl-3-(tetrahydro-3-furylmethyl)urea	Mammals, plants, soil (aerobic), water (hydrolysis+photolysis)	Ford and Casida 2006a, 2008; Rahman et al. 2013; FAO dinotefuran; USEPA 2004b
	DIN-dm-Urea, 3-(tetrahydro-3-furylmethyl)urea	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-2-OH	Mammals, plants, water (photolysis)	Ford and Casida 2006a; FAO dinotefuran; USEPA 2004b
	DIN-5-OH	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-4-OH	Mammals	Ford and Casida 2006a
	DIN-a, PHP, 1,3-diazinane aminocarbinal (derivative of DIN-2OH)	Mammals, plants	Ford and Casida 2006a; FAO dinotefuran
	DIN-b (derivative of DIN-dm)	Mammals	Ford and Casida 2006a
	DIN-e (guanidine derivative of DIN-a)	Mammals	Ford and Casida 2006a
	DIN-f (guanidine derivative fo DIN-b)	Mammals	Ford and Casida 2006a
	DIN-g (derivative of DIN-5-OH)	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-h (desmethyl DIN-g)	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-i (nitroso derivative of DIN-g)	Mammals, plants	Ford and Casida 2006a, 2008

Table 2 (continued)

Parent compound	Metabolites	Formation medium	Reference
Imidacloprid	DIN-j (nitroso derivative fo DIN-h)	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-k (guanidine derivative fo DIN-h)	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-l*, tetrahydrofuran carboxaldehyde, 3-Furfural	Mammals	Ford and Casida 2006a
	DIN-m, THFOL, tetrahydrofuran alcohol, 3-Furfuryl alcohol	Plants	Ford and Casida 2008
	DIN-n, THFCA, tetrahydrofuran-3-carboxylic acid	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-p, 4-hydroxy-tetrahydrofuran-3-carboxylic acid	Mammals, plants	Ford and Casida 2006a, 2008
	DIN-r, THFMA, tetrahydrofuran-3-yl-methylamine	Mammals, plants	Ford and Casida 2006a, 2008
	446-DO, 1-[4-hydroxy-2-(hydroxymethyl) butyl]-3-methyl-2-nitroguanidine	Mammals, plants	FAO dinotefuran
	DIN-3-OH	Mammals, plants, water (photolysis)	FAO dinotefuran; USEPA 2004b
	IMI-olefin, olefin derivative, 4, 5-dehydro-imidacloprid	Honeybee, housefly, drosophila, mice	Decourtye and Devillers 2010; Suchail et al. 2001; Nishiwaki et al. 2004; Sparks et al. 2012; Tomizawa and Casida 2003
	IMI-5-OH, 5-OH-imidacloprid, 5-hydroxy-imidacloprid, [(6-Chloro-3-pyridinyl) methyl]-4, 5-dihydro-2-(nitroamino)-1H-imidazol-5-ol	Honeybee, mice	Decourtye and Devillers 2010; Suchail et al. 2001; Tomizawa and Casida 2003
	IMI-de	Mice	Tomizawa and Casida 2003
	IMI-diol, 4,5-dihydroxy-imidacloprid	Honeybee, mice	Suchail et al. 2001; Tomizawa and Casida 2003
	IMI-NH, desnitro-imidacloprid	Honeybee, plants, mice	Suchail et al. 2001; Tomizawa and Casida 2003
Imidacloprid, Nitenpyram, Acetamiprid, Thiacloprid	IMI-urea, urea derivative, N-((6-Chloropyridin-3-yl)-methyl)-imidazolidinone	Honeybee, mice	Suchail et al. 2001; Tomizawa and Casida 2003
	6-CNA, 6-chloronicotinic acid	Animals, plants, soil	Suchail et al. 2001; Nishiwaki et al. 2004; Sparks et al. 2012; Ford and Casida 2008, 2006b; Casida 2011; Brunet et al. 2005; FAO acetamiprid; Lazic 2012; Tokieda et al. 1999; Phugare and Jadhav 2013; FAO thiacloprid
Nitenpyram	NIT-COOH	Mice	Ford and Casida 2008; Casida 2011
	NIT-deschloropyridine	Mice	Ford and Casida 2008; Casida 2011
	NIT-dm, N-desmethyl nitempyram	Mice	Ford and Casida 2008; Casida 2011
	NIT-CN	Mice	Ford and Casida 2008; Casida 2011
	NIT-deschloropyridine derivatives	Mice	Ford and Casida 2008; Casida 2011
Acetamiprid	Acetamiprid-D-desmethyl, N-desmethyl acetamiprid, IM-2-1, ACE-dm, N-(6-Chloro-3-pyridylmethyl)-N'-cyano-acetamidine	Animal, plants, soil (microbial)	FAO acetamiprid; Brunet et al. 2005; Casida 2011; Ford and Casida 2008; Chen et al. 2008; Wang et al. 2012; Wang et al. 2013a
	IM-1-3, N-[(6-chloro-3-pyridyl)methyl]-N-methylacetamide, ACE-acet, ACE-urea	Animal, plants, soil, water (hydrolysis)	Casida 2011; FAO acetamiprid; Brunet et al. 2005; Dai et al. 2010; Liu et al. 2011
	IM-2-3, N-[(6-chloro-3-pyridyl)methyl] acetamide, ACE-dm-acet	Mice, plants	Casida 2011; FAO acetamiprid
	IM-1-2, N2-carbamoyl-N1- [(6-chloro-3-pyridyl)methyl]-N1-methylacetamidine, ACE-NCONH2	Mice, plants, soil (microbial)	Casida 2011; FAO acetamiprid; Phugare and Jadhav 2013
	IM-2-2, N2-carbamoyl-N1- [(6-chloro-3-pyridyl)methyl]-acetamidine, ACE-dm-NCONH2	Mice, plants	Casida 2011; Ford and Casida 2008

Table 2 (continued)

Parent compound	Metabolites	Formation medium	Reference
Thiacloprid	IM-1-4, N-methyl(6-chloro-3-pyridyl)methylamine, N-methylpyridinylmethylamine	Animal (honeybees), plants, soil	Casida 2011; Ford and Casida 2006b; Brunet et al. 2005; FAO acetamiprid; Dai et al. 2010; Liu et al. 2011; Wang et al. 2013b; Tokieda 1999; Phugare and Jadhav 2013; Wang et al. 2013a
	IM-0, (6-chloro-3-pyridyl)methanol, CPOL	Animal (honeybees), plants	Brunet et al. 2005; FAO acetamiprid
	ACE-NH, descyano derivative	Plants, soil	Casida 2011; Wang et al. 2013a
	IM-2-5, N1-(6-Chloropyridin-3-ylmethyl)-acetamide, ACE-dm-NH	Animals	FAO acetamiprid
	IM-2-4, (6-chloro-3-pyridyl)methylamine, chloropyridinylmethylamine	Mice, plants	Casida 2011; Ford and Casida 2006a, 2008
	N-methylpyridinylmethylamine	Soil	Phugare and Jadhav 2013
	(E)-1-ethylideneurea	Soil	Phugare and Jadhav 2013
	ACE-w, N'-cyano-N-methylacetimidamide	Mice, plants	Casida 2011; Ford and Casida 2006b, 2008
	ACE-u, N'-cyanoacetimidamide	Mice, plants	Casida 2011; Ford and Casida 2006b, 2008
	THI-NH, M29, thiacloprid thiazolidinimine, 3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinimine, descyano derivative	Mice, plants, soil	Ford and Casida 2006b, 2008; FAO thiacloprid;
	THI-ole, M38, thiacloprid-olefin, {3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolylidene}cyanamide	Mice, plants	Ford and Casida 2006b, 2008; FAO thiacloprid;
	THI-ole-NH	Mice, plants	Ford and Casida 2006b, 2008
	THI-4-OH, 4-hydroxy-thiacloprid, {3-[(6-chloro-3-pyridinyl)methyl]-4-hydroxy-2-thiazolidinylidene}cyanamide	Animals, plants, soil (microbial)	Ford and Casida 2006b, 2008; FAO thiacloprid; Zhao et al. 2009
	Thiacloprid-amide, THI-NCONH2, 3-[(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene}urea, M02	Mice, plants, Soil (microbial)	Ford and Casida 2006b, 2008; FAO thiacloprid; Dai et al. 2010
	THI-4-OH-NCONH2, M37, {3-[(6-chloro-3-pyridinyl)methyl]-4-hydroxy-2-thiazolidinylidene}urea	Mice, plants	Ford and Casida 2006b, 2008; FAO thiacloprid; Casida 2011
	THI-SO	Mice, plants	Ford and Casida 2006b, 2008
	THI-SO3-H-NCONH2, Thiacloprid sulfonic acid, M30	Mice, plants, Soil	Ford and Casida 2006b, 2008; PPDB 2013; FAO thiacloprid
	THI-SOMe	Mice	Ford and Casida 2006b
Cycloxaprid	CYC-OH, hydroxy derivatives	Mice	Shao et al. 2013b
	CYC-(OH) ₂ , dihydroxy derivatives	Mice	Shao et al. 2013b
	CYC-NO, nitroso derivative	Mice	Shao et al. 2013b
	CYC-NH ₂ , amine derivative	Mice	Shao et al. 2013b
	NMI, nitromethylene imidazole	Mice	Shao et al. 2013b
	NMI-NO, nitroso derivative of NMI	Mice	Shao et al. 2013b

*not observed

thiamethoxam is almost as active as the insecticide imidacloprid (Karmakar et al. 2009). However, nitro reduction reverses the relative toxicity to insects and mammals, being a mechanism of detoxification for insects and bioactivation for mammals (Kanne et al. 2005; Honda et al. 2006; Casida 2011).

Thiamethoxam has been found to be a liver carcinogen in mice (*M. musculus*) (Green et al. 2005a, b; Tomizawa and

Casida 2005). Green et al. (2005a, b) proposed that TMX-dm may be a hepatotoxicant. This suggests that contrary to initial ideas, neonicotinoids may significantly affect the health of vertebrates including humans. A detailed review of such effects is, however, outside the scope of the present review.

Further steps in the metabolism pathway involve either phase I metabolites (N-methylene and C-methylene

Table 3 First-generation metabolites of fipronil in various media and organisms. Metabolites known to be active toward invertebrates or mammals are highlighted in bold

Parent compound	Metabolites	Formation medium	Reference
Fipronil	Fipronil detrifluoromethylsulphinyl, 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl) pyrazole, MB 45897	Mammals, soil, plants (photolysis)	FAO fipronil, Hainzl and Casida 1996; France 2005
	Fipronil-sulfide, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)thio]-1H-pyrazole-3-carbonitrile, MB45950	Mammals, soil, plants, water (photolysis)	FAO fipronil; Bobé et al. 1998; Aajoud et al. 2003; France 2005; Gunasekara et al. 2007
	Fipronil-sulfone, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole-3-carbonitrile, MB 46136	Mammals (milk), hens (eggs), soil, plants, water (incl. photolysis)	Hainzl and Casida 1996; Hainzl et al. 1998; Bobé et al. 1998; FAO fipronil, Tingle et al. 2003; Aajoud et al. 2003; France 2005
	Fipronil-desulfinyl, desthiofipronil, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)]-1H-pyrazole-3-carbonitrile, MB 46513	Soil, plants, water (photolysis)	Hainzl and Casida 1996; Hainzl et al. 1998; Bobé et al. 1998; FAO fipronil; Tingle et al. 2003; Aajoud et al. 2003; Gunasekara et al. 2007
	5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-pyrazole-4-sulfonic acid, RPA104615	Soil, water (photolysis)	Tingle et al. 2003; FAO fipronil
	5-amino-3-carbamyl-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfonylpyrazole, RPA105320	Soil, plants	FAO fipronil
	Fipronil-amide, 5-amino-3-carbamyl-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinylpyrazole, RPA 200766	Mammals, soil, plants, water (hydrolysis)	Bobé et al. 1998; Tingle et al. 2003; Aajoud et al. 2003; FAO fipronil
	5-amino-3-carbamyl-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinylpyrazole-3-carboxylic acid, RPA 200761	Mammals, soil, plants, water	FAO fipronil; France 2005
	Various conjugates in urine and bile (RPA 105048, UMET/10, UMET/3, FMET/9, UMET/4, FMET/7, FMET/10, UMET/15)	Mammals	FAO fipronil; France 2005
	MB 46400	Mammals, hens (eggs)	FAO fipronil; France 2005
	RPA 108058	Mammals, hens (eggs)	FAO fipronil
	Ring-opened 106889	Mammals, hens (eggs)	FAO fipronil
	RPA 106681	Soil	FAO fipronil

hydroxylation) leading to a wide range of nitroguanidine (NG) and chlorothiazolymethyl (CTM) cleavage products or oxidation to the urea derivatives (TMX-Urea, TMX-dm. Urea, CLO-Urea, CLO-dm-Urea) or phase II metabolites by adding pyruvate to give the methyltriazinones (TMX-dm-tri, CLO-tri, and CLO-dm-tri) (Chen et al. 2005; Ford and Casida 2006a).

While all CTM cleavage products are in common between thiamethoxam and clothianidin, only some NG cleavage

products are in common between the two insecticides (methylnitroguanidine (NG-E), methylguanidine (NG-F), and other NG compounds) (Yokota et al. 2003; Ford and Casida 2006a; Kim et al. 2012). Other NG metabolites are specific to thiamethoxam (NG-A, NG-B, NG-C, and NG-D). These compounds may continue their metabolism leading to a wide range of breakdown products.

Most of the metabolites of thiamethoxam and clothianidin are observed not only in small mammals, such as mice and

rats, but also in dogs and hens (USEPA 2000; Klein 2003; USEPA 2003b; Yokota et al. 2003; USEPA 2004a; Ford and Casida 2006a; Kim et al. 2012).

Dinotefuran differs from TMX and CLO by its tetrahydrofuranyl moiety instead of the chlorothiazolyl part. As for thiamethoxam and clothianidin, the principal metabolic pathways of dinotefuran (hereafter also DIN) in mammals involve N-demethylation, nitro reduction, and N-methylene hydroxylation accompanied by amine cleavage (Ford and Casida 2006a; Casida 2011). Common metabolites have been described (NG-E, NG-F, and other NG compounds) (FAO dinotefuran). The metabolism of dinotefuran differs from that of clothianidin and thiamethoxam by the ready hydroxylation and metabolism of the tetrahydrofuranyl moiety. The pharmacokinetics of dinotefuran are characterized by a rapid metabolism and excretion probably associated with its high polarity and fast metabolism of the hydrofuranyl moiety (Ford and Casida 2006a). As a result, DIN metabolites follow a similar pattern than those of TMX and CLO (DIN-dm, DIN-NNO, DIN-dm-NNO, DIN-NNH₂, DIN-dm-NNH₂, DIN-NH, DIN-dm-NH) and urea derivatives. Phase II metabolism, with pyruvate addition, produces methyltriazinones (DIN-tri and DIN-dm-tri) (Ford and Casida 2006a; Casida 2011). As already observed for thiamethoxam and clothianidin, the nitro reduction pathway causes a shift from insect-selective to vertebrate-selective action (Kanne et al. 2005; Honda et al. 2006; Casida 2011).

The tetrahydrofuran group may undergo metabolism including hydroxylation at 2, 5, and 4 positions, ring opening, N-acetylation, N-demethylation or nitro reduction (Ford and Casida 2006a).

Most of the metabolites are observed in both small mammals such as mice and rats but also in dogs and hens (Ford and Casida 2006a; USEPA 2003c; USEPA 2004b). Hydrolysis of the tetrahydrofuran ring to form 1-[4-hydroxy-2-(hydroxymethyl) butyl]-3-methyl-2-nitroguanidine (446-DO) has also been reported (FAO dinotefuran).

Plants

Clothianidin metabolism in plants has been evaluated in a variety of crops, including maize, sugar beet, fodder beet, apples, and tomatoes (EFSA 2010). Metabolism of thiamethoxam has been evaluated in maize, rice, pears, cucumbers, lettuce, and potatoes (FAO thiamethoxam). The plant enzymes responsible for the conversion of thiamethoxam and clothianidin into their metabolites have not been examined so far (Ford and Casida 2008).

Phase I metabolites in spinach, maize, and sugar beet were remarkably similar to those observed in small mammals (Chen et al. 2005; Ford and Casida 2006a, 2008), with the main metabolic pathways proceeding through N-demethylation and nitro reduction (FAO thiamethoxam; Ford and Casida 2008).

Thiamethoxam is rapidly metabolized to clothianidin in cotton plants, while TMX-dm is not significantly produced (Karmakar et al. 2009). EFSA (2010) describes clothianidin as being metabolized extensively in the leaves predominantly leading to CLO-NH and NG-F (Kim et al. 2012). Clothianidin is oxidatively cleaved in plants to the carboxylic acid derivative, among other metabolites and cleavage products (Ford and Casida 2008; Ford et al. 2010; FAO clothianidin). In spinach, thiamethoxam, clothianidin, and their N-demethylated products form nitrosoguanidine, guanidine, and urea derivatives (Ford and Casida 2008; FAO thiamethoxam; FAO clothianidin). Conjugated products from thiamethoxam and clothianidin have not been observed in spinach and neither have methylthio derivatives (Ford and Casida 2008). Contrary to the metabolism in mammals, clothianidin undergoes hydroxylation at the inner guanidine nitrogen atom leading to the N-OH derivative (N-2-chlorothiazol-5-ylmethyl-N'-hydroxy-N'-methyl-N''-nitroguanidine, THMN) followed by glycosylation (phase II metabolism) in maize, apple, and sugarbeet (FAO clothianidin).

Metabolism of dinotefuran in plants is similar to that in mammals, leading mainly to methylguanidine, nitroguanidine, and urea metabolites (Ford and Casida 2008; Casida 2011; Rahman et al. 2013; FAO dinotefuran). As for clothianidin, N-methylene hydroxylation yields either tetrahydrofurylmethylamine (THFMA/DIN-r), which could be further metabolized through phase I (e.g., N-acetylation, oxidation, reduction...) and/or phase II (glucoside derivative) reactions (Ford and Casida 2008). In plants, internal ring formation yields 6-hydroxy-5-(2-hydroxyethyl)-1-methyl-1,3-diazinane-2-ylidene-N-nitroamine (PHP). NG-E and NG-F are observed as major cleavage products (Ford and Casida 2008; FAO dinotefuran).

Water

In water, thiamethoxam is stable to hydrolysis in dark conditions at pH 1–7 (De Uderzo et al. 2007) while it is quickly hydrolyzed at pH 9 and 20 °C (European Commission 2006) and almost completely degraded (ca. 96 %) under UV radiation in about 10 min (De Uderzo et al. 2007). The main hydrolysis products are identified: TMX-Urea, clothianidin and its derivatives (N-(2-chlorothiazol-5-ylmethyl)-N'-nitrourea (CTNU), CTM-i, methylurea (MU), and NG-B) (FAO thiamethoxam).

Conversely, De Uderzo et al. (2007) proposed a photodegradation mechanism of thiamethoxam to form the guanidine derivatives (TMX-NH), with a loss of HNO₃. After that, a nucleophilic substitution of the Cl with OH in the thiazolic ring could occur, which then quickly decomposes to 5-methyl-2(3H)-thiazolone and NG-F (De Uderzo et al. 2007). 5-Methyl-2(3H)-thiazolone could in turn decompose to volatile products, such as carbonyl sulfide and isocyanic acid, already observed by Schwartz et al. (2000). Other observed photodegradation products include an oxazine derivative,

possibly formed by extrusion of S to generate an azetidinone intermediate, and an acrylonitrile derivative from hydrolysis of the imine group of the oxazol ring (De Uderzo et al. 2007).

No peer-reviewed literature could be found concerning clothianidin breakdown in water. However, the FAO mentions that this compound degrades by hydrolysis and/or photolysis into CLO-Urea, with further cleavage to methylurea (MU) and 2-chlorothiazol-5-yl-methylamine (ACT), (FAO clothianidin). Clothianidin could also be hydrolyzed to the nitro urea derivative (CTNU) and further cleaved into ACT. Nitro reduction, cleavage at the methylene bridge or complex cyclization reaction accompanied by loss of nitro group, chlorine elimination, and desulphuration convert the parent compound into CLO-NH, NG-F and forms 7-methylamino-4H-imidazo[5,1-b][1,2,5]thiadiazin-4-one (MIT). Successively, ring cleavage forms 2-methylamino-2-imidazolin-5-one (MIO), 4-hydroxy-2-methylamino-2-imidazolin-5-one (HMIO), NG-F and formamide (FA) with a final mineralization to carbon dioxide (FAO clothianidin).

Hydrolysis of dinotefuran in dark conditions and alkaline pH produces DIN-Urea. Photolysis on surface water produces DIN-Urea, DIN-NH, DIN-2-OH, and DIN-3-OH (USEPA 2004b).

Soil

No peer-reviewed literature could be found concerning thiamethoxam breakdown in soil. However, the FAO provides some information on this regard (FAO thiamethoxam). The metabolic pathways of thiamethoxam in soil, under aerobic conditions, lead to the conversion of TMX into CLO, which then is degraded to CLO-NH and CLO-Urea. CLO-dm is also observed as a degradation product. Nitro reduction of the parent compound also occurs, which finally forms TMX-Urea. The intermediate TMX-NH has been observed only in rice-paddies so far. NG-A cleavage product, from N-methylene hydroxylation, has also been observed as a major product in soil (FAO thiamethoxam). The main metabolite formed in anaerobic conditions is TMX-NH but TMX-Urea has been also observed (European Commission 2006).

The aerobic degradation of clothianidin in soil proceeds through three main pathways. The first pathway starts with N-demethylation of clothianidin to form CLO-dm and N-methylene hydroxylation to form nitroguanidine (NG-G). The second pathway starts with the N-methylene hydroxylation to form NG-F and proceeds through N-demethylation to form NG-G. A third route involves the formation of CLO-Urea via nitro reduction (FAO clothianidin). The metabolisation of clothianidin further progresses to carbon dioxide.

In soil incubated under aerobic conditions in the dark at 20 °C, dinotefuran degraded to NG-E and NG-F as major degradation products. Other minor observed metabolites were DIN-Urea and DIN-dm (FAO dinotefuran). Dinotefuran and its metabolites are further mineralized to carbon dioxide. It has been also found that

photolysis is not a significant degradation pathway of dinotefuran in soil (FAO dinotefuran). DIN-NH has been observed in soil under anaerobic conditions (USEPA 2004b).

Imidacloprid and nitenpyram

Animals (and plants)

The metabolic pathways of neonicotinoids present many similarities between insects and plants. In the honey bee, imidacloprid (hereafter also IMI) is transformed mainly to olefin, 5-hydroxy-imidacloprid (5-OH-imidacloprid), 4,5-dihydroxy-imidacloprid, desnitro-imidacloprid, urea derivative, and 6-chloronicotinic acid (6-CNA). Among these metabolites, olefin and 5-OH-imidacloprid exhibit toxicity both in acute and chronic exposures (Suchail et al. 2001). Thus, the biotransformation of imidacloprid leads to a metabolic activation and to the concentration of the toxic metabolites in the brain and thorax of the honey bee for more than 96 h (Suchail et al. 2004a, b). This results in a metabolic relay, in which imidacloprid induces first toxicity and then the toxic metabolites act in bees surviving the early action of imidacloprid. This leads to a lethal phenomenon that lasts more than 96 h, contrary to the other neurotoxic insecticides for which the maximum mortality rate is generally observed between 10 and 24 h (Suchail et al. 2001). The metabolism of imidacloprid is very similar in bees and flies with hydroxylated imidacloprid derivatives, olefin, 6-CNA, and the imidazoline moiety as main metabolites in the housefly and drosophila (Nishiwaki et al. 2004; Sparks et al. 2012). This suggests that insects may exhibit close neonicotinoid metabolic pathways. Thus, metabolic activation and sensitivity to certain plant metabolites might be a common feature in insects. That could be the reason for which the conserved toxicity profiles have been depicted in bees and in flies after chronic exposure to concentrations three to five orders of magnitude lower than LC₅₀ (Charpentier et al. 2014).

Much of the use of neonicotinoids takes advantage of the systemic properties of the active substances and involves plant treatments by seed dressing. As a result, humans and animals are exposed through consumption of vegetables containing neonicotinoid active substances taken up by plants, and their metabolites. Exposure through food should be taken into account, since studies have shown that nicotine and nicotine derivatives, such as the neonicotinoids imidacloprid, acetamiprid, and clothianidin, can be rapidly and efficiently absorbed through the intestine barrier (Yokota et al. 2003; Brunet et al. 2004; Brunet et al. 2008). Moreover, seven metabolites of these neonicotinoids have been found in human urine of sick patients (Taira et al. 2013). Among plant metabolites, desnitro-imidacloprid is of particular interest because it displays high toxicity to vertebrates associated with an agonist action on the $\alpha 4\beta 2$ nAChRs (Chao and Casida 1997; D'Amour

and Casida 1999; Tomizawa and Casida 2000; Tomizawa et al. 2001a). Desnitro-imidacloprid is also able to activate intracellular calcium mobilization and the extracellular signal-regulated kinase cascade through its interaction with the nAChR (Tomizawa and Casida 2002). In mice, imidacloprid is biotransformed into IMI-de, IMI-olefin, IMI-NH (desnitro-imidacloprid), IMI-urea, IMI-urea-gluc, IMI-urea-gent, IMI-diol, IMI-diol-gluc, IMI-5-OH, IMI-5-OH-gluc, IMI-NNO, 6-CNA and different imidazoline and pyridinyl derivatives. IMI-NH is generated by the action of cytochromes P450 on imidacloprid (Tomizawa and Casida 2003). The appearance of this metabolite can be considered a bioactivation, since IMI-NH exhibits toxicity to mammals due to its ability to bind to $\alpha 4\beta 2$ nAChR (Chao and Casida 1997; D'Amour and Casida 1999; Tomizawa and Casida 2000; Tomizawa et al. 2001a; Tomizawa and Casida 2003, 2005).

However, desnitro-imidacloprid is a detoxification derivative in insects. The 6-CNA is a metabolite common to chloropyridinyl neonicotinoids (Ford and Casida 2008; Casida 2011). Thus, the risk posed by 6-CNA to the honey bee might be common to the use of imidacloprid, thiacloprid, acetamiprid, and nitenpyram.

Nitenpyram (hereafter also NIT) is metabolized in mice into NIT-COOH, NIT-deschloropyridine, NIT-dm (N-desmethyl nitenpyram), NIT-CN, and different NIT-deschloropyridine derivative (Ford and Casida 2008; Casida 2011). The NIT metabolites have not been subjected to in-depth toxicological investigations. These metabolites can undergo an oxidation of the cyano group into a carboxylic acid (Ford and Casida 2008; Casida 2011).

Soil and water

Further to metabolites described for plants and animals, desnitro-olefin, 2,5 diketone, carbene dioxide, and 6-hydroxynicotinic acid have been described in soil (FAO imidacloprid).

Acetamiprid and thiacloprid

Animals

In mammals, acetamiprid (hereafter also ACE) undergoes a rapid and efficient intestinal absorption (Brunet et al. 2008). As for the other neonicotinoids, N-demethylation is the main metabolism pathway for acetamiprid and thiacloprid (hereafter also THI). In insects, acetamiprid undergoes a rapid biotransformation, which signals a high metabolic activity, being metabolized into IM2-1 (ACE-dm), IM1-3 (ACE-urea), IM1-4 (N-methyl-chloropyridinylmethylamine), IM0 (6-chloropicolyl alcohol), IC0 (6-CNA) and two unknown metabolites (Brunet et al. 2005; Ford and Casida 2006a; Casida 2011). The metabolite 6-CNA remains stable for more than

72 h in all biological compartments, except gut-free abdomen, which could explain the toxicity of acetamiprid (Brunet et al. 2005). Thiacloprid is transformed into THI-NH, THI-ole, THI-ole-NH (putative), THI-4-OH, THI-NCONH₂, THI-4-OH-NCONH₂, THI-SO, THI-SO₃H-NCONH₂, and THI-SMe (Ford and Casida 2006b; Casida 2011). Descyano-thiacloprid (THI-NH) is generated by the action of cytochromes P450 on thiacloprid in vivo (Tomizawa and Casida 2003, 2005). As for imidacloprid and desnitro-imidacloprid, the appearance of THI-NH can be considered as thiacloprid bioactivation because THI-NH exhibits a toxicity to mammals in relation with its ability to bind to $\alpha 4\beta 2$ nicotinic acetylcholine receptors (Chao and Casida 1997; D'Amour and Casida 1999; Tomizawa and Casida 2000; Tomizawa et al. 2001a; Tomizawa and Casida 2003, 2005). In insects, THI-NH is instead a detoxification metabolite.

Plants

As seen for the other neonicotinoids, metabolism of acetamiprid and thiacloprid is similar in plants and mammals. Acetamiprid metabolism involves several initial sites of attack: N-demethylation, cyano hydrolysis, cleavage of 6-CNA. Additionally, cleavage of N-CN linkage from acetamiprid, which yields the N-descyano compound (ACE-NH) also occurs (Ford and Casida 2008; Casida 2011).

Thiacloprid metabolism involves five different sites of attack: cyano hydrolysis (THI-NCONH₂), sulfoxidation (THI-SO, THI-SO₃H-NCONH₂), hydroxylation at the 4-position (THI-4-OH, THI-4-OHNCONH₂), conversion to the olefin (THI-ole) and loss of the cyano group (THI-NH, THI-ole-NH). The urea derivative (THI-4-OHNCONH₂) and THI-SO were the major metabolites observed (Ford and Casida 2008; Casida 2011).

Soil and water

Acetamiprid is stable to hydrolysis and photolysis, the main metabolite in soil being IM1-4 (FAO acetamiprid; Dai et al. 2010; Liu et al. 2011; Wang et al. 2013a; Wang et al. 2013b). Minor metabolites are ACE-urea and 6-CNA (FAO acetamiprid; Dai et al. 2010; Liu et al. 2011). Biotransformation of acetamiprid produces the N-demethylated derivative (Chen et al. 2008; Wang et al. 2012). Recently, Phugare and Jadhav (2013) evidenced the formation of ACE-NCONH₂ from microbial degradation in soil, which is then cleaved to N-methylpyridinylmethylamine and (E)-1-ethylideneurea with further oxidative cleavage to 6-CNA.

Thiacloprid is stable to hydrolysis (95–98 % recovery after 30 days). It can be degraded to THI-NCONH₂ in soil in both light and dark conditions (FAO thiacloprid), which can be further transformed into THI-NH and THI-SO₃-H-NCONH₂.

Cis-neonicotinoids and new-generation insecticides

Cycloxaprid, paichongding, imidaclothiz, and sulfoxaflor are newly developed neonicotinoid-like insecticides. Paichongding and cycloxaprid are *cis*-neonicotinoids (Li et al. 2011; Shao et al. 2011; Cui et al. 2012), imidaclothiz is a nitroguanidine thiazole neonicotinoid (Wu et al. 2010), and sulfoxaflor is a sulfoximine insecticide, whose insecticidal activity could be closely related to its very high efficacy at nAChRs (Watson et al. 2011). However, only a few studies have been published on the metabolism of these new substances in insects and mammals.

Animals

Cycloxaprid (hereafter also CYC) metabolism has been investigated in mice (Shao et al. 2013b). Five monohydroxy (CYC-OH) and one dihydroxy (CYC-(OH)₂) metabolites have been characterized, along with compounds resulting from modification of the NO₂ group into nitroso and amine derivatives (CYC-NO and CYC-NH₂, respectively). The next more abundant product was nitromethylene imidazole (NMI) and its NO derivative (NMI-NO). When they bind to housefly (*M. domestica* L.) head membranes, NMI and CYC exhibit dissociation constants of 1.1 and 28 nM, respectively. This indicates that, as imidacloprid, the degradation of CYC generates toxic metabolites with high affinity for receptors. As a result, metabolites could prolong their toxic effects. Should these metabolites be found on plants, insect exposure could occur.

Sulfoxaflor metabolism has been investigated in vitro on drosophila D.mel-2 cells transfected with CYP6G1 (Sparks et al. 2012). Compared to imidacloprid, acetamiprid, dinotefuran, thiamethoxam, and clothianidin for which the extents of metabolism are respectively 85.1, 95.5, 55.1, 46.8, and 45.6 % after 24 h, sulfoxaflor presents an almost undetectable metabolism. These results could explain the absence of cross-resistance to sulfoxaflor in insects resistant to neonicotinoids or other insecticides. However, because sulfoxaflor metabolism has been investigated only with CYP6G1, the extrapolation of the least metabolic susceptibility to the whole drosophila metabolism is difficult.

Fipronil

Animals

In mammals, fipronil can be metabolized at its trifluoromethylsulfinyl or cyano moieties through three major pathways: (1) oxidation at the sulfinyl moiety to form fipronil-sulfone; (2) reduction at the sulfinyl moiety yielding fipronil-sulfide; and (3) by hydrolysis of the cyano moiety to form fipronil-amide followed by further hydrolysis to the

corresponding carboxylic acid (5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinyl pyrazole-3-carboxylic acid) (France 2005).

Metabolism in rats has proved to be independent of dose level, regime, and sex (France 2005). In the rat, two urinary metabolites have been identified following deconjugation with glucuronidase and sulfatase, leading to pyrazole ring-opened compounds. Other compounds can be found in urine as the derivatives fipronil-amide, fipronil-sulfone, and fipronil-sulfide, and the metabolite of fipronil-sulfone, defluoromethylsulfinyl-fipronil (France 2005; FAO fipronil). Fipronil itself can also be found in urine. Fipronil-sulfone is the major metabolite and often the only one found in the tissues of the species examined: fat, adrenal gland, pancreas, skin, liver, kidney, muscle, thyroid, and ovaries and uterus, as well as in foodstuffs: milk and eggs (FAO fipronil). Fipronil, and its amide, sulfone, and sulfide derivatives are the main compounds recovered from fat tissues, consistently with their lipophilic nature. Fipronil and its amide, sulfone, and sulfide derivatives are the main components found in feces, together with seven other metabolites found at minimal quantities. At least 16 different derivatives are present in bile, including the fipronil-carboxylic acid metabolite (FAO fipronil).

Experiments on rats, goats, and hens with the photolytic metabolite of fipronil, desulfinyl-fipronil, yield numerous urinary metabolites mainly as a result of phase II metabolism. These metabolites result from the metabolism of radicals of the pyrazole ring different from the trifluoromethylsulfinyl or cyano moieties. Among others, the following have been described: (1) N-sulfate conjugate of desulfinyl-fipronil, (2) two amino acid conjugates resulting from the action of deconjugating enzymes glucuronidase and sulfatase followed by acidic hydrolysis, (3) 5-aminoglucuronide conjugate, (4) 5-(*N*-cysteinyl) conjugate of fipronil-desulfinyl, and (5) a 4-cyano- 5-(*N*-cysteinylglycine) conjugate, (4) and (5) linked through the cysteine residue. Metabolization of desulfinyl-fipronil leads to the amide derivative, 4-cyano-5-(*N*-cysteinyl) derivative, which in turn may result in the 4-carboxylic acid-fipronil (Totis 1996 in FAO fipronil). Ring-opened conjugates were observed in goat's liver (Johnson et al. 1996 in FAO fipronil).

Plants

Translocation studies carried out with [¹⁴C]fipronil on maize, sunflower, and sugar beet show uptake of about 5 %. Fipronil could be co-formulated with numerous polymers in order to enhance the systemicity of this active substance (Dieckmann et al. 2010c). Studies carried out in potatoes, rice, sunflower, sugar beet, cabbage, cotton, maize, showed metabolism of the mother compound in plants via hydrolysis to amide-fipronil, oxidation to the sulfone-fipronil and reduction to the sulfide-fipronil. Foliar application was also subject of photodegradation to desulfinyl-fipronil. Fipronil-sulfone can

undergo photolysis resulting in sulfonic acid (Roberts and Hutson 1999). This molecule may be target of cleavage and loss of the sulfone moiety, resulting in detrifluoromethylsulfinyl-fipronil. A carboxylic derivate of fipronil can be produced from the hydrolysis of the radical CONH_2 of fipronil-amida (FAO fipronil).

Residues of fipronil, fipronil-amida, fipronil-sulfone, and fipronil-carboxylic acid, as well as minor undetermined derivatives, have been found in boll components following seed dressing in cotton (France 2005). Fipronil and its desulfinyl and sulfone derivatives have been found in pollen loads and honey (Bonmatin et al. 2007; Chauzat et al. 2011).

Soil and water

Fipronil degrades in water and soil through various metabolic pathways: (1) hydrolysis to the amide metabolite; (2) oxidation to fipronil-sulfone; and (3) reduction to fipronil-sulfide, mainly under anaerobic conditions (Raveton et al. 2007). Photolysis may also occur, leading to desulfinyl-fipronil and other aniline derivatives (Raveton et al. 2006). A minor photoproduct both in water and soil surfaces is sulfonic acid. In aqueous surfaces, fipronil has proved to be stable in dark conditions. However, pH is a relevant factor determining metabolism. Hydrolysis kinetics at different pH values differ from half-lives of 770 h at pH 9 to 2.4 h at pH 12. Fipronil remains stable under acid (pH 5.5) and neutral conditions (Bobé et al. 1998). An amide derivate of the fipronil-sulfone can be present following hydrolysis or the cyano moiety (FAO fipronil), which can be further hydrolyzed rendering a carboxylic acid derivate. Photolysis of fipronil-sulfone results in the production of sulfonic acid. Fipronil-sulfide can follow hydrolyzes of its cyano moiety leading to a carboxylic acid derivate.

Detrifluoromethylsulfinyl-fipronil can appear in soil following cleavage of the trifluoromethylsulfinyl moiety (FAO fipronil).

Adsorption and leaching studies carried out in laboratory show that fipronil and its main metabolites are slightly mobile in soil (IUPAC 2014).

Conclusion

This paper summarizes some of the key reasons for the success of neonicotinoids and fipronil and documents their rapidly expanding share of the global insecticide market in the last 25 years. Their physicochemical characteristics (extensively covered in Bonmatin et al. (2014)), especially in terms of water solubility, pK_a , and K_{ow} s confer systemic properties enabling them to be absorbed and translocated within all plant tissues. They are persistent (e.g., imidacloprid half-life in soil is ca. 6 months) and neurotoxic. Neonicotinoids share greater affinity

toward arthropod nACh receptors than toward those of mammals and other vertebrates. Fipronil acts on insect specific receptors. This makes them highly efficient insecticides with reduced operator and consumer risk compared to some of their predecessors such as organophosphorous and carbamate insecticides. Furthermore, their mode of action enables new strategies for pest control that profit from the existing synergies between these substances and either other chemicals or micro-organisms. As a result, there are a wide range of uses available, including seed coating and root bathing, as invertebrate pest control in agriculture, horticulture, orchards, forestry, veterinary applications, and fish farming. However, these same properties have led to problems. Specifically, their widespread (Main et al. 2014) and prophylactic use, their systemic properties in plants, their broad spectrum of toxicity in invertebrates, and the persistence and environmental fate of parent compounds and metabolites renders them potentially harmful to a broad range of non-target organisms. Subsequent papers in this review of the global literature explore different aspects of these risks. Pisa et al. (2014) and Gibbons et al. (2014) extensively cover the potential impacts on non-target invertebrates and vertebrates, respectively. Chagnon et al. (2014) explore the risks of their large scale of use to ecosystem functioning and services. These papers show a growing body of evidence that persistent, low concentrations of these insecticides pose serious risks of undesirable environmental impacts (Tennekes and Sánchez-Bayo 2011; Roessink et al. 2013), and therefore the sustainability of the current heavy reliance upon these compounds is questionable considering the availability of existing alternative agricultural and forestry practices (Furlan and Kreutzweiser 2014).

Notes

Authors are in the alphabetic order with the exception of the corresponding author. They declare no competing conflict of interest. All authors are working for public agencies or academic institutions, except NSD working both for the Utrecht University and the technical center CARI (mainly supported by public funds), VAR is employed by Buglife (a UK charity devoted to the conservation of invertebrates), DWG is employed by RSPB (a UK nature conservation charity), and DN is financed by the Stirling Triodos Foundation.

Acknowledgments This manuscript benefited from the discussions in the International Task Force on Systemic Pesticides during its plenary meetings in Paris (2010), Bath (2011), Cambridge (2012), Montegrotto (Padova, 2012), Louvain-la-Neuve (2013), and Legnaro (Padova, 2013). The work has been funded by the Triodos Foundation's Support Fund for Independent Research on Bee Decline and Systemic Pesticides. This Support Fund has been created from donations by Adessium Foundation (The Netherlands), Act Beyond Trust (Japan), Universiteit Utrecht (The Netherlands), Stichting Triodos Foundation (The Netherlands), Gesellschaft fuer Schmetterlingsschutz (Germany), M.A.O.C. Gravin van Bylandt Stichting (The Netherlands), Zukunft Stiftung Landwirtschaft

(Germany), Study Association Storm (Student Association Environmental Sciences Utrecht University), Bee Life - European Beekeeping Coordination (Belgium), Deutscher Berufs- und Erwerbsimkerbund e.V. (Germany), Gemeinschaft der europäischen Buckfastimker e.V. (Germany), and citizens. Contributions of J. Settele and M. Wiemers were part of www.legato-project.net (funded by the BMBF, German Ministry for Education and Research). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Aajoud A, Ravanel P, Tissut M (2003) Fipronil metabolism and dissipation in a simplified aquatic ecosystem. *J Agric Food Chem* 51:1347–1352
- Abbas N, Khan HA, Shad S (2014) Cross-resistance, genetics, and realized heritability of resistance to fipronil in the house fly, *Musca domestica* (Diptera: Muscidae): a potential vector for disease transmission. *Parasitol Res* 113(4):1343–1352. doi:10.1007/s00436-014-3773-4
- Alyokhin A, Dively G, Patterson M, Castaldo C, Rogers D, Mahoney M, Wollam J (2007) Resistance and cross-resistance to imidacloprid and thiamethoxam in the Colorado potato beetle. *Pest Manag Sci* 63: 32–41
- Alyokhin A, Baker M, Mota-Sanchez D, Dively G, Grafius E (2008) Colorado potato beetle resistance to insecticides. *Am J Potato Res* 85:395–413. doi:10.1007/s12230-008-9052-0
- Apenet (2009) Effects of coated maize seed on honey bees. Report based on results obtained from the first year activity of the APENET project. Accessible at: <http://www.reterurale.it/apenet>
- Apenet (2010) Effects of coated maize seed on honey bees. Report based on results obtained from the second year (2010) activity of the APENET project. Accessible at: <http://www.reterurale.it/apenet>
- Apenet (2011) Effects of coated maize seed on honey bees. Report based on results obtained from the third year (2011) activity of the APENET project. Accessible at: <http://www.reterurale.it/apenet>
- Azzam S, Yang F, Wu JC, Geng J, Yang GQ (2011) Imidacloprid-induced transference effect on some elements in rice plants and the brown planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae). *Insect Sci* 18:289–297
- Babcock JM, Gerwick CB, Huang JX, Loso MR, Nakamura G, Nolting SP, Rogers RB, Sparks TC, Thomas J, Watson GB, Zhu Y (2011) Biological characterization of sulfoxaflor, a novel insecticide. *Pest Manag Sci* 67:328–334
- Balança G, de Visscher MN (1997) Effects of very low doses of fipronil on grasshoppers and non-target insects following field trials for grasshopper control. *Crop Prot* 16:553–564
- Barbara GS, Zube C, Rybak J, Gauthier M, Grünwald B (2005) Acetylcholine, GABA and glutamate induce ionic currents in cultured antennal lobe neurons of the honeybee, *Apis mellifera*. *J Comp Physiol A* 191:823–836
- Barbara G, Grünwald B, Paute S, Gauthier M, Raymond-Delpech V (2008) Study of nicotinic acetylcholine receptors on cultured antennal lobe neurones from adult honeybee brains. *Invert Neurosci* 8:19–29
- Barbee GC, Stout MJ (2009) Comparative acute toxicity of neonicotinoid and pyrethroid insecticides to non-target crayfish (*Procambarus clarkii*) associated with rice-crayfish crop rotations. *Pest Manag Sci* 65:1250–1256
- Bayer CropScience (2006) Confidor with Stress Shield inside. Courier - The Bayer CropScience Magazine for modern agriculture 2/06
- Bayer CropScience (2011) <http://www.bayercropscience.com/bcsweb/cropprotection.nsf/id/FactsFigures>. Visited 3 March 2011
- Bayer Healthcare (2011) <http://www.bayerhealthcare.com/scripts/pages/en/company/products/index.php>
- Beketov MA, Liess M (2008) Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. *Environ Toxicol Chem* 27:461–470
- Benizdane Y, Touinsi S, Motte E, Jadas-Hécart A, Communal P-Y, Leduc L, Thany SH (2010) Effect of thiamethoxam on cockroach locomotor activity is associated with its metabolite clothianidin. *Pest Manag Sci* 66:1351–1359
- Bergkvist P (2011) Chemical properties, toxicity and regulatory issues on neonicotinoids in Sweden and Europe. Accessible at: http://www.slu.se/PageFiles/103909/Peter_Bergkvist%20x.pdf. Accessed 22 Oct 2012
- Bobé A, Meallier P, Cooper JF, Coste CM (1998) Kinetics and mechanisms of abiotic degradation of fipronil (hydrolysis and photolysis). *J Agric Food Chem* 46(7):2834–2839
- Bonmatin J-M, Marchand PA, Cotte JF, Aajoud A, Casabianca H, Goutailler G, Courtiade M (2007) Bees and systemic insecticides (imidacloprid, fipronil) in pollen: subnano-quantification by HPLC/MS/MS and GC/MS. In: Del Re AAM, Capri E, Fragoulis, Trevisan M (eds) Environmental fate and ecological effects of pesticide. La Goliardica Pavese, Pavia, (It), pp. 827–824. <http://www.cabdirect.org/abstracts/20083103467.html;jsessionid=8EE58D309B91521CB0CFECD7D2568525>. Accessed 21 June 2014
- Bonmatin J-M, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, et al (2014) Environmental fate and exposure; neonicotinoids and fipronil. *Environ Sci Pollut Res*:1–33. doi:10.1007/s11356-014-3332-7
- Bordereau-Dubois B, List O, Calas-List D, Marques O, Communal P-Y, Thany SH, Lapié B (2012) Transmembrane potential polarization, calcium influx and receptor conformational state modulate the sensitivity of the imidacloprid-insensitive neuronal insect nAChR to the neonicotinoid insecticides. *J Pharmacol Exp Ther* 341(2):326–339. doi:10.1124/jpet.111.188060
- Brassard D (2012) Estimated incremental increase in clothianidin usage from pending registrations (DP404793). US Environmental Protection Agency memorandum, Washington
- Bromilow RH, Chamberlain K (1995) Principles governing uptake and transport of chemicals. Plant contamination: modelling and simulation. Lewis Publishers, London, pp 37–64
- Brunet JL, Maresca M, Fantini J, Belzunces LP (2004) Human intestinal absorption of imidacloprid with Caco-2 cells as enterocyte model. *Toxicol Appl Pharmacol* 194:1–9
- Brunet JL, Badiou A, Belzunces LP (2005) In vivo metabolic fate of [¹⁴C]-acetamiprid in six biological compartments of the honeybee, *Apis mellifera* L. *Pest Manag Sci* 61:742–748
- Brunet JL, Maresca M, Fantini J, Belzunces LP (2008) Intestinal absorption of the acetamiprid neonicotinoid by Caco-2 cells: transepithelial transport, cellular uptake and efflux. *J Environ Sci Health Part B* 43: 261–270
- Cahill M, Denholm I, Gorman K, Day S, Elbert A, Nauen R (1996) Baseline determination and detection of resistance to imidacloprid in *Bemisia tabaci* (Homoptera: Aleyrodidae). *B Entomol Resist* 86: 343–349
- Calas-List D, List O, Quinchard S, Thany SH (2013) Calcium pathways such as cAMP modulate clothianidin action through activation of α -bungarotoxin-sensitive and -insensitive nicotinic acetylcholine receptors. *Neurotoxicology* 37:127–133

- Casida JE (2011) Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance. *J Agric Food Chem* 59:2923–2931
- Casida JE, Durkin KA (2013) Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annu Rev Entomol* 58:99–117
- Casida JE, Quistad GB (2004) Why insecticides are more toxic to insects than people: the unique toxicology of insects. *J Pest Sci* 29:81–86
- CCM International Ltd (2011). Accessible at: http://www.researchandmarkets.com/reports/649028/production_and_market_of_imidacloprid_in_china. Accessed 21 Oct 2012
- Chagnon M, Kreutzweiser D, Mitchell EAD, Morrissey CA, Noome DA, et al (2014) Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environ Sci Pollut Res*:1–16. doi:10.1007/s11356-014-3277-x
- Chao SL, Casida JE (1997) Interaction of imidacloprid metabolites and analogs with the nicotinic acetylcholine receptor of mouse brain in relation to toxicity. *Pest Biochem Physiol* 58:77–88
- Charpentier G, Louat F, Bonmatin J-M, Marchand PA, Vannier F et al (2014) Lethal and sublethal effects of imidacloprid, after chronic exposure, on the insect model *Drosophila melanogaster*. *Environ Sci Technol* 48(7):4096–4102
- Chauzat MP, Martel AC, Cougoule N, Porta P, Lachaize J et al (2011) An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France. *Environ Toxicol Chem* 30:103–111
- Chen MF, Huang JW, Wong SW, Li GC (2005) Analysis of insecticide clothianidin and its metabolites in rice by liquid chromatography with a UV detector. *J Food Drug Anal* 13:279–283
- Chen T, Dai YJ, Ding JF, Yuan S, Ni JP (2008) N-demethylation of neonicotinoid insecticide acetamiprid by bacterium *Stenotrophomonas maltophilia* CGMCC 1.1788. *Biodegradation* 19:651–658
- Cole LM, Nicholson RA, Casida JE (1993) Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pestic Biochem Physiol* 46:47–54
- Courjaret R, Lapied B (2001) Complex intracellular messenger pathways regulate one type of neuronal alpha-bungarotoxin-resistant nicotinic acetylcholine receptors expressed in insect neurosecretory cells (dorsal unpaired median neurons). *Mol Pharmacol* 60:80–91
- Courjaret R, Grolleau F, Lapied B (2003) Two distinct calcium-sensitive and -insensitive PKC up- and down-regulate an alpha-bungarotoxin-resistant nAChR1 in insect neurosecretory cells (DUM neurons). *Eur J Neurosci* 17:2023–2034
- Cui L, Sun L, Yang D, Yan X, Yuan H (2012) Effects of cycloxaprid, a novel *cis*-nitromethylene neonicotinoid insecticide, on the feeding behaviour of *Sitobion avenae*. *Pest Manag Sci* 68:1484–1491
- Cutler P, Slater R, Edmunds AJF, Maienfisch P, Hall RG, Earley FGP, Pittema T, Pal S, Paul VL, Goodchild J, Blacker M, Hagmann L, Crosssthaite AJ (2013) Investigating the mode of action of sulfoxaflor: a fourth-generation neonicotinoid. *Pest Manag Sci* 69:607–619
- Dai YJ, Ji WW, Chen T, Zhang WJ, Liu ZH, Ge F, Yuan S (2010) Metabolism of the neonicotinoid insecticides acetamiprid and thiacloprid by the yeast *Rhodotorula mucilaginosa* strain IM-2. *J Agric Food Chem* 58:2419–2425
- D'Amour KA, Casida JE (1999) Desnitroimidacloprid and nicotine binding site in rat recombinant alpha *a4b2* neuronal nicotinic acetylcholine receptor. *Pest Biochem Physiol* 64:55–61
- De Freitas Bueno A, Batistela MJ, de Freitas Bueno RCO, de Barros Franca-Neto J, Nishikawa MAN, Filho AL (2011) Effects of integrated pest management, biological control, and prophylactic use of insecticides on the management and sustainability of soybean. *Crop Prot* 30:937–945
- De Uderzo APFM, Diniz MER, Nascentes CC, Catharino RR, Eberlin MN, Augusti R (2007) Photolytic degradation of the insecticide thiamethoxam in aqueous medium monitored by direct infusion electrospray ionization mass spectrometry. *Int J Mass Spectrom* 42:1319–1325
- Decourtye A, Devillers J (2010) Ecotoxicity of neonicotinoid insecticides to bees. Insect nicotinic acetylcholine receptors. Springer. pp. 85–95. Accessible at: http://link.springer.com/chapter/10.1007/978-1-4419-6445-8_8. Accessed 10 Jul 2014
- Dégise P, Grünewald B, Gauthier M (2002) The insecticide imidacloprid is a partial agonist of the nicotinic receptor of honeybee Kenyon cells. *Neurosci Lett* 321:13–16
- Denholm I, Cahill M, Dennehy TJ, Horowitz AR (1998) Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. *Philos Trans R Soc B* 353(1376):1757–1767. doi:10.1098/rstb.1998.0328
- Dick RA, Kanne DB, Casida JE (2005) Identification of aldehyde oxidase as the neonicotinoid nitroreductase. *Chem Res Toxicol* 18:317–323
- Dieckmann Y, Göth FC, Ishaque M, Kerl W, Köhle H, et al (2010a) Agrochemical Formulations Comprising Co-Polymers Based on Ethylenically Unsaturated Dicarboxylic Mono and Diesters. Patent No. US 2010/0063167 A1. 1–14
- Dieckmann Y, Göth FC, Ishaque M, Kerl W, Köhle H, et al (2010b) Agrochemical Formulations Comprising 1-vinyl-2-pyrrolidinone Co-Polymers. Patent No. US 2010/0075849 A1. 1–14
- Dieckmann Y, Ishaque M, Muenster I, Picard L, Benz A, et al (2010c) Systemicity enhancers. Patent No. US 2010/0204045 A1. 1–21
- DoW Agro Sciences (2013) DoW AgroSciences receives US EPA Registration for Sulfoxaflor
- Dupuis JP, Gauthier M, Raymond-Delpech V (2011) Expression patterns of nicotinic subunits $\alpha 2$, $\alpha 7$, $\alpha 8$, and $\beta 1$ affect the kinetics and pharmacology of ACh-induced currents in adult bee olfactory neuropiles. *J Neurophysiol* 106:1604–1613
- Elbert A, Nauen R (2000) Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. *Pest Manag Sci* 56:60–64
- Elbert A, Haas M, Springer B, Thielert W, Nauen R (2008) Applied aspects of neonicotinoid uses in crop protection. *Pest Manag Sci* 64:1099–1105
- European Commission, Directorate General of Health and Consumer protection (2006) Review report for the active substance thiamethoxam. Accessible at: http://ec.europa.eu/food/plant/protection/evaluation/newactive/thiamethoxam_en.pdf
- European Food Safety Authority (EFSA) (2010) Modification of the existing MRL for clothianidin in carrots. *EFSA Journal* 8:1515 [23 pp.]. Accessible at: <http://www.efsa.europa.eu/it/efsajournal/doc/1515.pdf>
- European Food Safety Authority (EFSA) (2012) Statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe. *EFSA J* 10(6):2752. doi:10.2903/j.efsa.2012.2752, 27 pp
- European Food Safety Authority (EFSA) (2014) Conclusion on the peer review of the pesticide risk assessment of the active substance sulfoxaflor. *EFSA J* 12:3692. doi:10.2903/j.efsa.2014.3692, 170 pp
- FAO acetamiprid. Accessible at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report11/Acetamiprid.pdf
- FAO clothianidin. Accessible at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation10/Chlothianidin.pdf
- FAO dinotefuran. Accessible at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation12/Dinotefuran.pdf
- FAO fipronil. Accessible at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation01/08_Fipronil.pdf
- FAO imidacloprid. Accessible at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation02/IMIDA_EVjib.pdf

- FAO thiacloprid. Accessible at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation06/Thiacloprid06.pdf
- FAO thiamethoxam. Accessible at: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation10/Thiamethoxam.pdf
- Feltham H, Park K, Goulson D (2014) Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology* 23:317–323. doi:10.1007/s10646-014-1189-7
- Fogel MN, Schneider MI, Desneux N, González B, Ronco AE (2013) Impact of the neonicotinoid acetamiprid on immature stages of the predator *Eriopis connexa* (Coleoptera: Coccinellidae). *Ecotoxicology* 22:1063–1071
- Ford KA, Casida JE (2006a) Unique and common metabolites of thiamethoxam, clothianidin, and dinotefuran in mice. *Chem Res Toxicol* 19:1549–1556
- Ford KA, Casida JE (2006b) Chloropyridinyl neonicotinoid insecticides: diverse molecular substituents contribute to facile metabolism in mice. *Chem Res Toxicol* 19:944–951
- Ford KA, Casida JE (2008) Comparative metabolism and pharmacokinetics of seven neonicotinoid insecticides in spinach. *J Agric Food Chem* 56:10168–10175
- Ford KA, Casida JE, Chandran D, Gulevich AG, Okrent RA, Durkin KA, Sarpong R, Bunnelle EM, Wildermuth MC (2010) Neonicotinoid insecticides induce salicylate-associated plant defense responses. *PNAS* 107:17527–17532
- France (2005) Draft Assessment Report (DAR). Initial risk assessment provided by the rapporteur Member State France for the existing active substance fipronil of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC
- Furlan L, Kreutzweiser DP (2014) Alternatives to neonicotinoid insecticides for pest control: case studies in agriculture and forestry. *Enviro Sci Pollut Res* (this issue)
- Georgiou GP, Mellon RB (1983) Pesticide resistance in time and space. *Pest resistance to pesticides*. Springer. pp. 1–46. Accessible: http://link.springer.com/chapter/10.1007/978-1-4684-4466-7_1. Accessed 7 Feb 2014
- Gibbons D, Morrissey C, Mineau P (2014) A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Enviro Sci Pollut Res*. doi:10.1007/s11356-014-3180-5
- Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. *J Appl Ecol* 50(4):977–987. doi:10.1111/1365-2664.12111
- Grafton-Cardwell EE, Lee JE, Robillard SM, Gorden JM (2008) Role of imidacloprid in integrated pest management of California citrus. *J Econ Entomol* 101:451–460
- Grant DB, Chalmers AE, Wolff MA, Hoffman HB, Bushey DF, Kuhr RJ, Motoyama N (1998) Fipronil: action at the GABA receptor. In: *Pesticides and the future: minimizing chronic exposure of humans and the environment*. Ios Press, Amsterdam, pp 147–156
- Greatti M, Barbattini R, Stravisi A, Sabatini AG, Rossi S (2006) Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho dressed seeds. *Bull Insectol* 59:99–103
- Green T, Toghill A, Lee R, Waechter F, Weber E, Noakes J (2005a) Thiamethoxam induced mouse liver tumors and their relevance to humans. Part 1: mode of action studies in the mouse. *Toxicol Sci* 86:36–47
- Green T, Toghill A, Lee R, Waechter F, Weber E, Peffer R, Noakes J, Robinson M (2005b) Thiamethoxam induced mouse liver tumors and their relevance to humans. Part 2: species differences in response. *Toxicol Sci* 86:48–55
- Guez D, Suchail S, Gauthier M, Maleszka R, Belzunces LP (2001) Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). *Neurobiol Learn Mem* 76:183–191
- Guez D, Belzunces LP, Maleszka R (2003) Effects of imidacloprid metabolites on habituation in honeybees suggest the existence of two subtypes of nicotinic receptors differentially expressed during adult development. *Pharmacol Biochem Behav* 75:217–222
- Gunasekara AS, Truong T, Goh KS, Spurlock F, Tjeerdema RS (2007) Environmental fate and toxicology of fipronil. *J Pest Sci* 32:189–199. doi:10.1584/jpestics.R07-02
- Hainzl D, Casida JE (1996) Fipronil insecticide: novel photochemical desulfinylation with retention of neurotoxicity. *PNAS* 93:12764–12767
- Hainzl D, Cole LM, Casida JE (1998) Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem Res Toxicol* 11:1529–1535
- Hasegawa K, Arakawa M, Funatsu K (1999) 3D-QSAR study of insecticidal neonicotinoid compounds based on 3-way partial least squares model. *Chemometr Intell Lab* 47:33–40
- Hayasaka D, Korenaga T, Suzuki K, Saito F, Sánchez-Bayo F et al (2012a) Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. *Ecotoxicol Environ Saf* 80:355–362
- Hayasaka D, Korenaga T, Suzuki K, Sánchez-Bayo F, Goka K (2012b) Differences in susceptibility of five cladoceran species to two systemic insecticides, imidacloprid and fipronil. *Ecotoxicology* 21:421–427
- Herron GA, Wilson LJ (2011) Neonicotinoid resistance in *Aphis gossypii* (Aphididae: Hemiptera) from Australian cotton. *Aust J Entomol* 50:93–98
- Honda H, Tomizawa M, Casida JE (2006) Insect nicotinic acetylcholine receptors: neonicotinoid binding site specificity is usually but not always conserved with varied substituents and species. *J Agric Food Chem* 54:3365–3371
- IUPAC (2014) <http://sitem.herts.ac.uk/aeru/iupac/316.htm> Accessed 3 Apr 2014
- Japan's National Institute for Environmental Studies database, provided by Mizuno, R. *in litt.*, 2012
- Jepson JEC, Brown LA, Sattelle DB (2006) The actions of the neonicotinoid imidacloprid on cholinergic neurons of *Drosophila melanogaster*. *Invert Neurosci* 6:33–40
- Jeschke P, Nauen R (2008) Neonicotinoids—from zero to hero in insecticide chemistry. *Pest Manag Sci* 64:1084–1098
- Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* 59:2897–2908
- Jeschke P, Nauen R, Beck ME (2013) Nicotinic acetylcholine receptor agonists: a milestone for modern crop protection. *Angew Chem Int Ed* 52:9464–9485
- Johnson KD, O'Neil ME, Ragsdale DW, Difonzo CD, Swinton SM, Dixon PM, Potter BD, Hodgson EW, Costamagna AC (2009) Probability of cost-effective management of soybean aphid (Hemiptera: Aphididae) in North America. *J Econ Entomol* 102:2101–2108
- Kagabu S (2008) Pharmacophore of neonicotinoid insecticides. *J Pest Sci* 33:9–13
- Kagabu S, Maienfisch P, Zhang AG, Granda-Minones J, Haettenschwiler J, Kayser H, Maetzke T, Casida JE (2000) 5-azidoimidacloprid and an acyclic analogue as candidate photoaffinity probes for mammalian and insect nicotinic acetylcholine receptors. *J Med Chem* 43:5003–5009
- Kagabu S, Nishiwaki H, Sato K, Hibi M, Yamaoka N, Nakagawa Y (2002) Nicotinic acetylcholine receptor binding of imidacloprid-related diaza compounds with various ring sizes and their insecticidal activity against *Musca domestica*. *Pest Manag Sci* 58:483–490
- Kagabu S, Nishimura K, Naruse Y, Ohno I (2008) Insecticidal and neuroblocking potencies of variants of the thiazolidine moiety of thiacloprid and quantitative relationship study for the key neonicotinoid pharmacophore. *J Pest Sci* 33:58–66

- Kagabu S, Takagi M, Ohno I, Mikawa T, Miyamoto T (2009) Crown-capped imidacloprid: a novel design and insecticidal activity. *Bioorg Med Chem Lett* 19:2947–2948
- Kanne DB, Dick RA, Tomizawa M, Casida JE (2005) Neonicotinoid nitroguanidine insecticide metabolites: synthesis and nicotinic receptor potency of guanidines, aminoguanidines, and their derivatives. *Chem Res Toxicol* 18:1479–1484
- Karatolos N, Denholm I, Williamson M, Nauen R, Gorman K (2010) Incidence and characterisation of resistance to neonicotinoid insecticides and pymetrozine in the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae). *Pest Manag Sci* 66:1304–1307
- Karmakar R, Kulshrestha G (2009) Persistence, metabolism and safety evaluation of thiamethoxam in tomato crop. *Pest Manag Sci* 65: 931–937
- Karmakar R, Bhattacharya R, Kulshrestha G (2009) Comparative metabolite profiling of the insecticide thiamethoxam in plant and cell suspension culture of tomato. *J Agric Food Chem* 57:6369–6374
- Kavi LAK, Kaufman PE, Scott JG (2014) Genetics and mechanisms of imidacloprid resistance in house flies. *Pest Biochem Physiol* 109: 64–69
- Kim BM, Park JS, Choi JH, El-Aty AMA, Na TW, Shim JH (2012) Residual determination of clothianidin and its metabolites in three minor crops via tandem mass spectrometry. *Food Chem* 131:1546–1551
- Kiriyama K, Nishimura K (2002) Structural effects of dinotefuran and analogues in insecticidal and neural activities. *Pest Manag Sci* 58: 669–676
- Kiriyama K, Nishiwaki H, Nakagawa Y, Nishimura K (2003) Insecticidal activity and nicotinic acetylcholine receptor binding of dinotefuran and its analogues in the housefly, *Musca domestica*. *Pest Manag Sci* 59:1093–1100
- Klein O (2003) Behaviour of clothianidin (TI-435) in plants and animals. *Pflanzenschutz-Nachr Bayer* 56:75–101
- Koch RL, Burkness EC, Hutchison WD, Rabaey TL (2005) Efficacy of systemic insecticide seed treatments for protection of early-growth-stage snap beans from bean leaf beetle (Coleoptera: Chrysomelidae) foliar feeding. *Crop Prot* 24:734–742
- Kuhar TP, Stivers-Young LJ, Hoffmann MP, Taylor AG (2002) Control of corn flea beetle and Stewart's wilt in sweet corn with imidacloprid and thiamethoxam seed treatments. *Crop Prot* 21:25–31
- Lansdell SJ, Millar NS (2000) The influence of nicotinic receptor subunit composition upon agonist, alpha-bungarotoxin and insecticide (imidacloprid) binding affinity. *Neuropharmacology* 39:671–679
- Lanzoni A, Sangiorgi L, De Luigi V, Consolini L, Pasqualini E, Burgio G (2012) Evaluation of chronic toxicity of four neonicotinoids to *Adalia bipunctata* L. (Coleoptera: Coccinellidae) using a demographic approach. *IOBC/wprs Bull* 74:211–217
- Lapied B, Lecorrone H, Hue B (1990) Sensitive nicotinic and mixed nicotinic-muscarinic receptors in insect neurosecretory cells. *Brain Res* 533:132–136
- Laurent FM, Rathahao E (2003) Distribution of C-14 imidacloprid in sunflowers (*Helianthus annuus* L.) following seed treatment. *J Agric Food Chem* 51:8005–8010
- Le Questel J-Y, Graton J, Cerón-Carrasco JP, Jacquemin D, Planchat A, Thany SH (2011) New insights on the molecular features and electrophysiological properties of dinotefuran, imidacloprid and acetamiprid neonicotinoid insecticides. *Bioorg Med Chem Lett* 19: 7623–7634
- Li J, Shao Y, Ding Z, Bao H, Liu Z, Han Z, Millar NS (2010) Native subunit composition of two insect nicotinic receptor subtypes with differing affinities for the insecticide imidacloprid. *Insect Biochem Mol Biol* 40:17–22
- Li C, Xu X-Y, Li J-Y, Ye Q-F, Li Z (2011) Synthesis and chiral purification of ¹⁴C-labeled novel neonicotinoids, paichongding. *J Label Comp Radiopharm* 54:775–779
- Lind RJ, Clough MS, Reynolds SE, Earley FGP (1998) [³H]-Imidacloprid Labels high- and low-affinity nicotinic acetylcholine receptor-like binding sites in the Aphid *Myzus persicae* (Hemiptera: Aphididae). *Pestic Biochem Physiol* 62:3–14
- Lind RJ, Clough MS, Earley FGP, Wonnacott S, Reynolds SE (1999) Characterisation of multiple [alpha]-bungarotoxin binding sites in the aphid *Myzus persicae* (Hemiptera: Aphididae). *Insect Biochem Mol Biol* 29:979–988
- Liu MY, Latli B, Casida JE (1995) Imidacloprid binding site in *Musca* nicotinic acetylcholine receptor: interactions with physostigmine and a variety of nicotinic agonists with chloropyridyl and chlorothiazolyl substituents. *Pest Biochem Physiol* 52:170–181
- Liu ZW, Williamson MS, Lansdell SJ, Denholm I, Han ZJ, Millar NS (2005) A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in *Nilaparvata lugens* (brown planthopper). *PNAS* 102:8420–8425
- Liu Z, Williamson MS, Lansdell SJ, Han Z, Denholm I, Millar NS (2006) A nicotinic acetylcholine receptor mutation (Y151S) causes reduced agonist potency to a range of neonicotinoid insecticides. *J Neurochem* 99:1273–1281
- Liu GY, Ju XL, Cheng J (2010) Selectivity of imidacloprid for fruit fly versus rat nicotinic acetylcholine receptors by molecular modeling. *J Mol Model* 16:993–1002
- Liu Z, Dai Y, Huang G, Gu Y, Ni J, Wei H, Yuan S (2011) Soil microbial degradation of neonicotinoid insecticides imidacloprid, acetamiprid, thiacloprid and imidaclothiz and its effect on the persistence of bioefficacy against horsebean aphid *Aphis craccivora* Koch after soil application. *Pest Manag Sci* 67:1245–1252
- Longhurst C, Babcock JM, Denholm I, Gorman K, Thomas JD, Sparks TC (2013) Cross-resistance relationships of the sulfoximine insecticide sulfoxaflor with neonicotinoids and other insecticides in the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum*. *Pest Manag Sci* 69:809–813
- Lu C, Warchol KM, Callahan RA (2012) In situ replication of honey bee colony collapse disorder. *Bull Insectol* 65:99–106
- Lu C, Warchol KM, Callahan RA (2014) Sub-lethal exposure to neonicotinoids impaired honey bees winterization before proceeding to colony collapse disorder. *Bull Insectol* 67:125–130
- Macfadyen S, Hardie DC, Fagan L, Stefanova K, Perry KD, DeGraaf HE, Holloway J, Spafford H, Umina PA (2014) Reducing insecticide use in broad-acre grains production: an Australian study. *PlosOne* 9: e89119
- Maienfish P, Angst M, Brandl F, Fischer W, Hofer D, Kayser H, Kobel W, Rindlisbacher A, Senn R, Steinemann A, Withmer H (2001a) Chemistry and biology of thiamethoxam: a second generation neonicotinoid. *Pest Manag Sci* 57:906–913
- Maienfish P, Huerlimann H, Rindlisbacher A, Gsell L, Dettwiler H, Haettenschwiler J, Sieger E, Wälti M (2001b) The discovery of thiamethoxam: a second-generation neonicotinoid. *Pest Manag Sci* 57:165–176
- Main AR, Headley JV, Peru KM, Michel NL, Cessna AJ et al (2014) Widespread use and frequent detection of neonicotinoid insecticides in Wetlands of Canada's Prairie Pothole Region. *PLoS One* 9: e92821
- Maini S, Medrzycki P, Porrini C (2010) The puzzle of honey bee losses: a brief review. *Bull Insectol* 63:153–160
- Marrs TC (1993) Organophosphate poisoning. *Pharmacol Ther* 58:51–66
- Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Grauso M, Sattelle DB (2001) Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharmacol Sci* 22:573–580
- Matsuda K, Shimomura M, Ihara M, Akamatsu M, Sattelle DB (2005) Neonicotinoids show selective and diverse actions on their nicotinic receptor targets: electrophysiology, molecular biology, and receptor modeling studies. *Biosci Biotechnol Biochem* 69:1442–1452

- Matsumoto T (2013) Short-and long-term effects of neonicotinoid application in rice fields, on the mortality and colony collapse of honeybees (*Apis mellifera*). *J Apic Sci* 57:21–35
- Matsumura M, Sanada-Morimura S (2010) Recent status of insecticide resistance in Asian rice planthoppers. *JARQ* 44:225–230
- Matsumura M, Takeuchi H, Satoh M, Sanada-Morimura S, Otuka A, Watanabe T, Van Thanh D (2008) Species-specific insecticide resistance to imidacloprid and fipronil in the rice planthoppers *Nilaparvata lugens* and *Sogatella furcifera* in East and South-east Asia. *Pest Manag Sci* 64:1115–1121
- Meredith RH, Heatherington PJ, Morris DB (2002) Clothianidin—a new chloronicotinyl seed treatment for use on sugar beet and cereals: field trial experiences from Northern Europe. The BCPC Conference: Pests and diseases, Volumes 1 and 2. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel, Brighton, UK, 18–21 November 2002. British Crop Protection Council. pp. 691–696. Accessible at: <http://www.cabdirect.org/abstracts/20033026728.html>. Accessed 4 Feb 2014
- Miyagi S, Komaki I, Ozoe Y (2006) Identification of a high-affinity binding site for dinotefuran in the nerve cord of the American cockroach. *Pest Manag Sci* 62:293–298
- Mole N, Williamson S, Lievens P, Tyrell K (2013) Neonicotinoid restrictions present a unique opportunity to introduce safer agro-ecological approaches to pest management. *Outlooks Pest Manag* 24:156–159
- Mori K, Okumoto T, Kawahara N, Ozoe Y (2002) Interaction of dinotefuran and its analogues with nicotinic acetylcholine receptors of cockroach nerve cords. *Pest Manag Sci* 58:190–196
- Narahashi T, Zhao X, Ikeda T, Nagata K, Yeh JZ (2007) Differential actions of insecticides on target sites: basis for selective toxicity. *Hum Exp Toxicol* 26:361–366
- Narahashi T, Zhao X, Ikeda T, Salgado VL, Yeh JZ (2010) Glutamate-activated chloride channels: unique fipronil targets present in insects but not in mammals. *Pestic Biochem Physiol* 97:149–152
- Nauen R, Denholm I (2005) Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. *Arch Insect Biochem Physiol* 58:200–215
- Nauen R, Tietjen K, Wagner K, Elbert A (1998) Efficacy of plant metabolites of imidacloprid against *Myzus persicae* and *Aphis gossypii* (Homoptera : Aphididae). *J Pest Sci* 52:53–57
- Nauen R, Ebbinghaus U, Tietjen K (1999) Ligands of the nicotinic acetylcholine receptor as insecticides. *J Pest Sci* 55:608–610
- Nauen R, Ebbinghaus-Kintscher U, Schmuck R (2001) Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera : Apidae). *Pest Manag Sci* 57:577–586
- Nauen R, Ebbinghaus-Kintscher U, Salgado VL, Kaussmann M (2003) Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pest Biochem Physiol* 76:55–69
- Nault BA, Taylor AG, Urwiler M, Rabaey T, Hutchison WD (2004) Neonicotinoid seed treatments for managing potato leafhopper infestations in snap bean. *Crop Prot* 23:147–154
- Nishimura K, Tanaka M, Iwaya K, Kagabu S (1998) Relationship between insecticidal and nerve-excitatory activities of imidacloprid and its alkylated congeners at the imidazolidine NH site. *Pest Biochem Physiol* 62:172–178
- Nishiwaki H, Nakagawa Y, Kuwamura M, Sato K, Akamatsu M, Matsuda K, Komai K, Miyagawa H (2003) Correlations of the electrophysiological activity of neonicotinoids with their binding and insecticidal activities. *Pest Manag Sci* 59:1023–1030
- Nishiwaki H, Sato K, Nakagawa Y, Miyashita M, Miyagawa H (2004) Metabolism of imidacloprid in houseflies. *J Pest Sci* 29:110–116
- Oliveira EE, Schleicher S, Büschges A, Schmidt J, Kloppenburg P, Salgado VL (2011) Desensitization of nicotinic acetylcholine receptors in central nervous system neurons of the stick insect (*Carausius morosus*) by imidacloprid and sulfoximine insecticides. *Insect Biochem Mol Biol* 41:872–880
- Orr N, Shaffner AJ, Watson GB (1997) Pharmacological characterization of an epibatidine binding site in the nerve cord of *Periplaneta americana*. *Pest Biochem Physiol* 58:183–192
- Pandey G, Dorrian SJ, Russell RJ, Oakeshott JG (2009) Biotransformation of the neonicotinoid insecticides imidacloprid and thiamethoxam by *Pseudomonas* sp. 1G. *Biochem Biophys Res Commun* 380:710–714
- Perry T, Chan JQ, Batterham P, Watson GB, Geng C, Sparks TC (2012) Effects of mutations in *Drosophila* nicotinic acetylcholine receptor subunits on sensitivity to insecticides targeting nicotinic acetylcholine receptors. *Pest Biochem Physiol* 102:56–60
- Pesticide Properties Data Base (PPDB) (2013) <http://sitem.herts.ac.uk/aeru/footprint/it/index.htm>. Accessed 5 Oct 2013
- Phugare SS, Jadhav JP (2013) Biodegradation of acetamiprid by isolated bacterial strain *Rhodococcus* sp. BCH2 and toxicological analysis of its metabolites in silkworm (*Bombax mori*). *CLEAN Soil Air Water*. doi:10.1002/clen.201200563
- Pisa L, Amaral-Rogers V, Belzunces LP, Bonmatin J-M, Downs C, Goulson D, Kreutzweiser DP, Krupke C, Liess M, McField M, Noome DA, Settele J, Simon-Delso N, Stark JD, Van der Sluijs JP, Van Dyck H, Wiemers M (2014) Effects of neonicotinoids and fipronil on non-target invertebrates. *Environ Sci Pollut Res* doi:10.1007/s11356-014-3471-x
- Pollack P (2011) Fine chemicals: the industry and the business – 2nd edition. John Wiley & Sons, New Jersey
- Prabhakar N, Toscano NC, Castle SJ, Henneberry TJ (1997) Selection for resistance to imidacloprid in silverleaf whiteflies from the Imperial Valley and development of a hydroponic bioassay for resistance monitoring. *J Pest Sci* 51:419–428
- Rahman MM, Park JH, Abd El-Aty AM, Choi JH, Yang A, Park KH, Nashir Uddin Al Mahmud M, Im GJ, Shim JH (2013) Feasibility and application of an HPLC/UV to determine dinotefuran and its shorter wavelength metabolites residues in melon with tandem mass confirmation. *Food Chem* 136:1038–1046
- Ratra GS, Casida JE (2001) GABA receptor subunit composition relative to insecticide potency and selectivity. *Toxicol Lett* 122:215–222
- Ratra GS, Kamita SG, Casida JE (2001) Role of human GABAA receptor $\beta 3$ subunit in insecticide toxicity. *Toxicol Appl Pharmacol* 172: 233–240. doi:10.1006/taap.2001.9154
- Raveton M, Aajoud A, Willison JC, Aouadi H, Tissut M et al (2006) Phototransformation of the insecticide fipronil: identification of novel photoproducts and evidence for an alternative pathway of photodegradation. *Environ Sci Technol* 40:4151–4157
- Raveton M, Aajoud A, Willison J, Cherifi M, Tissut M, Ravanel P (2007) Soil distribution of fipronil and its metabolites originating from a seed-coated formulation. *Chemosphere* 69(7):1124–1129
- Roberts T, Hutson D (1999) Metabolic pathways of agrochemicals. In: Terry R, David H (eds) *Insecticides and fungicides*. The Royal Society of Chemistry, Cambridge
- Roessink I, Merga LB, Zweers HJ, Van den Brink PJ (2013) The neonicotinoid imidacloprid shows high chronic toxicity to mayfly nymphs. *Environ Toxicol Chem* 32:1096–1100
- Royer TA, Giles KL, Nyamanzi T, Hunger RM, Krenzer EG et al (2005) Economic evaluation of the effects of planting date and application rate of imidacloprid for management of cereal aphids and barley yellow dwarf in winter wheat. *J Econ Entomol* 98:95–102
- Salgado VL, Saar R (2004) Desensitizing and non-desensitizing subtypes of alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in cockroach neurons. *J Insect Physiol* 50:867–879
- Sánchez-Bayo F, Goka K (2006) Ecological effects of the insecticide imidacloprid and a pollutant from antidandruff shampoo in experimental rice fields. *Environ Toxicol Chem* 25: 1677–1687

- Sánchez-Bayo F, Hyne RV (2014) Detection and analysis of neonicotinoids in river waters—development of a passive sampler for three commonly used insecticides. *Chemosphere* 99:143–151
- Sanchez-Bayo F, Tennekes AH, Goka K (2013) Impact of systemic insecticides on organisms and ecosystems. In: Trdan S, editor. *Insecticides—development of safer and more effective technologies*. InTech. Accessible at: <http://www.intechopen.com/books/insecticides-development-of-safer-and-more-effective-technologies/impact-of-systemic-insecticides-on-organisms-and-ecosystems>. Accessed 6 Jun 2014
- Schulz-Jander DA, Casida JE (2002) Imidacloprid insecticide metabolism: human cytochrome P450 isozymes differ in selectivity for imidazolidine oxidation versus nitroimine reduction. *Toxicol Lett* 132:65–70
- Schwartz BJ, Sparrow FK, Heard NE, Thede BM (2000) Simultaneous derivatization and trapping of volatile products from aqueous photolysis of thiamethoxam insecticide. *J Agric Food Chem* 48:4671–4675
- Seagraves MP, Lundgren JG (2012) Effects of neonicotinoid seed treatments on soybean aphid and its natural enemies. *J Pest Sci* 85:125–132
- Seifert J, Stollberg J (2005) Antagonism of a neonicotinoid insecticide imidacloprid at neuromuscular receptors. *Environ Toxicol Pharmacol* 20:18–21
- Shao X, Ye Z, Bao H, Li Z, Xu X, Li Z, Qian X (2011) Advanced research on *cis*-neonicotinoids. *Chimia* 65:957–960
- Shao X, Liu Z, Xu X, Li Z, Qian X (2013a) Overall status of neonicotinoid insecticides in China: production, application and innovation. *J Pest Sci* 38:1–9
- Shao X, Swenson TL, Casida JE (2013b) Cycloxaprid insecticide: nicotinic acetylcholine receptor binding site and metabolism. *J Agric Food Chem* 61:7883–7888. doi:10.1021/jf4030695
- Shi X, Dick RA, Ford KA, Casida JE (2009) Enzymes and inhibitors in neonicotinoid insecticide metabolism. *J Agric Food Chem* 57:4861–4866
- Shi X, Jiang L, Wang H, Qiao K, Wang D et al (2011) Toxicities and sublethal effects of seven neonicotinoid insecticides on survival, growth and reproduction of imidacloprid-resistant cotton aphid, *Aphis gossypii*. *Pest Manag Sci* 67:1528–1533
- Sparks TC, DeBoer GJ, Wang NX, Hasler JM, Loso MR, Watson GB (2012) Differential metabolism of sulfoximine and neonicotinoid insecticides by *Drosophila melanogaster* monooxygenase CYP6G1. *Pest Biochem Physiol* 103:159–165
- Sparks TC, Watson GB, Loso MR, Geng C, Babcock JM, Thomas JD (2013) Sulfoxaflor and the sulfoximine insecticides: chemistry, mode of action and basis for efficacy on resistant insects. *Pest Biochem Physiol* 107:1–7
- Stevens S, Jenkins P (2014) Heavy costs. Center for food safety. Accessible at: <http://www.centerforfoodsafety.org/issues/304/pollinators-and-pesticides/reports/2999/heavy-costs-weighing-the-value-of-neonicotinoid-insecticides-in-agriculture#>
- Stokstad E (2013) How big a role should neonicotinoids play in food security? *Science* 340(6133):675
- Suchail S, Guez D, Belzunces LP (2000) Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. *Environ Toxicol Chem* 19:1901–1905
- Suchail S, Guez D, Belzunces LP (2001) Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ Toxicol Chem* 20:2482–2486
- Suchail S, De Sousa G, Rahmani R, Belzunces LP (2004a) In vivo distribution and metabolism of [¹⁴C]-imidacloprid in different compartments of *Apis mellifera* L. *Pest Manag Sci* 60:1056–1062
- Suchail S, Debrauwer L, Belzunces LP (2004b) Metabolism of imidacloprid in *Apis mellifera*. *Pest Manag Sci* 60:291–296
- Syngenta (2012) 2011 Full Year Results. <http://www.syngenta.com/global/corporate/en/news-center/news-releases/pages/120208.aspx>. Accessed 29 Sept 2013
- Syngenta (2013) 2012 Full Year Results. <http://www.syngenta.com/global/corporate/SiteCollectionDocuments/pdf/media-releases/en/20130206-analyst-presentation-fyr2012.pdf>. Accessed 29 Sept 2013
- Szendrei Z, Grafius E, Byrne A, Ziegler A (2012) Resistance to neonicotinoid insecticides in field populations of the Colorado potato beetle (Coleoptera: Chrysomelidae). *Pest Manag Sci* 68:941–946
- Taira K, Fujioka K, Aoyama Y (2013) Qualitative profiling and quantification of neonicotinoid metabolites in human urine by liquid chromatography coupled with mass spectrometry. *PLoS One* 8:e80332
- Tan J, Galligan JJ, Hollingworth RM (2007) Agonist actions of neonicotinoids on nicotinic acetylcholine receptors expressed by cockroach neurons. *Neurotoxicology* 28:829–842
- Taniguchi T, Kita Y, Matsumoto T, Kimura K (2012) Honeybee colony losses during 2008–2010 caused by pesticide application in Japan. *Jpn J Apic* 27:15–27
- Tennekes HA, Sánchez-Bayo F (2011) Time-dependent toxicity of neonicotinoids and other toxicants: implications for a new approach to risk assessment. *J Environ Anal Toxicol* S4:001. doi:10.4172/2161-0525.S4-001
- Thany SH (2009) Agonist actions of clothianidin on synaptic and extrasynaptic nicotinic acetylcholine receptors expressed on cockroach sixth abdominal ganglion. *Neurotoxicology* 30:1045–1052
- Thany SH (2010) Neonicotinoid insecticides. Historical evolution and resistance mechanisms. In: Thany SH (Ed.) *Insect nicotinic acetylcholine receptors*. *Adv Exp Med Biol* 683:75–84
- Thany SH (2011) Thiamethoxam, a poor agonist of nicotinic acetylcholine receptors expressed on isolated cell bodies, acts as a full agonist at cockroach cercal afferent/giant interneuron synapses. *Neuropharmacology* 60:587–592
- Thany SH, Courjaret R, Lapied B (2008) Effect of calcium on nicotine-induced current expressed by an atypical alpha-bungarotoxin-insensitive nAChR2. *Neurosci Lett* 438:317–321
- Thielert W, Metzlaß M, De Block M (2006) Increase of stress tolerance by application of neonicotinoids on plants engineered to be stress tolerant. Patent No. European Patent Application EP1731037
- Thuyet DQ, Watanabe H, Motobayashi T (2011) Effect of formulations and treatment methods of nursery boxes applied with insecticide on the behavior of imidacloprid in rice paddy fields. *J Pest Sci* 36:9–15
- Thuyet DQ, Jorgenson BC, Wissel-Tyson C, Watanabe H, Young TM (2012) Wash off of imidacloprid and fipronil from turf and concrete surfaces using simulated rainfall. *Sci Total Environ* 414:515–524. doi:10.1016/j.scitotenv.2011.10.051
- Tingle CCD, Rother JA, Dewhurst CF, Lauer S, King WJ (2003) Fipronil: environmental fate, ecotoxicology and human health concerns. *Rev Environ Contam Toxicol* 176:1–66
- Tokieda M, Ozawa M, Gomyo T (1999) Methods of determination of acetamiprid and its degradation products in soil by gas chromatography. *J Pest Sci* 24:181–185
- Tomizawa M, Casida JE (1997) I-125 Azidonicotinoid photoaffinity labeling of insecticide-binding subunit of *Drosophila* nicotinic acetylcholine receptor. *Neurosci Lett* 237:61–64
- Tomizawa M, Casida JE (2000) Imidacloprid, thiacloprid, and their imine derivatives up-regulate the $\alpha 4\beta 2$ nicotinic acetylcholinereceptor in M10 cells. *Toxicol Appl Pharmacol* 169:114–120
- Tomizawa M, Casida JE (2001) Structure and diversity of insect nicotinic acetylcholine receptors. *Pest Manag Sci* 57:914–922
- Tomizawa M, Casida JE (2002) Desnitro-imidacloprid activates the extracellular signal-regulated kinase cascade via the nicotinic receptor and intracellular calcium mobilization in N1E-115 cells. *Toxicol Appl Pharmacol* 184:180–186
- Tomizawa M, Casida JE (2003) Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annu Rev Entomol* 48:339–364

- Tomizawa M, Casida JE (2005) Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annu Rev Pharmacol Toxicol* 45: 247–268
- Tomizawa M, Casida JE (2009) Molecular recognition of neonicotinoid insecticides: the determinants of life or death. *Acc Chem Res* 42: 260–269
- Tomizawa M, Casida JE (2011) Neonicotinoid insecticides: highlights of a symposium on strategic molecular designs. *J Agric Food Chem* 59:2883–2886
- Tomizawa M, Cowan A, Casida JE (2001a) Analgesic and toxic effects of neonicotinoid insecticides in mice. *Toxicol Appl Pharmacol* 177: 77–83
- Tomizawa M, Wen ZM, Chin HL, Morimoto H, Kayser H, Casida JE (2001b) Photoaffinity labeling of insect nicotinic acetylcholine receptors with a novel H-3 azidoneonicotinoid. *J Neurochem* 78: 1359–1366
- Tomizawa M, Zhang NJ, Durkin KA, Olmstead MM, Casida JE (2003) The neonicotinoid electronegative pharmacophore plays the crucial role in the high affinity and selectivity for the drosophila nicotinic receptor: an anomaly for the nicotinoid cation- π interaction model. *Biochemistry* 42:7819–7827
- Tomizawa M, Talley TT, Maltby D, Durkins KA, Medzihradsky KF, Burlingame AL, Taylor P, Casida JE (2007) Mapping the elusive neonicotinoid binding site. *PNAS* 104:9075–9080
- Tomizawa M, Maltby D, Talley TT, Durkin KA, Medzihradsky KF, Burlingame AL, Taylor P, Casida JE (2008) Atypical nicotinic agonist bound conformations conferring subtype selectivity. *PNAS* 105:1728–1732
- Tomlin CDS (2000) The pesticide manual. 12th Ed., The British Crop prot Council, Surrey, UK, pp.413–415
- Toshima K, Ihara M, Kanaoka S, Tarumoto K, Yamada A, Sattelle DB, Matsuda K (2008) Potentiating and blocking actions of neonicotinoids on the response to acetylcholine of the neuronal $\alpha 4 \beta 2$ nicotinic acetylcholine receptor. *J Pest Sci* 33:146–151
- United States Department of Agriculture (USDA) - National Agriculture Statistics Service (NASS), (2013) online database: Crop production 2013 summary. <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=10471>
- US Environmental Protection Agency (USEPA) (2000) Thiamethoxam; pesticide tolerance. *Fed Regist* 65:80343–80353
- US Environmental Protection Agency (USEPA) (2003a) Factsheet - Clothianidin. 19 pp
- US Environmental Protection Agency (USEPA) (2003b) Clothianidin; pesticide tolerance. *Fed Regist* 68:32390–32400
- US Environmental Protection Agency (USEPA) (2003c) Dinotefuran; notice of filing a pesticide petition to establish a tolerance for a certain pesticide chemical in or on food. *Fed Regist* 68:39547–39554
- US Environmental Protection Agency (USEPA) (2004a) Clothianidin; notice of filing a pesticide petition to establish a tolerance for a certain pesticide chemical in or on food. *Fed Regist* 69:33635–33638
- US Environmental Protection Agency (USEPA) (2004b) Dinotefuran; conditional registration. Pesticide Fact Sheet, 63 pp
- US Environmental Protection Agency (USEPA) (2013) Registration of the new active ingredient sulfoxaflor for use on multiple commodities, turfgrass and ornamentals. United States Environmental Protection Agency <http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2010-0889-0396>. Accessed 7 Mar 2014
- US Geological Survey (2014) Pesticide National Synthesis Project, Pesticide Use Maps Imidacloprid 2011 http://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2011&map=IMIDACLOPRID&hilo=L&disp=Imidacloprid. Accessed 7 March 2014
- Van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin J-M, Belzunces LP (2013) Neonicotinoids, bee disorders and the sustainability of pollinator services. *Curr Opin Environ Sustain* 5:293–305. doi:10.1016/j.cosust.2013.05.007
- van Rozen K, Ester A (2010) Chemical control of *Diabrotica virgifera virgifera* LeConte. *J Appl Entomol* 134:376–384
- Wakita T (2010) Molecular design of dinotefuran with unique insecticidal properties. *J Agric Food Chem* 59:2938–2942
- Wakita T, Kinoshita K, Yamada E, Yasui N, Kawahara N, Naoi A, Nakaya M, Ebihara K, Matsuno H, Kodaka K (2003) The discovery of dinotefuran: a novel neonicotinoid. *Pest Manag Sci* 59:1016–1022
- Wakita T, Kinoshita K, Kodaka K, Yasui N, Naoi A, Banba S (2004a) Synthesis and structure-activity relationships of dinotefuran derivatives: modification in the tetrahydro-3-furylmethyl part. *J Pest Sci* 29:356–363
- Wakita T, Kinoshita K, Yasui N, Yamada E, Kawahara N, Kodaka K (2004b) Synthesis and structure-activity relationships of dinotefuran derivatives: modification in the nitroguanidine part. *J Pest Sci* 29: 348–355
- Wang KI, Guo QL, Xia XM, Wang HY, Liu TX (2007) Resistance of *Aphis gossypii* (Homoptera: Aphididae) to selected insecticides on cotton from five cotton production regions in Shandong, China. *J Pest Sci* 32:372–378
- Wang Y, Chen J, Zhu YC, Ma C, Huang Y, Shen J (2008) Susceptibility to neonicotinoids and risk of resistance development in the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Pest Manag Sci* 64:1278–1284
- Wang J, Hirai H, Kawagishi H (2012) Biotransformation of acetamiprid by the white-rot fungus *Phanerochaete sordida* YK-624. *Appl Microbiol Biotechnol* 93:831–835
- Wang G, Yue W, Liu Y, Li F, Xiong M, Zhang H (2013a) Biodegradation of the neonicotinoid insecticide Acetamiprid by bacterium *Pigmentiphaga* sp. strain AAP-1 isolated from soil. *Bioresour Technol* 138:359–368
- Wang G, Zhao Y, Gao H, Yue W, Xiong M, Li F, Zhang H, Ge W (2013b) Co-metabolic biodegradation of acetamiprid by *Pseudoxanthomonas* sp. AAP-7 isolated from a long-term acetamiprid-polluted soil. *Bioresour Technol* 150:259–265
- Watson GB, Loso MR, Babcock JM, Hasler JM, Letherer TJ, Young CD, Zhu Y, Casida JE, Sparks TC (2011) Novel nicotinic action of the sulfoximine insecticide sulfoxaflor. *Insect Biochem Mol Biol* 41: 432–439
- Wiesner P, Kayser H (2000) Characterization of nicotinic acetylcholine receptors from the insects *Aphis craccivora*, *Myzus persicae*, and *Locusta migratoria* by radioligand binding assays: relation to thiamethoxam action. *J Biochem Mol Toxicol* 14:221–230
- Wu M, Cai J, Yao J, Dai B, Lu Y (2010) Study of imidacloprid residues in cabbage and soil by HPLC with UV detection. *Bull Environ Contam Toxicol* 84:289–293
- Ying GG, Kookana RS (2006) Persistence and movement of fipronil termiticide with under-slab and trenching treatments. *Environ Toxicol Chem* 25:2045–2050
- Yokota T, Mikata K, Nagasaki H, Ohta K (2003) Absorption, tissue distribution, excretion, and metabolism of clothianidin in rats. *J Agric Food Chem* 51:7066–7072
- Žabar R, Komel T, Fabjan J, Kralj MB, Trebše P (2012) Photocatalytic degradation with immobilised TiO_2 of three selected neonicotinoid insecticides: imidacloprid, thiamethoxam and clothianidin. *Chemosphere* 89:293–301
- Zhang NJ, Tomizawa M, Casida JE (2002) Structural features of azidopyridinyl neonicotinoid probes conferring high affinity and selectivity for mammalian $\alpha 4 \beta 2$ and *Drosophila* nicotinic receptors. *J Med Chem* 45:2832–2840
- Zhang NJ, Tomizawa M, Casida JE (2003) 5-azidoepibatidine: an exceptionally potent photoaffinity ligand for neuronal $\alpha 4 \beta 2$ and

- alpha 7 nicotinic acetylcholine receptors. *Bioorg Med Chem Lett* 13: 525–527
- Zhang Y, Liu S, Gu J, Song F, Yao X, Liu Z (2008) Imidacloprid acts as an antagonist on insect nicotinic acetylcholine receptor containing the Y151M mutation. *Neurosci Lett* 446:97–100
- Zhang X, Liu X, Zhu F, Li J, You H, Lu P (2014) Field evolution of insecticide resistance in the brown planthopper (*Nilaparvata lugens* Stål) in China. *Crop Prot* 58:61–66. doi:[10.1016/j.cropro.2013.12.026](https://doi.org/10.1016/j.cropro.2013.12.026)
- Zhao Y-J, Dai Y-J, Yu C-G, Luo J, Xu W-P et al (2009) Hydroxylation of thiacloprid by bacterium *Stenotrophomonas maltophilia* CGMCC1.1788. *Biodegradation* 20:761–768. doi:[10.1007/s10532-009-9264-0](https://doi.org/10.1007/s10532-009-9264-0)
- Zhu Y, Loso MR, Watson GB, Sparks TC, Rogers RB et al (2010) Discovery and characterization of sulfoxaflor, a novel insecticide targeting sap-feeding pests. *J Agric Food Chem* 59:2950–2957. doi:[10.1021/jf102765x](https://doi.org/10.1021/jf102765x)

Environmental fate and exposure; neonicotinoids and fipronil

J.-M. Bonmatin · C. Giorio · V. Girolami · D. Goulson · D. P. Kreutzweiser ·
C. Krupke · M. Liess · E. Long · M. Marzaro · E. A. D. Mitchell ·
D. A. Noome · N. Simon-Delso · A. Tapparo

Received: 27 May 2014 / Accepted: 11 July 2014 / Published online: 7 August 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract Systemic insecticides are applied to plants using a wide variety of methods, ranging from foliar sprays to seed treatments and soil drenches. Neonicotinoids and fipronil are among the most widely used pesticides in the world. Their popularity is largely due to their high toxicity to invertebrates, the ease and flexibility with which they can be applied, their long persistence, and their systemic nature, which ensures that they spread to all parts of the target crop. However, these properties also increase the probability of environmental contamination and exposure of nontarget organisms. Environmental contamination occurs via a number of routes including dust generated during drilling of dressed seeds,

contamination and accumulation in arable soils and soil water, runoff into waterways, and uptake of pesticides by nontarget plants via their roots or dust deposition on leaves. Persistence in soils, waterways, and nontarget plants is variable but can be prolonged; for example, the half-lives of neonicotinoids in soils can exceed 1,000 days, so they can accumulate when used repeatedly. Similarly, they can persist in woody plants for periods exceeding 1 year. Breakdown results in toxic metabolites, though concentrations of these in the environment are rarely measured. Overall, there is strong evidence that soils, waterways, and plants in agricultural environments and neighboring areas are contaminated with variable levels of

Responsible editor: Philippe Garrigues

J.-M. Bonmatin (✉)
Centre National de la Recherche Scientifique,
Centre de Biophysique Moléculaire, Rue Charles Sadron,
45071 Orléans cedex 02, France
e-mail: bonmatin@cnrs-orleans.fr

C. Giorio
Department of Chemistry, University of Cambridge, Lensfield Road,
CB2 1EW Cambridge, UK

V. Girolami · M. Marzaro
Dipartimento di Agronomia Animali Alimenti Risorse Naturali e
Ambiente, Università degli Studi di Padova, Agripolis, viale dell'
Università 16, 35020 Legnaro, Padova, Italy

D. Goulson
School of Life Sciences, University of Sussex, Falmer, Sussex BN1
9QG, UK

D. P. Kreutzweiser
Canadian Forest Service, Natural Resources Canada, 1219 Queen
Street East, Sault Ste Marie, ON, Canada P6A 2E5

C. Krupke · E. Long
Department of Entomology, Purdue University, West Lafayette,
IN 47907-2089, USA

M. Liess
Department of System-Ecotoxicology, Helmholtz Centre for
Environmental Research - UFZ, 04318 Leipzig, Germany

E. A. D. Mitchell
Laboratory of Soil Biology, University of Neuchâtel, Rue Emile
Argand 11, 2000 Neuchâtel, Switzerland

E. A. D. Mitchell
Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58,
2000 Neuchâtel, Switzerland

D. A. Noome
Task Force on Systemic Pesticides, Perthuis-du-Sault,
2000 Neuchâtel, Switzerland

D. A. Noome
Kijani, Kasungu National Park, Private Bag 151, Lilongwe, Malawi

N. Simon-Delso
Environmental Sciences, Copernicus Institute, Utrecht University,
Heidelberglaan 2, 3584 CS Utrecht, The Netherlands

A. Tapparo
Dipartimento di Scienze Chimiche, Università degli Studi di Padova,
via Marzolo 1, 35131 Padova, Italy

neonicotinoids or fipronil mixtures and their metabolites (soil, parts per billion (ppb)-parts per million (ppm) range; water, parts per trillion (ppt)-ppb range; and plants, ppb-ppm range). This provides multiple routes for chronic (and acute in some cases) exposure of nontarget animals. For example, pollinators are exposed through direct contact with dust during drilling; consumption of pollen, nectar, or guttation drops from seed-treated crops, water, and consumption of contaminated pollen and nectar from wild flowers and trees growing near-treated crops. Studies of food stores in honeybee colonies from across the globe demonstrate that colonies are routinely and chronically exposed to neonicotinoids, fipronil, and their metabolites (generally in the 1–100 ppb range), mixed with other pesticides some of which are known to act synergistically with neonicotinoids. Other nontarget organisms, particularly those inhabiting soils, aquatic habitats, or herbivorous insects feeding on noncrop plants in farmland, will also inevitably receive exposure, although data are generally lacking for these groups. We summarize the current state of knowledge regarding the environmental fate of these compounds by outlining what is known about the chemical properties of these compounds, and placing these properties in the context of modern agricultural practices.

Keywords Neonicotinoid · Fipronil · Water · Soil · Dust · Plant · Guttation · Pollen · Nontarget · Bee · Invertebrates · Vertebrates

Introduction

Currently licensed for the management of insect pests in more than 120 countries, the class of insecticides known as neonicotinoids represent some of the most popular and widely used insecticides in the world (Jeschke et al. 2011; Van der Sluijs et al. 2013; Simon-Delso et al. 2014, this issue). Neonicotinoids are an acetylcholine-interfering neurotoxic class of insecticides (Matsuda et al. 2005) that are utilized in a variety of venues ranging from veterinary medicine, urban landscaping, and use in many agricultural systems as agents of crop protection. They can be applied by multiple methods as foliar sprays to above-ground plants, as root drenches to the soil, or as trunk injections to trees. However, it is estimated that approximately 60 % of all neonicotinoid applications globally are delivered as seed/soil treatments (Jeschke et al. 2011).

A key characteristic distinguishing neonicotinoids from other currently popular insecticide classes is their systemic nature. Neonicotinoids are relatively small molecules and are highly water soluble. Upon uptake by the plant, these compounds and their metabolites circulate (primarily via xylem transport) throughout plant tissues and provide a period of

protection against a number of sap-feeding insects/arthropods (Nauen et al. 2008; Magalhaes et al. 2009). This systemic action is a key characteristic of the neonicotinoids and also fipronil, a phenylpyrazole insecticide largely used for crop protection that allows for great flexibility in methods of application. Additionally, neonicotinoids and fipronil are highly toxic to many classes of insects and exhibit relatively low vertebrate toxicity when compared with other insecticide classes currently in use (US EPA 2003). Therefore, these compounds are able to act specifically on insect pests while reducing impacts on some nontarget organisms (Tomizawa and Casida 2003, 2005; Tingle et al. 2003). However, in the last decade, concerns regarding the environmental fate and effects of these compounds—including soil persistence, effects on managed and wild pollinator species and other nontarget invertebrates, and the potential for contamination of untreated areas during sowing of treated seeds—have highlighted some of the pitfalls associated with the widespread use of these synthetic pesticides (Goulson 2013). Most recently, acute intoxication sources for bees associated with the use of seed-coating insecticides have been identified, specifically via contaminated guttation droplets (Girolami et al. 2009; Tapparo et al. 2011) and direct exposure of flying bees to dusts emitted by the drilling machine during sowing of treated seeds (Girolami et al. 2012; Krupke et al. 2012; Tapparo et al. 2012). Given the increasing evidence that these systemic insecticides pose serious risk of impacts on some nontarget organisms (Bijleveld van Lexmond et al. 2014, this issue), a review and synthesis of the literature describing the environmental fate and routes of exposure for these compounds is warranted.

Chemical properties

Volatility (air)

None of the systemic pesticides considered in this assessment (the neonicotinoids and fipronil) have a high vapor pressure. In general, values range between 2.8×10^{-8} and 0.002 mPa at 25 °C for these compounds. The low potential for volatilization of these substances indicates that these pesticides will most likely only be present in gaseous form for a short period during spray applications.

Sorption to soil particles (soil)

Neonicotinoids and fipronil can bind to soil particles and this reduces their potential to be leached through the soil profile. Imidacloprid sorption was found to correlate positively to soil organic matter and mineral clay content, while desorption was lower at low temperature and at low pesticide concentration (Cox et al. 1997, 1998a, b, c; Broznic and Milin 2012; Broznic

et al. 2012). The comparative study of four soils of contrasted texture and a reference sandy column revealed 27 to 69 % of imidacloprid leaching (97 % in the sand column) (Selim et al. 2010). Lowest mobility was observed in the soil with highest organic matter content (3.5 %), an effect attributed to the existence of hydrophilic bonding on functional groups of the pesticide which may bind to the phenolic hydroxyl and carboxylic acidic groups of soil organic matter. Studies on the effects of peat and tannic acid on mobility illustrate the importance of organic matter quality on imidacloprid dynamics in soil (Flores-Céspedes et al. 2002). Sorption coefficients differ between fipronil and its metabolites (desulfinyl, sulfide, and sulfone) (Ying and Kookana 2006). Neonicotinoids and fipronil and their metabolites also bind to particles in sediments that form the floor of freshwater and marine water bodies (e.g., Bobe et al. 1997; Baird et al. 2013). Bobe et al. (1997) observed that fipronil residues move from water to sediment within 1 week of application.

Solubility (water)

In general terms, the systemic activity of compounds increases with increasing solubility due to improved uniformity in the distribution of the active ingredient in the formulation (Koltzenburg et al. 2010) and increased bioavailability of the pesticide (Pierobon et al. 2008). Transport and translocation are positively correlated with solubility (Chamberlain 1992). The solubility of neonicotinoids in water depends on multiple factors such as water temperature and pH as well as the physical state of the pesticide applied. The molecular weight of the neonicotinoids is between 250 and 300 g/mol, and solubility ranges between 184 (moderate) and 590,000 mg/L (high) for thiacloprid and nitenpyram, respectively, at 20 °C and at pH 7 (Carbo et al. 2008; Jeschke et al. 2011; PPDB 2012) (Table 1). When compared to the neonicotinoids, fipronil has a low solubility at 3.78 mg/L under the same conditions and has a larger molecular weight (437.15 g/mol)

(Tingle et al. 2003). However, even lower solubilities ranging between 1.90 and 2.40 mg/L at pH 5 and pH 9, respectively were also reported.

It should be noted that commercial formulations often contain additional substances that alter the behavior of the active substance. For example, certain copolymers are used to increase the solubility or systemicity of fipronil (Dieckmann et al. 2010a, b, c) (US patents). In an experiment to determine leaching behavior, Gupta et al. (2002) consistently found commercially available formulas to have a higher leaching potential than analytical grade imidacloprid. This may be explained by the added surfactants, which keep the insecticide soluble or suspended for a longer period of time.

Environmental fate—abiotic

Air—environmental exposure by neonicotinoid and fipronil, contaminated dust

Seed coating/dressing is the leading delivery method for neonicotinoids in agriculture throughout the world. This method of pesticide application was initially considered to be a “safer” option for minimizing impacts on nontarget organisms by reducing drift (Ahmed et al. 2001; Koch et al. 2005). While it seems counterintuitive that environmental contamination could result from the use of treated seeds, mounting evidence indicates that the liberation of pesticides applied to seeds can and does arise via this widely used application method. We review research that has focused upon the dust generated during the sowing of neonicotinoid-treated seeds and highlight the risk of acute toxicity posed to honeybees that encounter dispersing dust. We further review current efforts to mitigate the drift of these compounds to nontarget areas.

Table 1 Leaching properties of various systemic insecticides (PPDB 2012)

Insecticide	Solubility in water at 20 °C at pH 7 (mg/L)	GUS leaching potential index	Aqueous photolysis DT50 (days) at pH 7	Water-sediment DT50 (days)
Acetamiprid	2,950 (high)	0.94 (very low)	34 (stable)	—
Clothianidin	340 (moderate)	4.91 (very high)	0.1 (fast)—Stable ^a	56.4 (moderately fast)
Dinotefuran	39,830 (high)	4.95 (very high)	0.2 (fast)	—
Fipronil	3.78 (low)	2.45 (moderate)	0.33 (fast)	68 (moderately fast)
Imidacloprid	610 (high)	3.76 (high)	0.2 (fast)	129 (slow)
Nitenpyram	590,000 (high)	2.01 (moderate)	—	—
Thiacloprid	184 (moderate)	1.44 (low)	Stable	28 (fast)
Thiamethoxam	4,100 (high)	3.82 (high)	2.7 (moderately fast)	40 (moderately fast)

^a USEPA (2010)

History and background

Concerns regarding pesticide-contaminated dust from neonicotinoid- or fipronil-treated seeds originated from reports of atypical levels of honeybee losses in several countries following the planting of treated maize in spring. These incidents have been reported in Italy, France, Slovenia, Germany, USA, and Canada dating as far back as 1999 and as recently as 2013 (Greatti et al. 2003; Pistorius et al. 2009; Krupke et al. 2012; Van der Geest 2012; PMRA 2013). In all cases, a great number of dead and dying bees were found near the hive entrance. Many of these bees were foragers; however, in incidents reported in the USA in 2010 and 2011, many of the dead bees had the characteristic pubescence associated with newly eclosed nurse bees (C. Krupke, unpublished data) and neonicotinoids used in seed treatments were consistently found in pollen stored in affected hives (Krupke et al. 2012). Given that bee deaths have occurred in conjunction with the sowing of treated seeds, much attention has focused on possible routes of exposure for honeybees, both during and shortly after the planting period.

Contaminated dust was first implicated as a potential route of honeybee exposure to neonicotinoid residues following a study by Greatti et al. (2003). This work demonstrated that high levels of neonicotinoid-active ingredients occurred in the exhaust of modern pneumatic planters during seed sowing, and the same active ingredients were detectable on the vegetation surrounding recently planted areas, although at very low concentration levels (ng/g). Based on these findings, it was proposed that the contamination of the air and surrounding environment was the result of the abrasion and separation of the insecticide coating away from seed kernels during planting, and the subsequent expulsion of insecticide particles into the environment via the exhaust fan system of the sowing machine. This discovery forms the basis for the now widely accepted mechanism of pesticide drift from neonicotinoid-treated seeds. Indeed, more recent work has further demonstrated that the sowing of treated seeds results in the development of a “toxic” dust cloud around the planting machine, where concentrations of insecticide particles reach levels of up to 30 $\mu\text{g}/\text{m}^3$, a concentration sufficient to kill bees passing through in a single flight (Girolami et al. 2012, 2013). In contrast, water droplets (both guttations and dew) collected from exposed vegetation adjacent to sown areas would not present acute risk of toxicity to bees (Marzaro et al. 2011).

Developments

It is now known that the dissemination of neonicotinoid-contaminated dust is exacerbated by the addition of seed lubricants during planting. In North America, for instance, talc, graphite, or a combination of these minerals in a finely powdered form is typically mixed with seeds to minimize

friction and ensure smooth seed flow during planting (Krupke et al. 2012). Lubricants are added directly into the planter with pesticide-treated seeds; inevitably some amount of lubricant powder fails to adhere to seeds during the sowing process. This residual lubricant remains behind in the planter to be exhausted, either immediately (i.e., during seed sowing) or later during routine cleaning of planting equipment. Because this powder comes into direct contact with treated seeds, it can act as a carrier of abraded seed coating. In fact, residual talc lubricant has been shown to contain high concentrations of seed treatment compounds, including the protectant fungicides metalaxyl and trifloxystrobin, and up to 15,000 $\mu\text{g}/\text{g}$ of neonicotinoid active ingredients (Krupke et al. 2012), a concentration several orders of magnitude above the contact lethal dose for honeybees.

Neonicotinoid-contaminated dust poses a risk to nontarget organisms through a variety of mechanisms. For instance, abraded insecticide particles that settle on surrounding vegetation can contaminate flowering plants (including insect-pollinated crops, cover crops, and weeds), and thus provide a means of exposure for pollinators utilizing these floral resources (Greatti et al. 2003). In fact, residues of the neonicotinoid clothianidin have been detected (up to 9 ng/g) on dandelions, a key early season resource for honeybees, following the planting of clothianidin-treated maize (Krupke et al. 2012). Exposure to contaminated dust could pose risks for nontarget organisms whether they are exposed to insecticides by contact (dust cloud or deposition on vegetation) or through the ingestion of contaminated plant products (pollen, nectar, etc.). Indeed, high concentrations (above 20 ng/g) of seed treatment pesticides (clothianidin and thiamethoxam) have been detected in samples of stored pollen taken from colonies experiencing losses during corn planting in the USA (Krupke et al. 2012). It is important to note that the reported pesticide concentrations from the flowers and nectar of seed-treated crops are below levels that would induce acute toxicity in honeybees foraging in recently planted areas. Therefore, this exposure mechanism is unlikely to explain the high incidence of bee deaths during the seed planting period. However, a possibly complementary exposure route for nontarget organisms during the planting period is via direct contact with contaminated dust in-flight (e.g., during pollinator foraging flights that pass through areas being sown with treated seeds). In-flight exposure could be of special consequence for organisms like honeybees that possess abundant pubescence on their body surface. This pubescence renders bees more likely to accumulate and retain small particles dispersing in the air, and furthermore creates electrostatic-friction with the air which can enhance the attraction of small particles by bees (Vaknin et al. 2000). By conditioning honeybees to fly through planter-generated dust clouds, Girolami et al. (2012) and Tapparo et al. (2012) unequivocally demonstrated that honeybee foragers can acquire lethal doses of neonicotinoid

residues in-flight, with concentrations ranging from 50–1,200 ng/bee (Girolami et al. 2012; Tapparo et al. 2012). The latter value of 1,200 ng/bee is 60 times the lethal dose of 20 ng/bee (US EPA 1993). As such, exposure to pesticide residues at the concentrations documented by Tapparo et al. (2012) would undoubtedly elicit acute toxicity in honeybees, and furthermore this in-flight mechanism of exposure to contaminated dust could explain the observations of dead and dying bees during the planting of neonicotinoid-treated seeds in various jurisdictions worldwide. Moreover, the sheer magnitude and frequency of crop treatment with neonicotinoid insecticides (e.g., the majority of maize, soybeans, wheat, and rapeseed), combined with the coincidence of seed sowing and the flush of spring blossoms may create scenarios where the flight paths of bees are likely to overlap, both in time and space, with planting activities in many areas. As a result, bees may be at greater risk of in-flight exposure to lethal doses of insecticides in planter exhaust as they forage near agricultural areas that increasingly dominate many landscapes.

Given the widespread risks posed to pollinators, efforts have been made to mitigate the dispersion of contaminated dust in recent years. These include modifications to planting equipment using a variety of devices (collectively known as “deflectors”) that direct seed dust down into the seed furrow before it is closed, as well as improvements to the quality of seed treatment formulations. Although these measures have the potential to reduce dust movement away from the planter (Nikolakis et al. 2009; Balsari et al. 2013), field experiments suggest that neither alterations to seed coating quality nor modifications to drilling machines eliminate the incidence of honeybee deaths during the sowing of treated seeds (Girolami et al. 2012, 2013; Tapparo et al. 2012). In addition, modifying equipment by adding deflectors can be laborious, time consuming, and potentially counter-productive if these changes affect the accuracy and precision of seed placement (Pochi et al. 2012). Taken together, these factors make this option less appealing to growers and planter manufacturers alike. Furthermore, because the seed lubricants used in North American planting equipment (talc and graphite) have been found to abrade pesticides from the seed coat during planting, efforts have been made to transition to less abrasive lubricants. Bayer CropSciences has recently developed a novel lubricant powder to reduce the development of dust during the sowing of treated seeds. This powder, known as “fluency agent” has been tested in North American production fields, but there are currently no published data regarding planting efficacy and/or dust reduction. However, in acknowledging that most incidents of acute honeybee poisonings in recent years were the result of contact with planter dust, the Canadian Pest Management Regulatory Authority (PMRA) recently specified that all treated corn and soybean seed must be sown using “fluency agent”, beginning in 2014 (PMRA 2013). The European Food Safety Authority (EFSA) has recently

acknowledged that bees can be directly contaminated by poisoned dust around the drilling machine during seed sowing (EFSA 2013a, b, c, d). Similarly, the United States Environmental Protection Agency (EPA) has highlighted planter dust as an area of concern and a relevant exposure route in a recent white paper proposing a risk assessment for pollinators (US EPA 2013).

Conclusions

The relative importance of contaminated planter dust containing neonicotinoids and other seed treatment pesticides and its corresponding impacts on the health of honeybees and other nontarget organisms has been debated since these products were first registered for use (Schnier et al. 2003). While it is now generally accepted that honeybees encountering contaminated dust will experience mortality events, recent overviews of seed treatments and their impacts on honeybee health differ in the degree of importance they assign to this source of pesticide exposure (Cresswell 2011; Goulson 2013; Nuytens et al. 2013). While the impacts of contaminated planter dust have been studied closely for managed pollinators like honeybees, this area remains largely unexplored in the case of other pollinators, particularly solitary species, and species with small foraging radii. The degree to which the dispersion of contaminated dust affects nontarget lands, waterways, and the organisms living there in both the short- and long-term is currently unclear; however, given the millions of hectares of treated seed planted annually worldwide, neonicotinoid-contaminated dust stands out as a key route of pesticide exposure for nontarget organisms.

Soil—environmental fate and exposure of neonicotinoid insecticides in soils

Introduction

As outlined above, the primary method for application of the systemic neonicotinoids and fipronil for agricultural pest control is the planting of seeds that are coated with the insecticide. For other pest control uses, insecticides can be applied directly to soils for uptake by plants or to the plants themselves by stem injections (Tattar et al. 1998; Kreutzweiser et al. 2009). The subsequent breakdown of plant material containing insecticide residues can release concentrations back into the soils, thereby providing a further route of soil contamination (Horwood 2007).

Neonicotinoid and fipronil insecticides have been shown to pose a risk of harm to earthworms and other soil invertebrates (Pisa et al. 2014, this issue). In doing so, they have the potential to adversely affect soil ecosystem services (Chagnon et al. 2014, this issue). Therefore, an understanding of the fate and dynamics of insecticide residues in soils is

necessary for an environmental risk assessment. Below, we review the literature on the fate of neonicotinoids in soils.

Temporal dynamics

Neonicotinoids are applied directly to the soil or are released from seed coatings into the soil where they are available to be taken up by plant roots and incorporated into plant tissues (Mullins 1993). Plant uptake processes together with natural degradation of these pesticides is believed to cause soil concentrations to rapidly decrease over time (Horwood 2007). For example, in a field experiment, imidacloprid concentration declined from 652 µg/kg 30 days after seeding to 11 µg/kg by the time of harvest (130 days after seeding), by which time it was not significantly higher than in untreated soils (5 µg/kg) (Donnarumma et al. 2011). Natural degradation was also reported for several insecticides, including imidacloprid and fipronil used to fight termites in Australia with 95 % loss measured after 1 year in situ at one site and 50 % at another site (Horwood 2007).

Nevertheless, neonicotinoids can remain present in measurable concentrations for long periods (months to years) in the soil. Bonmatin et al. (2005a) analyzed the concentration of imidacloprid in 74 soils covering a broad range of climates, soil type, and agricultural practices in France. Imidacloprid was detected in 91 % of the samples (>0.1 µg/kg), although only 15 % of the sites had been planted with treated seeds during the same year. Imidacloprid could be detected in 100 % of the soils seeded with treated seeds in the same year. Imidacloprid was detected in 97 % of soils seeded with treated seed 1 or 2 years before the study. Interestingly, the concentrations were higher in the soils that had been treated consecutively during 2 years before the analysis than in those that received treated seed only 1 year before the analysis (Bonmatin et al. 2005a), indicating that imidacloprid can accumulate over time in soils. These observations are in line with others who have reported a long persistence of neonicotinoids in the environment (Fossen 2006; Gupta and Gajbhiye 2007). In contrast, Bonmatin et al. (2005a) found no detectable residues of neonicotinoids in soils of agricultural fields under organic farming practices.

Half-life—ranges (soil)

Degradation of neonicotinoids and fipronil in soils depends on factors such as soil type (especially texture and organic matter content), ultraviolet radiation (for surface degradation), moisture, temperature, and pH and will therefore vary from place to place. In the mid and higher latitudes, the half-life will be longer than in tropical regions because of fewer sun hours, lower sun light intensity, and lower temperatures.

Calculated half-lives of imidacloprid in soil range over 1 order of magnitude from 100 to 1,230 days following

application (Baskaran et al. 1999). The shortest recorded half-life of imidacloprid in the field is 107 days in turf-covered soils in the humid subtropical climate of Georgia, USA (Cox 2001), while according to Belzunces and Tasei (1997), the half-life of imidacloprid ranges between 188 and 249 days. However, ranges of 27 to 229 days, 997–1,136 days (in laboratory studies) (Scorza et al. 2004; Fossen 2006), 455–518 days (Fernandez-Bayo et al. 2009), 28–46 days (in India) (Sarkar et al. 2001), and even 1,000 days in soil and bedding material (Baskaran et al. 1999) have been reported. The half-life for imidacloprid in soils of seed-treated fields was about 270 days in France (Bonmatin et al. 2005a). However, no decrease in concentration was observed over a 1-year period following treatment in a field test in Minnesota (Cox 2001). Half-life of imidacloprid ranged from 3 to 4 months to over 1 year in soils in the USA (US EPA 1993a) and was longer under higher pH conditions (Sarkar et al. 2001). Based on data in Anon (2006), Goulson (2013) calculated the half-life of 1,250 days for loam in the UK.

The calculated half-life of clothianidin in soil varies even more than that of imidacloprid and ranges between 148 and ca. 7,000 days (DeCant 2010). However, degradation is higher at soil surfaces owing to UV degradation (Gupta et al. 2008a). Goulson (2013) reviewed estimated DT50 (half-life) in soil for the other neonicotinoids as well and reported 31–450 days for acetamiprid, 75–82 days for dinotefuran, 8 days for nitenpyram, 3.4–>1,000 days for thiacloprid, and 7–335 days for thiamthoxam.

For fipronil, half-life times in soil range between 122 and 128 days in lab studies (sandy loam). In field studies, the half-life time ranges from 3 to 7.3 months (US EPA 1996) although a half-life 24 days was reported in a cotton field experiment (Gunasekara et al. 2007; Chopra et al. 2011).

Effect of water content (soil)

Although these half-life ranges seem very broad, they can be explained to some extent by environmental conditions. Acetamiprid half-life is known to depend strongly on soil conditions, being almost 10 times longer under dry conditions (150.5 and 125.4 days for air-dried soils for 1 and 10 µg/g dosage, respectively) than at field capacity moisture (17.4 and 15.7 days) and submerged conditions (19.2 and 29.8 days) (Gupta and Gajbhiye 2007). Similar results were obtained in lab studies for thiamethoxam, with half-life increasing from submerged conditions to field capacity and to dry conditions (46.3–75.3, 91.2–94.1, and 200.7–301 days, respectively) (Gupta et al. 2008b).

Similarly, fipronil half-life in Australian Red Earth loam soils increased from 68 days at 60 % maximum water-holding capacity (MWHC) to 198 days when the moisture content was 15 % MWHC. By contrast, no significant difference was

observed between MWHC of 90 and 165 % (Ying and Kookana 2006).

These results suggest that degradation is related to microbial activity, which is strongly reduced in dry soil conditions and somewhat reduced in saturated soil conditions as a result of low oxygen. In addition, lower concentrations in soils of higher water content may also be due to dilution effects. The concentrations of other chemical compounds in the soil are known to vary in relation to soil moisture content (Misra and Tyler 1999), and this is likely also true for neonicotinoids, but to our knowledge not studied directly. Such changes in concentrations of solutes can in turn affect soil organisms and the concentrations of pesticides in guttation fluid from vascular plants. In support for this view, thiamethoxam concentrations in guttation liquid collected from corn plants were indeed shown to be higher in low soil moisture conditions than in high soil moisture conditions (Tapparo et al. 2011).

Dose dependency of decay

Decay of pesticides has been shown to depend on the dose applied. We did not find any studies on this topic for neonicotinoids, but, in the case of fipronil, dissipation was shown to be rapid (24 days) at relatively low dose (56–112 g active ingredient/ha) (Chopra et al. 2011). Fipronil was also found to exhibit a dose-dependent rate of decay within a similar range (0.15, 0.75 and 3.0 g active ingredient/m²) in Australian Red Earth loam soils (Ying and Kookana 2006). The time for 50 % loss of active ingredients to occur increased approximately fourfold from low to high application rates (145–166 days at lowest rate to 514–613 days at highest rate). Although we did not find published reports of dose-dependent decay among neonicotinoid insecticides, we raise this as a possible further factor affecting concentrations in soils.

Effect of temperature on decay

Imidacloprid degradation was temperature-dependent in a lab incubation experiment (clay soil). Half-lives decreased from 547 to 153 days and finally to 85 days at incubation temperatures of 5, 15, and 25 °C, respectively (Scorza et al. 2004). The same authors report results from a field experiment in which imidacloprid concentrations declined rapidly at first (50 % between May and September) but then no significant change could be detected during the cold months of the year, suggesting a temperature effect (Scorza et al. 2004). High temperature (experimental site in Hisar, 100 km NW of new New Delhi, India) was shown to increase the degradation of fipronil (Chopra et al. 2011).

Leaching and other causes of concentration changes

Independently from uptake by plants or microbial breakdown, concentrations of neonicotinoids and fipronil may change owing to movement in the soil. Two main factors determine such movements: (1) the concentration or identity of dissolved molecules in the soil solution and (2) the sorption on soil particles. Neonicotinoids are mobile in the soil and thus represent a potential contamination threat to surface water and groundwater.

Leaching of pesticides is one of the main mechanisms responsible for the contamination of groundwater and surface water. The leaching process is highly variable across different soil types, pesticide formulations, and application methods (Gupta et al. 2002; Huseeth and Groves 2014). The presence of cracks or other macropores in the soil (earthworm burrows, root channels, etc.), or less-structured soil can lead to preferential flows that bypass the most chemically and biologically reactive topsoil, thus facilitating the high mobility of pesticides (Scorza et al. 2004).

One way of determining the leaching potential of a substance is by calculating the Groundwater Ubiquity Score (GUS). It is calculated from the sorption coefficient (K_{oc}) and the soil half-time (DT50) in the following manner (Gustafson 1989):

$$GUS = \log_{10}(DT50) \times (4 - \log_{10}(K_{oc}))$$

As seen in Table 1 and according to GUS, dinotefuran and clothianidin have a very high leaching potential, imidacloprid and thiamethoxam have a high leaching potential, while fipronil and nitenpyram are classified as possible leachers (PPDB 2012). Contrary to the other systemic pesticides, acetamiprid and thiacloprid break down readily in soil, thereby decreasing the risk of leaching. But the most commonly used agricultural neonicotinoids (imidacloprid, clothianidin, and thiamethoxam) each have a GUS leaching potential index greater than 3.7.

Imidacloprid is known to leach more rapidly through soil columns than other tested pesticides, including common water contaminants such as the organophosphate insecticides chlorpyrifos and diazinon and the herbicide diuron (Vollner and Klotz 1997; Cox 2001). Comparative modeling conducted by the US EPA have shown that imidacloprid had the highest leaching potential among 14 turf insecticides (US EPA 1993b). This high mobility was also confirmed in a field experiment in which imidacloprid was shown to be very mobile in irrigated soil (Felsot et al. 1998). This is also the case for greenhouse soil; Gonzalez-Pradas et al. (2002) report that imidacloprid penetrates the first 40 cm of soil within 2 years of the first application in greenhouses. Gupta et al. (2002) investigated the leaching behavior of different imidacloprid formulations and found that imidacloprid

recovery in 25 cm column leachate varied between 28.7 (analytical grade) and 44.3 % (water-dispersible powder). The heightened leaching potential in commercially available formulations is attributed to the surfactants that were added to the product. Indirect evidence of leaching is also shown by a nearly 50 % drop of imidacloprid concentration (120 vs. 220 ppb) in Hemlock tissue when applied to soil in autumn versus spring (Cowles et al. 2006). Thiamethoxam was also shown to be highly mobile in soil. In a soil column leaching experiment, the equivalent of 65 cm of rainfall caused leaching of 66–79 % of the applied thiamethoxam and no residues could be detected in the soil (Gupta et al. 2008b). These results clearly show that neonicotinoids have a high potential to leach vertically down the soil profile or laterally through soil flow paths and contaminate surface and groundwater.

Mobility of fipronil and of its metabolites (desulfinyl, sulfide, and sulfone derivatives) was observed down to 15 cm, but only traces were found at higher depths (15–30 cm) in three Australian Red Earth loam soils (sandy, loamy, and clay) overlain by 5 cm of quartzite sand. However, experimental plots were covered by plastic liners and fiber cement during the course of the experiment, thus limiting the leaching due to rain (Ying and Kookana 2006). The same authors reported an experiment on two repacked soils (sandy loamy and clay, respectively) with alternative wet-dry weekly cycles (7 days dry followed by 20 mm of rain). Fipronil was added at a high concentration (3 g/m² active ingredient, which in a parallel experiment was shown to result in longest half-life), and bromide was used as a tracer. Mobility was minimal in both soils and not related to the behavior of bromide (highly leached in the sandy loamy soil but not in the clay soil) (Ying and Kookana 2006). Limited fipronil mobility was also demonstrated in Australian soils despite rather dry conditions: although measured annual rainfall was only 432.1 mm, mostly falling during the second half of the experiment, significant downward movement of fipronil was measured (Ying and Kookana 2006). Fipronil was found to bind to soil organic matter, increasing in the range 0.1–6.5 % (Bobé et al. 1997; Gunasekara et al. 2007) and this may explain the low bioaccumulation measured in fungi grown on compost with different concentration of fipronil (Carvalho et al. 2014).

Conclusions

Neonicotinoid and fipronil concentrations in soils typically decline rapidly after application, by hydrolytic, photolytic, and microbial degradation, by plant uptake, by sorption to soil particles, and by leaching to receiving waters. However, in some soil conditions, neonicotinoid and fipronil concentrations can persist, and possibly accumulate, for months or years. Persistence is highest under cool, dry conditions and, at least for neonicotinoids but possibly also for fipronil, in

soils with high organic matter content. Given that neonicotinoids and fipronil are widely used in agricultural settings and can persist in drier, organic-enriched soils, which are common in agricultural fields, their residues in agricultural soils may pose a risk to soil organisms (Pisa et al. 2014, this issue). The uptake of soil-borne residues by plants expands this risk of exposure to other nontarget organisms such as those feeding on living or decomposing plant material, and those collecting nectar and pollen, although little is known about biologically-relevant concentrations found in nontarget plants and the effects of these concentrations upon other organisms.

While the environmental fate of neonicotinoids and fipronil in soils has been examined in several field and laboratory studies, some uncertainties remain. It is not always clear to what process the half-lives correspond. Half-life values are clear for imidacloprid hydrolysis (33 to 44 days at pH 7 and 25 °C) and photolysis (under 3 h) (Fossen 2006), but the term “half-life” is also used when discussing decreasing concentrations over time in soil regardless of the mechanism. For example, Cox writes “*The shortest half-life (the amount of time required for half of an applied pesticide to break down or move away from the test site) was 107 days in turf-covered soil in Georgia.*” (Cox 2001). There are several possible ways by which pesticide concentrations in soils can decrease including uptake by plants, leaching through the soil profile (a demonstrated important process), lateral drainage (in cases of sloping terrain), abiotic or biotic degradation, evaporation (although unlikely given to the low volatility of at least imidacloprid (Fossen 2006)), and dilution (if soil moisture content increases between measurements).

Although some of the mechanisms of dissipation or breakdown have been shown for parent compounds, little is known about the concentrations and dynamics of neonicotinoid and fipronil degradation products and metabolites. Progress on characterizing and tracking metabolites in soils is impeded by the lack of sensitive analytical methodology, and by the fact that information on the chemical structure of metabolites and the availability of reference materials is often proprietary and not available to researchers. Early indications from unpublished studies on metabolites of imidacloprid suggest that several metabolites can be found and they can be more toxic to invertebrates than the parent compound (Suchail et al. 2001; Simon-Delso et al. 2014, this issue).

Water—environmental fate and exposure of neonicotinoid and fipronil insecticides in water and sediments

Introduction

The contamination of surface water with pesticides is an ongoing concern worldwide. Innovations in pesticide composition and application methods present new solutions as well

as challenges. The invention of neonicotinoids and fipronil heralded a new era of pest management, with a higher versatility in application methods and a high target specificity for invertebrates (Jeschke and Nauen 2008). However, these new pesticides present their own set of problems. There are numerous ways for systemic pesticides such as neonicotinoids and fipronil to contaminate groundwater or surface water. The increasing use of these compounds worldwide therefore raises concerns about higher and more widespread contamination of aqueous environments (Overmyer et al. 2005; Tišler et al. 2009). In addition to toxicity, pesticide persistence, metabolite characteristics, the source of contamination and level of exposure are all important for determining the impact of these compounds on aquatic organisms and ecosystems. The persistence of systemic pesticides in the aqueous environment varies with field conditions. These include exposure to sunlight, pH, temperature, the composition of the microbial community, and also the formulation and quantity of the pesticide.

Photodegradation When studied under laboratory conditions, photolysis plays a major role in degradation of systemic pesticides in water (Table 1). Imidacloprid undergoes photolytic degradation rapidly (CCME 2007). However, it proves difficult to find consistent data. Tišler et al. (2009), for example, stored analytical-grade imidacloprid in distilled water (varying concentrations, 8.75–140 mg/L) in the dark at cold temperatures (3 ± 2 °C) and in room light at 21 ± 1 °C. The samples stored in the cold temperature showed no variation during 22 days, while the samples stored at room temperature showed decreasing levels of imidacloprid during this period, dependent on the initial concentration. The higher concentrations (105 and 140 mg/L) decreased by up to 24 % in this period, while levels of 70 mg/L and lower stayed the same. Although the authors hypothesize that this can be attributed to photolytic breakdown in light, the large temperature difference between the two methods is not taken into account in this statement.

In the absence of light, the DT50 of neonicotinoids and fipronil in sediments varies considerably. Thiacloprid is reported to have the shortest DT50, 28 days, while imidacloprid persists the longest at 130 days (PPDB 2012). This last finding on imidacloprid is confirmed by Spiteller (1993) and Krohn and Hellpointner (2002), and cited in Tišler et al. (2009), who found DT50 values of 130 and 160 days for different types of sediments.

Temperature The rate of hydrolysis of imidacloprid increases with temperature (Zheng and Liu 1999; Scorza et al. 2004). The first authors reported an effect of temperature on half-life times of imidacloprid in soil for example (547 days at 5 °C to 89 days at 25 °C).

pH The degradation rates of neonicotinoids and fipronil in water also vary with pH. PPDB (2012) and US EPA (2005)

reports that imidacloprid is stable at a pH between 5 and 7, while the half-life time at pH 9 is about 1 year at 25 °C, thereby indicating a decreasing DT50 with increasing pH. Thuyet et al. (2013) studied degradation of imidacloprid and fipronil at pH levels relevant for rice paddies. Kept at 18.2 ± 0.4 °C and in the dark, the initial concentrations of 60 and 3 µg/L, respectively, for analytical-grade imidacloprid and fipronil, were based on field-realistic concentrations found in paddy fields after application of these pesticides. After an initial decrease in concentration on the first 7 days, the concentration of imidacloprid remained stable at pH 7, but continued to decrease at pH 10. The authors estimated a DT50 of 182 and 44.7 days for imidacloprid at pH 7 and 10. However, Sarkar et al. (1999) found an average half-life of 36.2 days at pH 4, which increased to 41.6 days at pH 9. It should be noted that these results were obtained with commercial formulations (Confidor and Gaucho) at an ambient temperature of 30 ± 5 °C, which is a very wide range. The relatively high temperature will increase the degradation rate, making these results difficult to translate to the majority of field conditions.

Guzsvány et al. (2006) studied the effect of pH on degradation of four different neonicotinoids (at 23 °C) and found that imidacloprid and thiamethoxam degraded more rapidly in alkaline media, while staying relatively stable at pH 7 and 4. Likewise, fipronil degradation is strongly pH dependant, with hydrolysis half-life declining from >100 days at pH 5.5 and 7 to 2.4 h at pH 12 (Bobé et al. 1997). In contrast, acetamiprid and thiacloprid degraded more rapidly in acidic conditions while remaining stable for about 30 days in alkaline conditions. In contrast, several sources indicate that imidacloprid more readily degrades under alkaline conditions (Zheng and Liu 1999; US EPA 2005 in CCME 2007). An experiment determined that, while no hydrolysis products were detected at pH 5 and 7 at any sampling intervals, imidacloprid transformed slightly at pH 9, with a calculated half-life of 346.5 days (Yoshida 1989 report in CCME 2007). Based on these results, the compound is stable to hydrolysis at environmentally relevant pH (CCME 2007).

Field conditions Although most neonicotinoids and fipronil degrade in sunlight, in field conditions, the proportion of transmitted sunlight in water depends on water depth, turbidity, and the wavelength of the incident radiation (Peña et al. 2011). Overall, degradation under field conditions results in variable concentrations through time. In a field experiment, Sanchez-Bayo and Goka (2006) observed an initial decrease of imidacloprid in rice paddies with a starting concentration of 240 µg/L, but the concentration stabilized at 0.75 µg/L for the entire 4-month duration of the experiment. Kreutzweiser et al. (2007) report a declining rate of degradation over time for imidacloprid (initial doses, 0.001–15.4 mg/L) in water of laboratory microcosms, with a dissipation of about 50–60 % after 14 days for the higher doses. The authors conclude that

aqueous imidacloprid concentrations could therefore persist in natural water bodies for several weeks at measurable concentrations. Others have reported surface water concentrations of imidacloprid that persist under field conditions (Van Dijk et al. 2013; Main et al. 2014). However, in a study to aid registration of imidacloprid as a potential control measure for burrowing shrimp, imidacloprid was applied to tidal mudflats in Willapa Bay, USA, in three application rates (0.28, 0.56, and 1.12 a.i./ha). After 28 days, imidacloprid was still detectable in the sediment (limits of detection (LOD) of 2.5 ng/g). However, it dissipated very quickly from the water, being detectable only in one of the three test blocks the day after application. This was attributed to the fast dilution and low sorption potential of imidacloprid (Felsot and Ruppert 2002).

In urban areas, most pesticide runoff is collected in a sewage system and will often undergo treatment at a wastewater plant before being returned to the surface water. Although degradation of thiamethoxam does take place in wastewater, with a half-life of 25 days while in the dark, this is not the case for all neonicotinoids. For example, thiacloprid concentrations in wastewater remained stable whether exposed to sunlight or not, over a 41-day period (Peña et al. 2011). Imidacloprid has also been detected in wastewater treatment plants in Spain (Masiá et al. 2013).

Despite laboratory studies suggesting that clothianidin is susceptible to rapid degradation or dissipation through photolysis (aqueous photolysis DT₅₀<1 day), the slow rate of dissipation in field conditions indicates that photolysis in natural systems does not play a large role in the degradation process (US EPA 2010). Peña et al. (2011) demonstrated the susceptibility of thiamethoxam to direct photolysis, but found clothianidine and thiacloprid to be stable under direct sunlight. Clothianidin is reported to be stable under environmentally realistic pH and temperatures (US EPA 2010).

Metabolites Degradation of neonicotinoids often produces secondary metabolites in water, some of which have been proven to have an equal or greater toxicity than their parent compounds (Suchail et al. 2001). An example is clothianidin, a metabolite of thiamethoxam, which is itself commercially available as an insecticide. For an overview, see Simon-Delso et al. (2014, this issue).

Sources of contamination in water

Systemic pesticides used on agricultural fields, grass, turf, or hard surfaces such as lawns, golf courses, or concrete may contaminate surface and/or groundwater through (foliar) runoff, as well as through leaching, (subsurface) drains, spillage, greenhouse wastewater, and spray or dust drift (Gerecke et al. 2002). In addition, water on the soil surface of treated fields, temporary pondage, may contain high concentrations of systemic pesticides (Main et al. 2014). In sporadic events, flooding of greenhouses

and the subsequent emptying thereof into surface water may result in severe contamination locally. In addition, when applied as stem injection to trees, the falling leaves in autumn may provide a source of contamination to water bodies (Kreutzweiser et al. 2007). Figure 1 provides an overview.

Spray or dust drift Spray application may lead to direct contamination of surface water. This may be caused by unintentional overspray, careless application, or wind dispersal. In addition, dust emission from treated seeds during planting has the potential to drift to adjacent areas. EFSA (2013b, f) gives the percentage of dust drift deposition on the surrounding vegetation from 0.01 % in sugar beet to 7.0 % for maize. Although surface water does not have the three-dimensional catchment properties of surrounding vegetation, it still indicates that measureable amounts of these pesticides may potentially contaminate surface water directly through drift. For example, Tapparo et al. (2012) carried out particulate matter emission tests with different types of commercially available treated maize seeds. While the exact distance that the dust travels depends on atmospheric conditions, it is reasonable to assume that such particulate matter can drift to nearby surface water.

Runoff Neonicotinoids and fipronil are often used to control insect pests in urban or residential areas. Use of these insecticides on ornamental plants or near impervious surfaces creates a potential mode of contamination for aquatic ecosystems through runoff during rainfall or irrigation (Armbrust and Peeler 2002; Haith 2010; Thuyet et al. 2012). Runoff may include dissolved, suspended particulate and sediment-adsorbed pesticides (van der Werf 1996). Imidacloprid and fipronil runoff from turf and concrete surfaces was studied by Thuyet et al. (2012). During their experiment, they subjected turf and concrete surfaces to simulated rainfall at different points in time and with different treatments (turf, granular imidacloprid; concrete, emulsifiable concentrate of imidacloprid and suspension concentrate of fipronil). Their findings indicate a high runoff of imidacloprid on concrete surfaces following 1.5 h after application, with peaks up to 3,267.8 µg/L, 57.3 % of the amount applied. However, percentages dropped between 1.0 and 5.9 % 1 day after the application. No imidacloprid was detected in runoff 7 days after application. Mass losses of fipronil from concrete surface runoff were comparable to imidacloprid with 0.9 to 5.8 %. However, the concentration of toxic byproducts from fipronil runoff was high in all samples. The findings on turf surfaces for imidacloprid varied largely between repeated samples, with between 2.4 and 6.3 % of applied mass product detected in the runoff.

Runoff of these pesticides can also occur in agricultural settings. Residues can occur on plant surfaces after foliar applications or accumulation of pesticide-contaminated dust,

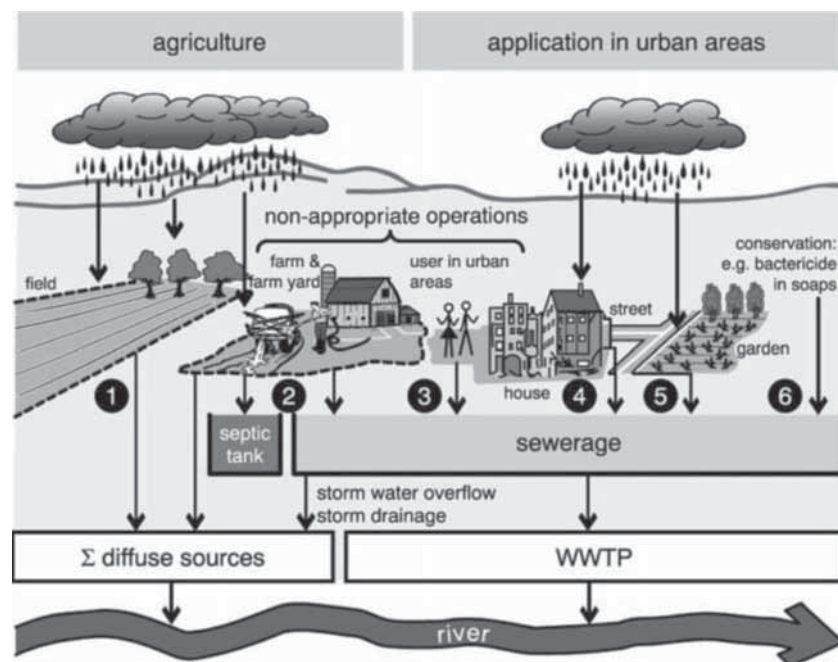


Fig. 1 Important applications and major pathways for pesticide transport into surface waters. 1 Field—spray and dust drift during application, surface runoff, and leaching with subsequent transport through drainage channels during rain events. 2 Farm and farmyard—improper operations (e.g., filling of sprayers, washing of measuring utilities, disposing of packing material, driving with seeping sprayers, and cleaning of spraying equipment). These operations are done either at locations, which are

drained to the sewerage, to the septic tank or into surface waters. 3 Like 2 for pesticide users in urban areas. 4 Pesticides in building material—leaching during rain events. 5 Applications on lawns, streets, and road embankments—runoff during rain events. 6 Protection of materials—e.g., products containing antifouling ingredients that get into the sewerage (e.g., detergents and cosmetics) (source, Gerecke et al. 2002)

and these residues can be washed off during rain events leading to contamination of surface waters. Climate change is expected to play a role in altering pesticide environmental fate in the future. The likelihood of runoff increases with precipitation levels, with increased frequency and intensity of storm events and with increasing pest pressure under climate change effects. As a consequence, the risk of pesticide runoff is likely to be elevated (Kattwinkel et al. 2011). Bloomfield et al. (2006) examined the impacts of this for pesticide behavior in groundwater and surface water in the UK. Pesticide mobility is expected to increase through more frequent heavy rainfall events, increased soil erosion, and cracking of soils leading to faster by-pass flows in winter. In the drier periods, lower flow in rivers also has the potential to increase pesticide concentration and accumulation in sediments (Masiá et al. 2013). On the other hand, higher soil and surface water temperatures due to climate change will decrease some pesticide half-life times. While the overall impact is difficult to predict, increased transport to surface and groundwater of soluble substances such as several neonicotinoids seems likely. For clothianidin, for example, increased mobility is expected, but not the predicted decrease in half-life time as clothianidin is not sensitive to temperature changes. The future increased potential of such pesticides to reach and accumulate in surface and groundwater is an aspect that requires attention and warrants further research. Similarly,

increases in the risk of flooding, especially in greenhouses, could result in washing out of systemic pesticides to the environment (Blom et al. 2008).

Drainage Systemic pesticides are also used in greenhouses, where application techniques include drenching of flower bulbs or chemigation (adding chemicals to irrigation water). The wastewater drainage from these greenhouses is often released into surface water and contains high levels of neonicotinoids. Kreuger et al. (2010) studied pesticides in surface water next to vegetable crops and greenhouses in different regions in Sweden. The authors found imidacloprid present in 36 % of the samples, including all samples taken from stream water draining areas with greenhouse cultivation. The highest concentration of imidacloprid was 9.6 µg/L, substantially higher than in other areas with outdoor cultivation of vegetables. Acetamiprid and thiametoxam were also detected, in 9 and 3 % of the samples, respectively. Only a trace of thiacloprid was found once.

Exposure

Environmental concentrations Contamination of surface water with neonicotinoids or fipronil has been reported in various countries as early as the 1990s. In the Netherlands, imidacloprid was one of the top three of the substances

exceeding the ecotoxicological limit (13 ng/L) since 2004, and has been shown to occur in surface water at up to 25,000 times that amount (Van Dijk et al. 2013). In 2010 and 2011, 75 surface water samples were taken from agricultural regions in California. Imidacloprid was detected in 89 % of the samples and the US EPA toxicity benchmark of 1.05 µg/L was exceeded in 19 % of the samples (Starner and Goh 2012). In a more recent study, Main et al. (2014) surveyed levels of neonicotinoids in water and sediment in the Canadian Prairie Pothole Region. A total of 440 samples were taken before seeding (2012 and again in 2013), during the growing season (2012) and after the harvest of crops in fall (2012). At least one of the following neonicotinoids, clothianidin, thiamethoxam, imidacloprid, or acetamiprid was found in 16 to 91 % of the samples, depending on the time of sampling. Clothianidin was the most commonly detected chemical of the group during three of the four sampling periods, while thiamethoxam was predominant in water samples during the fourth sampling period (after harvest 2012). Maximum concentrations detected in the water were 256 ng/L for imidacloprid (mean, 15.9 ng/L; wheat crops after seeding 2012), 1,490 ng/L for thiamethoxam (mean, 40.3 ng/L; canola after seeding 2012), 3,110 ng/L for clothianidin (mean, 142 ng/L; canola after seeding 2012), and 54.4 ng/L for acetamiprid (mean, 1.1 ng/L; canola after seeding 2012).

Concentrations in soil water exceeding 20 times the permitted level in groundwater (EU directive at the time of the study 1997–1999, i.e., 91/414) were measured in greenhouse soil in Almeria, Spain (Gonzalez-Pradas et al. 2002). A large-scale study of the Guadalquivir River Basin in Spain by Masiá et al. (2013) detected imidacloprid in 58 % (2010) and 17 % (2011) of the samples, with concentrations in these 2 years ranging between 2.34 and 19.20 ng/L. The situation is comparable in Sweden, where imidacloprid was detected in 36 % of the points sampled by Kreuger et al. (2010). The Swedish guideline value of 13 ng/L was exceeded 21 times, with a maximum concentration of 15,000 ng/L, which is 1,154 times over the guideline value. Acetamiprid was also detected, exceeding the guideline value of 100 ng/L twice, with a maximum value of 410 ng/L. Concentration of imidacloprid at 1 µg/L was reported by Bacey (2003) in California groundwater. Concentration reaching 6.4 µg/L were measured from wells in potato-growing areas in Quebec with detection of imidacloprid and three of its metabolites in 35 % of these wells (Giroux 2003). Detections ranging from 0.2 to 7 µg/L were measured in New York State (US EPA 2008).

Fipronil was detected in the Mermentau and Calcasieu River Basins in the USA, in more than 78 % of water samples from the study area. The metabolites fipronil sulfone and fipronil sulfide were detected more often than the parent compound in 81.7 and 90.0 % of the samples, respectively (Mize et al. 2008). In an earlier report by Demcheck et al.

(2004), the accumulation of fipronil degradates in sediment in the same area was reported (100 % of samples). Both authors report that higher concentrations of fipronil and its metabolites were connected to changes in aquatic invertebrate communities, notably a decrease in abundance and diversity. Contamination with fipronil has also an impact on fish as exemplified by Baird et al. (2013).

The contamination of groundwater is also a concern. With the large-scale use of these systemic insecticides and the increasing evidence of their presence in surface water, it should be taken into account that the time lapse between first application of a pesticide and its measured presence in groundwater is, on average, 20 years. Atrazine, for example, is only recently being discovered in groundwater despite having been registered in 1958. Detection of contamination of groundwater with neonicotinoids and fipronil is only a matter of time (Kurwadkar et al. 2013) as this is also the case for lindane (Gonçalves et al. 2007). This is supported by levels measured for thiamethoxam in 2008 and 2009 where several wells in Wisconsin had values above 1 µg/L, with a maximum at 9 µg/L (Huseth and Groves 2013, 2014). Following these results, imidacloprid (average, 0.79; range, 0.26–3.34 µg/L), clothianidin (average, 0.62; range, 0.21–3.34 µg/L), and thiamethoxam (average, 1.59; range, 0.20–8.93 µg/L) were detected at 23 monitoring locations over a 5-year period.

Exposure routes Exposure of nontarget organisms in aqueous environments can take place through different scenarios. Baird et al. (2013) studied toxicity and exposure levels of fipronil on fathead minnow (*Pimephales promelas*), and stated that although waterborne fipronil can be toxic to larval fish, this would only be of concern at high concentrations. The authors conclude that it is the exposure through sediment that presents the real threat to aquatic organisms, including bioaccumulation of fipronil, fipronil sulfone, and/or fipronil sulfate in fish. The fact that systemic pesticides are more persistent in low-light conditions draws further attention to the importance of this exposure route.

Other exposure routes could include the use of contaminated water as drinking water. For example, honeybees (*Apis mellifera*) use water in the hive for cooling and for preparing liquid food for the brood (Kühnholz and Seeley 1997). In extreme conditions (desert), water foraging bees can collect water from up to 2 km from their colony (Visscher et al. 1996). EFSA (2012a) reports 20–42 L per colony per year, and up to 20 L a week or 2.9 L a day in summer. They draw attention to the lack of data on the exposure of honeybees to water through surface water, puddles, and in leaves and/or axils, and recommends that this should be taken into consideration when determining the level of exposure to honeybees.

Conclusion

The high to moderate solubility, leaching potential, and persistence of most of the neonicotinoids and fipronil pose a continuing and increasing risk to aqueous environments. Detections of (high) concentrations in groundwater and surface water are becoming more widespread around the globe. With an ever-increasing scale of use and a relatively high toxicity for aquatic invertebrates, severe impacts on aquatic ecosystems can be expected, and are indeed being discovered (Skrobialowski et al. 2004, cited by Mize et al. 2008; Goulson 2013; van Dijk et al. 2013; Pisa et al. 2014, this issue).

Environmental fate and exposure in plants

Introduction

The efficacy of neonicotinoid insecticides is due in part to the moderate to high water solubility (PPDB 2012); a factor which enhances the uptake and translocation of active ingredients. An advantage associated with using these systemic products is that treated plants are resistant to pests much longer than those treated with nonsystemic products (Dieckmann et al. 2010b).

Neonicotinoids and fipronil are taken up by plants, e.g., by the roots or the leaves, and then transported along the phloem or the xylem to distal tissues different from those where the product was applied (Nauen et al. 2001; Dieckmann et al. 2010a; Aajoud et al. 2008), including the flowers (Bonmatin et al. 2003, 2005b), their pollen (Bonmatin et al. 2007; Krupke et al. 2012), and nectar (Stoner and Eitzer 2012; Paradis et al. 2014). Thus, no matter where a pest or nontarget organism attacks the treated plant it is likely to come in contact with these chemicals. This chapter aims to provide an overview on the environmental fate of neonicotinoids and fipronil in plants and subsequent exposure routes for nontarget organisms.

Uptake by the roots and leaves

Prediction of translocation of pesticides in plants is difficult. Plant morphology and physiology as well as chemical properties of the specific compounds are highly variable and the mechanisms behind translocation processes are often poorly known (Trapp 2004). This chapter focuses on several physical-chemical characteristics of neonicotinoid insecticides and fipronil, aiming to describe the translocation of these pesticides within treated plants after their application.

Systemicity depends on the physical-chemical parameters of the chemicals including water solubility, the partition

coefficient octanol/water ($\log P_{ow}$ or K_{ow}) and the coefficient of dissociation (pK_a). The values of these parameters for the molecules of interest (neonicotinoids and fipronil) can be found in Table 2. However, there are ways to render nonsystemic products, such as fipronil, systemic, by adding copolymers to the pesticide formulation (e.g., Dieckmann et al. 2010a, b; Ishaque et al. 2012).

Partition coefficient octanol/water ($\log K_{ow}$) This parameter indicates the lipophilicity of substances which is related to the ability of substances to penetrate through bio-membranes (Trapp 2004). In order to enter into the plant, chemicals need to cross the plant cuticle. The coefficient cuticle/water is closely linked to the $\log K_{ow}$ (Trapp 2004). However, it is difficult to predict cuticle uptake as it depends on many other factors such as the chemical ingredient, the contact area, the cuticle surface, etc.

When used as root, soil, or seed applications, the sorption of organic chemicals to plant tissues depends on the root concentration factor (RCF) which is the ratio between the concentration in the root (g/g) and the concentration in solution (g/mL). The dependency of the RCF on the K_{ow} has been empirically estimated by Briggs et al. (1983). Maximal cuticle permeability occurs with neutral lipophilic compounds (Trapp 2004), $\log K_{ow}$ being around between 1 and 2.5. Compounds can be considered systemic when their partition coefficient octanol/water goes from 0.1 to 5.4 (Dieckmann et al. 2010a). Certain experts (ICPPR: International Commission for Plant-Pollinator Relationships, <http://www.uoguelph.ca/icpbr/index.html>) have proposed to consider a molecule as systemic if the partition coefficient lays underneath 4 because of hydrosolubility. A parameter that may influence the uptake of pesticides by the roots is the adsorption of chemicals by the soil. However, the final determination of the systemic character should be based on residue analyses or fate analyses in order to reduce uncertainties.

Similarly, when applied as foliar spray, the $\log K_{ow}$ and the concentration of the applied formulation also influence uptake via the leaves. Buchholz and Nauen (2002) describe two additional parameters that alter cuticle permeability of systemic insecticides: molecular mass and temperature. Molecules with high molecular mass at low temperatures tend to penetrate less (Baur et al. 1997). However, cuticle specific characteristics are determinant for pesticide uptake.

Dissociation coefficient (pK_a) This parameter indicates if the diluted form of the molecule is a weak or a strong acid. A $pK_a < 4$ indicates a strong acid, while $pK_a > 5$ indicates a weak one. It is important to note that the phloem pH of plants is around 8 and the xylem pH is around 5.5. Almost all systemic compounds are weak electrolytes (Trapp 2004). The pK_a of neonicotinoids and fipronil (many in their undissociated form) are shown in Table 2. Roots tend to show higher uptake rates

Table 2 Physical-chemical parameters of neonicotinoids and fipronil determining their translocation capacity within the plant

Active substance	Molecular weight (g/mol)	Water solubility (g/L)	Octanol/water partition coefficient (log P_{ow})	Dissociation constant (pK_a)
Fipronil	437.15	0.00378	3.75	No dissociation
Imidacloprid	255.7	0.61	0.57	No dissociation
Thiamethoxam	291.71	4.1	−0.13	No dissociation
Thiacloprid	252.72	0.184	1.26	No dissociation
Clothianidin	249.7	0.34	0.905	11.1
Acetamiprid	222.67	2.95	0.8	0.7
Nitencyram	270.72	590	−0.66	3.1
Dinotefuran	202.21	39.83	−0.549	12.6

at reduced pH (Rigitano et al. 1987), with uptake increasing around pK_a 3 and partition coefficients between 1 and 3.

Apart from the inherent systemic properties exhibited by pesticide active substances, a wide variety of options have been patented in order to increase uptake—by increasing systemicity, solubility, etc.—which are mainly based on a co-formulation of pesticides with copolymers (e.g., Dieckmann et al. 2010a, b; Ishaque et al. 2012). Cell wall permeability of pesticides might also be increased due to the use of polymers (Chamberlain 1992). As a result, uptake by plants, either via the roots or the leaves, is enhanced when polymers are applied.

Imidacloprid and acetamiprid show different uptake capacities by cabbage (70–80 % recovered activity at day 1) and cotton (30–40 % penetration at day 1), respectively. However, both compounds still exhibit 100 % efficacy 12 days following foliar application (Buchholz and Nauen 2002). Non-absorbed active ingredients remain on the surface of the leaves or get associated with epicuticular waxes. Eventually, given their water solubility, these residues could be redissolved into guttation water or morning dew water and could be available to insects.

Imidacloprid uptake via the roots has been shown to range from 1.6 to 20 %, for aubergine and corn, respectively (Sur and Stork 2003). The remainder of the applied active substances is left behind in the soil and should be explored to determine its environmental fate.

The draft assessment report (DAR) of thiamethoxam in 2001 (EFSA 2013b) includes studies of distribution and metabolism of ^{14}C -oxadiazin- and ^{14}C -thiazol-thiamethoxam investigated in corn (seed treatment); pear and cucumber (foliar application); lettuce, potato, tobacco, and rice (soil and foliar treatment). All applications show high and fast uptake (e.g., 23 % recovered activity in the plant within day 1, 27 % of the applied amount being found after 28 h in leaves), where the product is continuously taken up from the soil reservoir for at least 100 days. The metabolism of thiamethoxam is very rapid, both inside the plant and following foliar application

(photodegradation, 30 % degradation in 12 h of sun). Clothianidin is the main metabolite of this active ingredient.

Field experiments show that neonicotinoids tend to have good systemic properties (Maienfisch et al. 2001; Sur and Stork 2003). Fipronil is often described as being less systemic than the neonicotinoids. However, uptake and translocation of this active ingredient following granular application on sugar beets has been confirmed (fipronil DAR from EFSA 2013d). Following a rate application of 2,000 g a.i./ha, 10 times more recovered activity was found in leaves (0.66 mg/kg fipronil equivalents) than in roots 6 months after soil treatment, where 0.06 mg/kg fipronil equivalents were found. In the roots, fipronil sulphone was the main component (64 % of total radioactive residue (TRR), followed by fipronil (14 % TRR) and its amide derivative (RPA200766) (5 % TRR)), while the leaves contained fipronil sulphone (31 % TRR), followed by RPA105320 (18 % TRR) and to a lesser extent MB45950, MB45897, and the amide derivative (less than 0.03 $\mu\text{g/g}$ and 4 % TRR) (see Simon-Delso et al. 2014 for definition of metabolites). Fipronil was found at lower amounts in these leaves. Experiments carried out on corn (420 g a.i./ha) have also shown the systemic activity of fipronil with 0.16, 0.18 and 3.93 ppm of fipronil equivalents being recovered 42, 98, and 106 days after treatment, respectively. Fipronil, its sulfone derivative and its amide derivative were the main components found (fipronil DAR from EFSA 2013d).

Transport of products within the plant

When systemic products are taken up by the roots, the acropetal translocation of pesticides via the xylem sap follows. Translocation into the shoots is described by the transpiration stream concentration factor (TSCF), which is the ratio between the concentration in xylem sap (g/mL) and the concentration in the solution (g/mL). Briggs et al. (1983) found that the translocation of neutral chemicals is most effective for compounds with intermediate lipophilicity. Pesticides with intermediate lipophilicity tend to be xylem mobile. For this

reason, they tend to accumulate in the stem cells and show a decreasing acropetal gradient. However, if polarity or lipophilicity increases, permeability tends to decrease (Briggs et al. 1983). Woody stems retain chemicals more effectively than younger stems due to the lignin content of cells.

The pK_a of imidacloprid (14) indicates that it remains in its undissociated form, despite any pH variations within the plant, diffusing freely within the plant transportation system. As a result, a good membrane penetration and a high xylem mobility can be predicted for imidacloprid ($\log K_{ow}=0.57$). Imidacloprid is therefore expected to be found in the xylem and not in the phloem because of the weak acidity/nondissociation and a TSCF of 0.6 (Sur and Stork 2003). Translocation into the xylem is mainly driven by water flow from the roots to the upper parts of the plant. However, its polarity and solubility in water (0.61 g/L) results in limited retention by tissues and no accumulation in roots (Alsayed et al. 2008). Thiamethoxam is also likely to be translocated (mainly acropetally) via the xylem sap (Maienfisch et al. 2001).

Theoretically, systemic products taken up by the leaves circulate to the rest of the plants mainly via phloem transport. However, translaminar and acropetal mobility have also been observed, with radiolabeled imidacloprid being shown to move toward the leaf tips and margins following foliar application (data from DAR). Aphid mortality tests confirmed the rapid systemic translocation of imidacloprid and acetamiprid within 1 day of application. Following foliar application, thiamethoxam also tends to accumulate in the leaf tips. This might be the reason that guttation water (excreted from the leaf margin) is so concentrated with neonicotinoid active ingredients (Girolami et al. 2009).

Phloem mobility tends to occur with compounds of intermediate lipophilicity ($\log K_{ow}$ between 1 and 3) and weak acidity (pK_a between 3 and 6) (Rigitano et al. 1987; Trapp 2004). The ion trap theory has been proposed for polar undissociated molecules, which exhibit intermediate permeability through cell walls and being translocated in the phloem immediately after application.

Imidacloprid exhibits xylem translocation, meaning that it is found mainly in the shoots and leaves. Following foliar application of a spray formulation of imidacloprid, a maximum of 0.1 % recovered activity could be found in fruits (Sur and Stork 2003). Imidacloprid is not translocated via the phloem; therefore, in theory, the amount of residues found in roots, fruits, and storage organs should be minimal (imidacloprid DAR 2006). However, some of its metabolites meet the physical-chemical conditions to be basipetally translocated, as for example 6-chloronicotinic acid. As a result, this compound or others with the same characteristics can be found in plant parts different from the site of application (Chamberlain et al. 1995).

Soil applications to potato and cucumber confirm the systemic property and acropetal mobility of thiamethoxam and show that the degree of uptake depends upon the method of application as well as the plant species and that this product tends to accumulate at the leaf tips and borders (thiamethoxam DAR). Leaf application confirms the acropetal translocation with relatively high concentrations of thiamethoxam in leaf tips. Small basipetal mobility can also be observed confirming phloem mobility of this compound.

In fact, the amount of imidacloprid, thiamethoxam, clothianidin, or their active metabolites translocated by the phloem seems to be high enough to achieve effective aphid mortality, considering that these insects are mainly phloem feeders (Nauen et al. 2003).

Exposure

As shown in Simon-Delso et al. (2014, this issue), the systemic properties of neonicotinoids and fipronil ensure that these compounds are taken up in all parts of the treated plant. There is much variability in pesticide dissipation (half-lives) in plants, as shown in a review by Fantke and Juraske (2013). The authors examined 811 scientific literature sources providing 4,513 dissipation times (half-lives) of 346 pesticides, measured in 183 plant species.

Foliage

Exposure of nontarget organisms to neonicotinoids and fipronil can occur via the ingestion of unintentionally treated plant parts (i.e., leaves, flowers, etc.). Depending on the application method, potential exposure by consuming contaminated foliage can take place after seed sowing or after spray treatment and exposure could potentially persist up to point of harvest or beyond. This risk of exposure will differ with crop type and chemical application method. In agricultural production, aerial part of crops is often a major by-product or waste component following the harvest of various crops. These products are often sold and used for varying purposes (livestock feed, industrial products, biofuel production, etc.) but may also be left in or next to the field where the crop is harvested. Again, depending on the crop and application method, this may be an exposure route for nontarget organisms. For example, Bonmatin et al. (2005b) evaluated imidacloprid content in the stems and leaves of maize treated with imidacloprid (Gaucho seed treatment, 1 mg/seed). The average concentration detected in the mixture of stems and leaves at the time of tasseling was 4.1 $\mu\text{g/kg}$, with 76 % of the samples containing more than 1 $\mu\text{g/kg}$.

Another example is sugar beet foliage, which is separated from the beet during harvesting and may be left on the field. Westwood et al. (1998) found that 3 weeks after spray treatment at a rate of 0.9 mg/seed of imidacloprid, leaves of sugar

beet seedlings contained an average of 15.2 µg/kg. Rouchaud et al. (1994) applied imidacloprid in the form of a seed dressing at 90 g/ha. The highest concentration of 12.4 mg/kg fresh weight was found in sugar beet leaves in the first week after sowing and concentrations remained greater than 1 mg/kg for 80 days after sowing. However, imidacloprid was not detected in the roots or leaves of sugar beets at harvest (LOD, 10 µg/kg). Similarly, imidacloprid was not detected in grape leaves at the time of harvest (Mohapatra et al. 2010).

These varying results indicate that exposure of nontarget organisms to parent compounds via contact with treated foliage will depend on the crop, application method, and also the time period following treatment. However, the levels of metabolites are often not taken into account. Sur and Stork (2003) found the main metabolites of imidacloprid in a wide variety of crops including maize, eggplant, cotton, potatoes, and rice. These included the olefin and hydroxyl metabolites of imidacloprid, which are known to have similar levels of toxicity in *A. mellifera* as the parent compound (Suchail et al. 2001). Based on the overview of parent compounds and metabolites found in nectar and pollen (*vide supra*), contact with or ingestion of treated foliage may indeed represent a route of exposure to nontarget organisms. This is further substantiated in the case of fipronil-contaminated silage (maize, dry material) which was found to contain 0.30 ng/g of fipronil and 0.13 ng/g of the metabolite sulfone-fipronil (sulfide-fipronil <0.025 ng/g). Furthermore, this indirectly led to the contamination of cow milk with sulfone-fipronil, at an average value of 0.14 ± 0.05 µg/L (0.14 ± 0.05 ppt) (Le Faouder et al. 2007).

Tree treatment

Imidacloprid is currently used to protect trees against wood-boring insects such as the emerald ash borer (*Agrilus planipennis fairmare*) or the Asian longhorned beetle (*Anoplophora glabripennis motschulsky*). It can be applied either through soil injection (drenching) at the base of the tree or through trunk injection, with the systemic action of imidacloprid providing protection for the entire tree (Cowles et al. 2006; Poland et al. 2006; Kreutzweiser et al. 2009).

Cowles et al. (2006) studied the concentrations of imidacloprid in Hemlock (*Tsuga* spp.) needles, twigs, and sap using soil and trunk injection methods and found residues after 1 month and up to 3 years after application. The detected concentration of imidacloprid in needles and twigs ranged from stable to increasing at times during the 3 years after application. This was more often the case when a soil injection was used, possibly due to continued uptake through the roots. These findings indicate the relative stability of imidacloprid once it is absorbed by the tree. Tattar et al. (1998) studied imidacloprid translocation in Eastern Hemlock (*Tsuga canadensis*), White Pine (*Pinus strobus*), and Pin Oak

(*Quercus palustris*) using soil and trunk applications. Although a continuous increase in imidacloprid concentration was observed in *Q. palustris* and *T. canadensis* after soil application, the restricted sample size ($n=6$) and sampling period render these results inconclusive with regard to the persistence of imidacloprid in these tree species. In addition, the concentration of imidacloprid in *P. strobus* needles began to decrease 12 weeks after treatment, indicating that the degradation of imidacloprid in tree foliage may be species-dependent. Multiple factors can be hypothesized to play a role in this mechanism including exposure to light, temperature differences, and the efficiency of translocation within the tree.

The efficacy of fipronil, acetamiprid, and imidacloprid as tree treatments were studied by Grosman and Upton (2006). In contrast to imidacloprid, fipronil appeared to take more than 1 month to disperse throughout all tree parts in *Pinus taeda* L. The authors hypothesized that fipronil could protect these trees for more than 1 year, again indicating this compound may be quite stable once acquired by tree tissues. The use of other neonicotinoids for tree treatment has not been documented, and therefore cannot be taken into account.

Guttation and related risk for honeybees

Guttation (Burgerstein 1887) is a natural phenomenon observed in a wide range of plant species (Bugbee and Koerner 2002; Singh and Singh 2013). Guttations are water droplets that are exuded from specific secretory tissues (hydathodes) located along the margins and tips of leaves in response to root pressure or excess water conditions (Goatley and Lewis 1966; Koulman et al. 2007; Katsuhara et al. 2008; Duby and Boutry 2009). These aqueous solutions may contain a variety of both organic and inorganic compounds (Singh et al. 2009a; Singh et al. 2009b). This phenomenon is mainly observed during the first hours of the morning; however, it can also occur throughout the day depending on environmental conditions. Guttations are also a mechanism by which plants regulate leaf turgidity (Curtis 1944; Knipfer et al. 2011).

In a comprehensive review of guttations, Singh and Singh (2013) reported that different secretory organs such as nectaries, hydathodes, and trichomes, produce secretions with varying functions including the disposal of solutes, improvement of hormone and nutrient acquisition, attraction (i.e., for pollination) or repulsion (for defense purposes). However, these liquid secretions are not to be confused with guttations, which are much more prominent. In addition, adult plants do not produce guttations regularly, while young plants tend to produce guttations frequently and at greater volumes.

As for the presence of insecticide residues in guttations, adult plants are normally treated with spray formulations which lead to active ingredient concentrations in the ppb range or below (Shawki et al. 2005). Conversely, guttations produced by seedlings grown from coated seeds can reach

insecticide concentrations of hundreds of ppm (Girolami et al. 2009; Tapparo et al. 2011). In our opinion, it is crucial to distinguish the risk posed by contaminated guttations arising from young versus mature plants, so as to accurately estimate the risk of acute intoxication for bees via ingestion and/or contact with guttations from insecticide-treated plants such as cereals. Moreover, in regions dominated by cereal production, the land area devoted to these crops is often greater than that of other noncereal crops. As a consequence, cereal guttations (i.e., maize guttations) may be produced across millions of hectares (Girolami et al. 2009).

The production of guttations by corn plants in southern Europe occurs during the first 3 weeks after seedling emergence. The produced amount is not well quantified; a first estimation indicates that each seedling produces 0.1–0.3 mL per day of guttations during the initial period of high guttation production, and less than 0.1 mL per day during the final days in which the phenomenon occurs (Girolami et al. 2009).

These aqueous solutions have not been considered as a potential source of contamination for insects since 2005. Shawki et al. (2005) evaluated the guttations of adult plants sprayed with an organophosphate insecticide and detected sub-ppb levels of active ingredient in droplets. The translocation of neonicotinoid insecticides from coated seeds to young plant guttations (at ppm levels) was observed for the first time in maize seedlings in spring 2008 (Girolami et al. 2009). Because neonicotinoids are water soluble and circulate systemically, residues or high concentrations of active ingredients can be found in guttation drops (Tapparo et al. 2011). The time at which samples are collected for analysis can strongly influence the detection of neonicotinoids in guttations. For example, the same authors show that 1 month after sowing, the concentration of insecticides in guttations decreases dramatically to a few ppb.

In general, neonicotinoid concentrations in guttation drops of corn seedlings show very high variability, and are only partially influenced by the amount of insecticide coating on the surface of the seed (Tapparo et al. 2011). The systemic properties and chemical stability of neonicotinoids in the soil and also within the plant seem to have strong effects on concentrations in guttation droplets. Values of a few ppm have been measured in Northern Europe (Reetz et al. 2011; Pistorius et al. 2012) while values of 10–1,000 ppm have been observed for at least 2 weeks by Girolami and co-workers in Italy (Girolami et al. 2009; Tapparo et al. 2011).

In addition, several climatic variables can affect neonicotinoid concentration in guttation drops of corn seedlings. Preliminary experiments in Italy demonstrate that under high humidity conditions (close to saturation, a situation that often occurs during the morning in spring) insecticide concentrations can be 10 times lower than those observed in guttations formed during the following sunny hours. This difference could be relevant especially in the warmer area of

Europe. Moreover, guttation production by corn seedlings may be dramatically reduced or ended under low humidity conditions (RH 50–60 %). Rain can reduce the concentration of insecticide in guttations by about 10 times with respect to the values observed the day before a rainfall event. Sunny conditions and a moderate wind can promote water evaporation and affect the concentration of insecticide in guttation drops. On the contrary, strong winds can dislodge droplets off leaves, eliminating any concentration effects that would otherwise occur if droplets remained on the leaves. Finally, soil moisture and composition only moderately affect the insecticide concentration of guttation droplets (APENET 2011), suggesting that air humidity is a significant environmental factor to consider in the case of guttations.

Guttations contaminated by high levels of neonicotinoids can also be produced by other insecticides. For instance, clothianidin can be applied in granular form directly to the soil during corn sowing, giving concentration levels of the same order of magnitude (or slightly lower) of those observed in guttations produced from coated seeds (Pistorius et al. 2012) and with almost identical levels of acute toxicity for bees. Another interesting case concerns the massive use of insecticide applied directly to the soil with irrigation water (fertigation) and inducing concentrations of neonicotinoids in guttations of cucurbitaceae in the range of a few ppm (Stoner and Eitzer 2012; Hoffman and Castle 2012). Thus, environmental contamination is possible, but it is not comparable to guttations from young plants obtained from coated seeds.

It is worth noting that corn guttations may show concentrations of insecticide higher than 1,000 ppm (mg/L); these values match the insecticide content (about 1‰) of the aqueous solutions used for foliar spray treatments. Despite the high levels of contamination, the influence of toxic guttations on spring losses of bees appears to be limited, as reported in Girolami et al. (2009) and Tapparo et al. (2011). Generally, bees collect water from spontaneous vegetation, well before maize emergence, and they do not require guttation droplets from maize fields. Although some individual explorer may drink guttations from the maize field, it would die in a few minutes (due to high pesticide concentration, lethal for bees even by contact only) and not have the time to communicate the presence of the water source to the colony. This does not exclude that the large extensions of poisonous drops cannot constitute a problem for other pollinators that nest in the ground (*Andrena* spp., *Halictus* spp.) or have an erratic behavior (*Bombus* spp. for example), resulting from the fact that they do not have communication ability through dance like bees. Those species would be killed by contact with contaminated guttations.

Concerning other systemic insecticides, the absence of relevant literature hinders any solid conclusion. As preliminary data, we can report that guttations of corn seedlings obtained from seeds coated with fipronil contain lower

concentrations of the insecticide (ppb levels) with respect to those obtained with neonicotinoid seed coating. Nevertheless, if administered to bees (solution with 15 % honey), these guttations are lethal within minutes, indicating the possible presence of metabolites with high acute toxicity (Girolami et al. 2009).

Resin (propolis)

Resin is harvested by honeybees (*A. mellifera*) and used as propolis for sealing holes and evening out surfaces within the beehive. Sources of propolis are tree buds and exudates from plants. Although pesticide residues have been reported in propolis, no information is available about neonicotinoids or fipronil.

Pareja et al. (2011) hypothesize that sunflower resin can be used by honeybees, thereby making it a possible source of pesticide exposure. The authors took five propolis samples from depopulated hives located near sunflower crops, which were also the only crops in the area to be previously treated with imidacloprid. Imidacloprid was detected in two of the samples at 20 and 100 ng/g, respectively, which supports the hypothesis that sunflower resin may be a potential exposure route for honeybees and other nontarget organisms that collect resin.

Presence in plant reproductive organs and fruits

Intake of systemic insecticides through residues in fruits and vegetables is a potential risk to invertebrates and vertebrates alike. Fruit and vegetables deemed unfit for human consumption may be discarded in piles that are easily accessible to various organisms. In addition, inadequate storage methods may provide further means of exposure to these insecticides.

The concentration of residues in the reproductive organs of plants following treatment varies with plant species and application method. Translocation studies show imidacloprid residues in plant reproductive organs ranging from 0.7 to 12 % of the originally applied soil treatments in rice and potato plants, respectively (Sur and Stork 2003). Sunflower treated with fipronil through soil treatment shows 0.2 % of the applied product in flower heads and seeds (EFSA 2013d, fipronil DAR).

Concerns regarding the contamination of fruits and vegetables with regard to human health are beyond the scope of the present study. However, the translocation of residues of systemic products into fruits can be achieved either by their transport through the xylem or phloem (Alsayeda et al. 2008), although the mechanisms of accumulation in fruits are not yet fully understood. Juraske et al. (2009) studied the human intake fraction of imidacloprid for unwashed tomatoes and found that it varies between 10^{-2} and 10^{-3} ($\text{kg}_{\text{ingested}}/\text{kg}_{\text{applied}}$) depending on the time of consumption. This was the case for tomato plants

treated with the recommended doses in spray application as well as chemigation. Sur and Stork (2003) found that tomato and apple exhibit 21 and 28 % recovery of applied compounds following a foliar application. More than two thirds of this recovery was located on the surface of the fruits. A study by Zywitz et al. (2004), examined a range of fruit and vegetable groups for which neonicotinoid residues could be detected ($\text{LOD}=3$ ng/g) and quantified (limits of quantification ($\text{LOQ}=5$ ng/g) (Table 3). Fruiting vegetables (tomatoes, pepper, cucumbers, courgettes, and melon) exhibited the highest number of positive samples (46.7 %), followed by leafy vegetables and fresh herbs (lettuce, cress, spinach, dill, chives, and parsley; 10 %), stone fruits (peach, nectarine, apricot, and cherry; 4.5 %), pome fruits (apple and pear; 2.9 %), and berries (strawberry, raspberry, currant, blueberry, and grape; 2.2 %). No information was provided on the method of application of neonicotinoids or the doses used. More recently, 22 % of fruits sampled in India showed the presence of imidacloprid and 2 % were above the maximum residue level (MRL) (Kapoor et al. 2013). A similar situation has been described in Turkey, with levels of acetamiprid in vegetables occurring above the allowable MRL (Sungur and Tunur 2012).

The contamination of nectar and pollen following treatment with neonicotinoids and fipronil is well known. Sunflowers seed-treated with imidacloprid have been shown to contain an average of 4.6 ng/g in the stems and leaves, 8 ng/g in flowers, and 3 ng/g in pollen (Bonmatin et al. 2003). In maize, Bonmatin et al. (2005b), showed a mean recovery of 4.1 ng/g in stems and leaves (max 10 ppb), 6.6 ng/g in male flowers (panicles, max 33.6 ng/g), and 2.1 ng/g in pollen (max 18 ng/g) following seed dressing at a rate of 1 mg/seed. Monitoring studies in Austria reported thiacloprid levels in nectar or honey to be between 11.1 and 81.2 ng/g (Tanner 2010). An extensive review of the contamination of pollen and nectar is given below.

Pollen and nectar

Pollen and nectar from flowers are collected by bees and form an integral component of their diet. Pollen and nectar also constitute the feeding resources of many nontarget insects of less economic importance. The contamination of pollen and nectar has been measured mainly for honeybees and bumble bees. However, these measurements also represent valuable starting points for assessing exposure risks of other nontarget species.

Pollen can be sampled in different forms—it can be obtained directly from flowers, by trapping from bee hives (bee-collected pollen pellets), or from bee bread (bee-mixed pollen and nectar). Nectar is converted by bees into raw/fresh honey and it is also a component of bee bread. Obviously, contamination of these matrices depends heavily on the presence of residues in flowers (Bonmatin et al. 2003; Aajoud et al. 2008)

Table 3 Quantity of positive samples of neonicotinoids in multiple fruit groups

Group	Commodities analyzed	Nb. of samples	Nb. positive samples	Nb. samples > MRL
Citrus fruits	Lemon, orange, mandarin, grape fruit	177	2	0
Stone fruits	Peach, nectarine, apricot, cherry	111	5 (4.5 %)	0
Pome fruits	Apple, pear	175	5 (2.9 %)	0
Berries	Strawberry, raspberry, currant, blueberry, grape	556	12 (2.2 %)	3 (0.5 %)
Tropical and subtropical fruits	Pineapple, kiwi, kaki, mango, kumquat	101	1	1
Leafy vegetables and fresh herbs	Lettuce, cress, spinach, dill, chives, parsley	231	24 (10.4 %)	3 (1.3 %)
Fruiting vegetables	Tomato, pepper, aubergine, courgette, melon, cucumber, chili pepper	540	252 (46.7 %)	104 (19.3 %)
Brassica vegetables	Cauliflower, Chinese cabbage, Brussels sprout, kohlrabi, white cabbage	47	1	0
Root and tuber vegetables	Carrot, radish, swede	39	0	0
Dietary foods, cereals and cereal products	Maize, wheat, commel, maize semolina, bran, rice and other	50	0	0
Legume and stem vegetables	Asparagus, bean, pea, celery	33	0	0
Miscellaneous	Rape, tea, dried fruit, leek, must mash, potato, (concentrated) fruit juice and other	64	0	0

Source, Zywitz et al. (2004)

but also upon the presence of residues found and collected directly in the environment of the bees (water, dust, etc.). Residues are defined as active ingredients used in crops and/or their active metabolites (Simon-Delso et al. 2014, this issue), although other compounds may be present (adjuvants or synergistic compounds). These other compounds are generally not considered for analysis or assessment, but could be of importance for toxicity toward nontarget species (Mesnage et al. 2014). However, it is often only the active ingredient which is measured in the majority of cases. Residues contained in pollen and nectar can be transformed or metabolized by bees, inside and outside the hive. Such complex processes are not well understood. Furthermore, these residues can cross-contaminate other matrices (bees, pollen, bee bread, nectar, honey, wax, propolis, royal jelly, etc.) (Rortais et al. 2005; Chauzat et al. 2006; Mullin et al. 2010). The routes of exposure for honeybees, bumble bees, and solitary bees were identified by the European Food Safety Authority (EFSA 2012a) and ranked from 0 (no route of exposure) to 4 (highly relevant route of exposure). Although some of these routes will need to be re-evaluated as new evidence comes to light, nectar and honey, pollen, and bee bread all share the highest scores and are therefore the most likely routes of exposure for bees.

Assessment The ecological risks of active ingredients are assessed using the hazard quotient (HQ) calculation. This approach estimates whether harmful effects of the contaminate in question may occur in the environment by comparing the Predicted Environmental Concentrations (PEC) to the Predicted No Effect Concentration (PNEC). HQ calculations

do not consider the mode of insecticide application, the systemic properties, routes of exposure, or the persistence or metabolism of pesticides. Historically, these calculations have been inaccurate due to a lack of adequate analytical techniques for the quantification of residues in matrices like pollen and/or nectar. This was the case for imidacloprid and fipronil in the 1990s—the initial risk assessment assumed that flowers were not significantly contaminated with respect to the LD₅₀ values for bees and so the PEC was underestimated at the time of registration (Maxim and van der Sluijs 2007). However, with the improvement of analytical techniques, the detection of residues in pollen/bee bread and nectar/honey have become more accurate (Bonmatin et al. 2005a; Dively and Kamel 2012; Paradis et al. 2014), and show that the PEC values are actually significantly higher. Meanwhile, new understanding of the sublethal and chronic exposure effects on bees has improved the PNEC value, and demonstrates that this value was clearly overestimated during the registration of these products (Suchail et al. 2001; Whitehorn et al. 2012). It was only in the early 2000s that assessments were conducted for imidacloprid using accurate data (Rortais et al. 2005; Halm et al. 2006). This work considered both (1) different exposure pathways and (2) relative needs in food among various castes of honeybees (foragers, nurses, larvae, winter bees, etc.).

The risk assessment of pesticides on bees has recently been completed in the EU. Currently, the risk of pesticides to bumble bees and solitary bees is taken into account (EFSA 2012a; EFSA 2013f) and different exposure forms are considered: (a) ingestion, (b) contact, and (c) inhalation. Additionally, bees are now assessed for (1) exposure inside the hive including food (mainly honey and bee bread), nest

(including wax and propolis), and other bee products and (2) exposure outside the hive including water, plants (considering several matrices such as nectar and pollen as a food supply), guttation, air, dust, soil, etc. The same approach could be used for any other species feeding on pollen and/or nectar.

Variability One of the main difficulties is the variability of measured data in these relevant matrices which depends significantly on the dose and mode of treatment, the studied crop, season, location, soil, weather, time, bees, etc. Even different crop varieties can induce significant variability in the residue content of pollen and nectar (Bonmatin et al. 2007). Additional sources of variability include variations in the amount of contaminated versus uncontaminated food harvested by bees (e.g., the proportion of treated pollen/total pollen and the proportion of treated nectar/total nectar); differences in metabolism between foragers and in-hive bees; the availability of alternative plant resources; the “filter” effects made by bees (e.g., trapped pollen is only brought back by nonlost foragers); the distance between treated crops and hives; effects of mixture (e.g., mixing nectar and pollen to produce bee bread) and the effects of concentration (e.g., reducing water content to produce honey from nectar); this list being non-exhaustive. Furthermore, measurements are not always performed on the same matrices or are influenced by the choice of samples and their location (experimental area) by the experimenters, which make comparisons of risk difficult. This is particularly relevant for water contamination, as water resources can differ significantly in their composition (surface water, ephemeral pooling, guttation etc.; EFSA 2013f) and because the concentration of contaminants in surface water can vary within the same area of foraging, from a few nanogram per liter (ppt) to a few nanogram per milliliter (ppb) (Starter and Goh 2012; Van Dijk et al. 2013; Goulson 2013; Main et al. 2014; Bonmatin, personal communication).

The contamination of fresh and stored honey originates from the presence of residues in nectar. Honey in beehives can be less contaminated than nectar. This situation was reported from sunflowers treated by seed dressing (Schmuck et al. 2001), but could have been due to a dilution effect, whereby mixture of treated and untreated nectar yields lower levels of contamination, as in the case of mixing pollen (*vide supra*). The opposite situation has also been described for citrus trees treated with soil applications (Byrne et al. 2014). Although the sum of processes remains poorly understood, it is known that there is an initial metabolism during transport and diverse chemical reactions and processing are conducted by workers—where the concentration factor is affected by the amount of water in the nectar (Winterlin et al. 1973) and by degradation over time leading to metabolites (Simon-Delso et al. 2014, this issue). Because foragers and in-hive bees participate in these metabolic processes, it can be assumed that in cases of high contamination of nectar, honey would not

be stored in the hive so efficiently, due to deleterious effects on the global functioning of the beehive (Bogdanov 2006; EFSA 2012a).

In pollen, differences have been reported between samples directly taken from crops and pollen pellets brought back by bees to the beehive. These differences in contamination are mainly due to significant dilution effects when bees mix pollen from treated crops with that of untreated crops (Bonmatin et al. 2003, 2005b). Furthermore, when pollen is stored in the beehive to constitute bee bread, a range of chemical and biochemical processes occur which can contribute to the differences in residue levels between pollen types.

Another important source of variability comes directly from sampling protocols and analytical methods. It is clear that the latter are not harmonized, as evidenced earlier by the calculation of the HQ values. In the early 1990s, analytical techniques had not been improved sufficiently to measure contamination levels in the range of nanograms per gram (ppb). LOD and LOQ were higher than at the present time, by 2 orders of magnitude. Chromatography was generally coupled to a less sensitive detection system than those used currently (e.g., UV/Vis spectroscopy versus mass-tandem spectrometry) and the ambiguous statement “nd” (not detected) often suggested the absence of residues. Additionally, it was usually the stems and leaves which were analyzed, flowers being analyzed to a lesser extent. Nectar and pollen were rarely analyzed because extraction methods and detection methods were not efficient or sensitive enough for these particular matrices. More sensitive methods should have been set up more quickly by stakeholders.

The use of improved extraction methods and high-performance chromatography coupled with tandem-mass spectrometry allowed LOQ values to reach the range of 1 ng/g in the early 2000s. These methods were fully validated for the matrices of interest, with an LOD of a few tenths of ppb (Schmuck et al. 2001; Laurent and Rathahao 2003; Bonmatin et al. 2003; Chauzat et al. 2006; Mullin et al. 2010; Wiest et al. 2011; Paradis et al. 2014). Analysis can be further refined by focusing on one compound or a very limited number of compounds within a chemical class. This results in a significantly lower LOD and LOQ than normal screening methods, which are designed for numerous active ingredients. Moreover, extraction yields can be relatively low for some compounds in screening methods, and results are often underestimated because published data are generally not corrected with respect to the yield for each compound. Also, general screening methods are not relevant for risk assessment because this strategy aims to identify and quantify as many active ingredients as possible regardless of whether the active ingredients are pertinent to agricultural practices or not. For these reasons, risk assessment should always use specific targeted methods, whereas screening methods are more appropriate for gaining initial evidence of contamination (e.g., in

unspecific monitoring studies). Recently, intermediate multiresidue methods (analyzing about 10 to 100 active materials) were published and present the advantage of being sensitive over a relatively wide range of residues in matrices such as nectar or honey (Wiest et al. 2011; Paradis et al. 2014). These methods are far better designed for detecting multiple exposures of bees than for risk assessment of one pesticide and are very useful in determining the presence of several pesticides within the same class of chemicals (e.g., neonicotinoids) or between various chemical classes (nicotinoids, phenylpyrazoles, and pyrethroids for instance). This is of particular interest when considering the possibility of additive toxicity or, in some cases, potential synergies.

For all the reasons listed above, it is not surprising that such high variability exists in the measurement of residues in the relevant matrices and this justifies the need for assessments to be based on the worst case scenario when data are lacking. However, there now exists for pollen/beebread and nectar/honey a body of data which allows for defining ranges of contamination of these matrices by the neonicotinoids and fipronil. Because this description is not limited to honeybees, this review focuses on the common food supply that can induce oral and contact toxicity to various types of pollinators.

Pollen and bee bread Data reported by recent scientific reviews, scientific literature, some relevant Draft Assessment Reports (DAR) and other relevant reports, are presented in Table 4 (Johnson et al. 2010; EFSA 2012a; Thompson 2012; EFSA 2013a, c, e; Sanchez-Bayo and Goka 2014). These recent reviews were undertaken to assess pesticide residue levels including neonicotinoids and fipronil. To avoid repetition in the data (e.g., data issuing from citations in cascade), we indicate the original sources in Tables 4 and 5.

According to a global analysis by Sanchez-Bayo and Goka (2014), which does not distinguish between the routes of exposure, crop species, or the mode of insecticide application, the detection rate of various agrochemicals in pollen/beebread were as follows: acetamiprid at 24 %, thiacloprid at 18 %, imidacloprid at 16 %, thiamethoxam at 13 %, clothianidin at 11 %, fipronil at 3 %, and dinotefuran at 1 % (although Dively and Kamel (2012) reported 100 % for dinotefuran). While the active ingredients were not detected or quantified in most of the samples analyzed, the results also show that the oldest measurements often had the lowest occurrence rate, confirming the influence of the sensitivity of analytical techniques on this parameter.

Interestingly, the maximum residue levels in Table 4 are thiacloprid (1,002 ng/g), imidacloprid (912 ng/g), dinotefuran (168 ng/g), acetamiprid (134 ng/g), thiamethoxam (127 ng/g), clothianidin (41 ng/g), and fipronil (29 ng/g). For each of these compounds, these values must be interpreted with respect to the corresponding data for toxicity. However, these

values represent the worst case scenarios. Further examination of exposure data shows that average levels in pollen/beebread are lower than these maximums, due to some data issuing from various types of application techniques (soil treatment, injection, spray, seed dressing, etc.). For example, it has been reported that aerial treatments represent a significantly higher source of contamination than seed-dressing treatments (Thompson 2012; EFSA 2012a). This explains the high variability of results when concentrations are ranked by decades. However, when imidacloprid was used as a seed dressing, mean residue levels were mostly found to be in the range of 1–10 ng/g and variability among crops was not so high (sunflower, maize, and canola), whereas spray or soil application led to higher values, by 1 order of magnitude. To a lesser extent, this was also observed for clothianidin and thiamethoxam. Therefore, averaged data must also be considered to gain a better idea of the average contamination of pollen/beebread: thiacloprid (75 ng/g), dinotefuran (45 ng/g), thiamethoxam (29 ng/g), imidacloprid (20 ng/g), clothianidin (9 ng/g), acetamiprid (3 ng/g), and fipronil (1.6 ng/g) (Sanchez-Bayo and Goka 2014). As a consequence, the latter values are the most relevant for toxicity studies for nontarget species.

Nectar and honey The work conducted by the EFSA (2012b) generally reported lower neonicotinoid concentrations in nectar than in pollen (see also Goulson 2013). Data reported by scientific reviews, scientific literature, and some relevant DARs are presented in Table 5 (Thompson 2012; EFSA 2012a, 2013a, b, d, e; Sanchez-Bayo and Goka 2014). Relatively recent reviews were done for the purpose of assessing neonicotinoids and fipronil. According to a global analysis by Sanchez-Bayo and Goka (2014), thiamethoxam was detected in 65 % of nectar/honey samples, followed by thiacloprid at 64 %, acetamiprid at 51 %, imidacloprid at 21 %, clothianidin at 17 %, and fipronil at 6.5 %. Note that the study of Dively and Kamel (2012) showed that dinotefuran was always detected (100 %) in pumpkin nectar samples in 2009. Contrary to the pollen/beebread case, three neonicotinoids were found in most of the nectar/honey from treated crops (Sanchez-Bayo and Goka 2014). However, the higher proportion of neonicotinoids in nectar/honey than in pollen/beebread could be linked to the higher sensitivity of the analytical techniques used. Validation of analytical methods for nectar/honey generally lead to LOD and LOQ values which are lower than in the case of pollen/beebread (Mullin et al. 2010; Lambert et al. 2013; Thompson et al. 2013), the latter being a difficult matrix to analyze due to the encapsulated nature of pollen and other interferences.

The values of Sanchez-Bayo and Goka (2014) for maximum levels in nectar/honey are thiacloprid (209 ng/g), imidacloprid (73 ng/g), dinotefuran (22 ng/g), thiamethoxam (17 ng/g), acetamiprid (13 ng/g), and clothianidin (10 ng/g).

Table 4 Residues (neonicotinoids and fipronil) in pollen or in pollen-derived matrices (pollen/beebread)

Insecticide ^a	Detection rate ^b (%)	Range ^c (ng/g)	Mean ^d or magnitude ^{e,f} (ng/g)	Maximum ^f (ng/g)	Reference ^g
Acetamiprid	24.1	1–1,000	3	134	Sanchez-Bayo and Goka (2014)
	45	0.1–100	4.1	26.1	Pohorecka et al. (2012)
	3.1	10–1,000	59.3	134	Mullin et al. (2010)
Clothianidin	11	1–100	9.4	41.2	Sanchez-Bayo and Goka (2014)
		0.1–100	0.1 ^h to 17.1 ^h	21.1 ^h	Dively and Kamel (2012)
		1–10	1 ⁱ to 4 ⁱ	7	Pilling et al. (2014)
	11	1–10	1.8	3.7	Pohorecka et al. (2012)
		1–100	3.9	10.7	Krupke et al. (2012)
		1–100			In EFSA (2013a):
			7.38-	36.88	See estimate for maize
			5.95-	19.04	See estimate for rape
				3.29	See estimate for sunflower
				15	See Schöning 2005 (DAR)
		1–10		2.59	Cutler and Scott-Dupree (2007)
		1–10		2.8	Scott-Dupree and Spivak (2001)
		1–10			In EFSA (2012a):
				10.4	See Nikolakis et al. (2009) (DAR)
			2.6-	2.9	See Maus and Schöening (2001) (DAR)
Dinotefuran	1	10–1,000	45.3	168.1	Sanchez-Bayo and Goka (2014)
	100	10–1,000	11.2 to 88.3+17.1 ^j	147+21.1 ^j	Dively and Kamel (2012)
	1	1–10	4	7.6	Stoner and Eitzer (2013)
Imidacloprid	16.2	1–1,000	19.7	912	Sanchez-Bayo and Goka (2014)
		0.1–1,000	0.1 to 80.2+19.1 ^k	101+27.5 ^k	Dively and Kamel (2012)
	9.1	1–1,000	30.8	216	Rennich et al. (2012)
	2.9	1–1,000	39	206+554 ^l +152 ^l	Mullin et al. (2010)
	40.5	0.1–10	0.9	5.7	Chauzat et al. (2011)
		1–100	14	28	Stoner and Eitzer (2012)
	12.1	1–100	5.2+5.6 ^l	70+5.6 ^l	Stoner and Eitzer (2013)
		10–100	13	36	Laurent and Rathahao (2003)
	87.2	0.1–100	2.1	18	Bonmatin et al. (2005)
		1–100	9.39	10.2	Byrne et al. (2014)
		1–100	2.6	12	Wiest et al. (2011)
	83	0.1–100	3	11	Bonmatin et al. (2003)
		1–100			In EFSA (2013c):
			3-	15	See Stork (1999) (Germany 2005, DAR)
			3.45-	4.6	See Germany 2005 (DAR)
		1–10			In EFSA (2012a):
			1.56-	8.19	See Schmuck et al. (2001) (DAR)
				3.3	See Stork (1999) (Germany 2005, DAR)
Thiacloprid		1–10	4.4-	7.6	Scott-Dupree and Spivak (2001)
	49.4	1–10	1.2		Chauzat et al. (2006)
		1–10	3.3-	3.9	Schmuck et al. (2001)
	0.8	1–10	1.35	<12	Lambert et al. (2013)
		0.1–1		<0.5	Thompson et al. (2013)
	17.7	100–1,000	75.1	1,002.2	Sanchez-Bayo and Goka (2014)
	62	1–1,000	89.1	1,002.2	Pohorecka et al. (2012)
	2	1–1,000	187.6	326	Rennich et al. (2012)
	5.4	1–1,000	23.8	115	Mullin et al. (2010)

Table 4 (continued)

Insecticide ^a	Detection rate ^b (%)	Range ^c (ng/g)	Mean ^d or magnitude ^{e,f} (ng/g)	Maximum ^f (ng/g)	Reference ^g
Thiamethoxam	1.3	1–100	22.3	68	Stoner and Eitzer (2013)
		1–1,000			In EFSA (2012a):
			150–	277	See Von der Ohe (DAR)
	12.8		9–	36	See Schatz and Wallner (2009) (DAR)
		1–100	10 to 30	90	Skerl et al. (2009)
		10–1,000	28.9	127	Sanchez-Bayo and Goka (2014)
		0.1–1,000	0.1 to 95.2+26.8 ^h	127+35.1 ^h	Dively and Kamel (2012)
	0.3 %	10–100	53.3	53.3	Mullin et al. (2010)
		1–100	12	35	Stoner and Eitzer (2012)
		1–10	3.8	9.9	Pohorecka et al. (2012)
	37	1–10	2.8	4.1	Stoner and Eitzer (2013)
	1	1–100	3 ⁱ to 7 ⁱ	12	Pilling et al. (2014)
		1–100	1.7	6.2 to 20.4	Krupke et al. (2012)
		1–100			In EFSA (2013b):
			13.41–	21.51	See estimate for maize
			2.37–	3.02	See estimate for sunflower
			4.59–	19.29	See estimate for rape
			4–	12	See Hecht-Rost (2007); Hargreaves (2007) (DAR)
		1–10	2.3 to 2.7		Thompson et al. (2013)
		0.1–10			In EFSA (2012a):
			2.5–	4.2	See Schuld (2001a) (DAR)
6-CNA	33	0.1–10	1.2	9.3	See Schuld (2001b) (DAR)
	57.3	0.1–10	1.2		See Barth (2001) (DAR)
	44.4	0.1–10	1.2		See Balluf (2001) (DAR)
Fipronil	2.8 and 3.7 ^m	1–100	1.6	29	See Schur (2001c) (DAR)
	0.3	1–100	28.5	28.5	Chauzat et al. (2011)
	6.5	0.1–10	1.2+1.0+1.7 ^m	0.3+1.5+3.7 ^m	Chauzat et al. (2009)
	0.6	1–10	2.8	3.5	Chauzat et al. (2006)
	3.7 ^m	1–10	2 to 2.3 ^m	4	Sanchez-Bayo and Goka (2014)
	49 ^m	0.1–10	0.8 ^m	8.3 ^m	Mullin et al. (2010)
	12.4	0.1–10	1.2	1.2+1.7+1 ^m	Chauzat et al. (2011)
		1–10		1.9 and 6.4	Stoner and Eitzer (2013)

6-CNA (6-chloro-nicotinic acid)

^a Active ingredient^b Proportion of positive analyses (see text)^c Classified by decade^d Mean value from positive analyses^e The lowest value of quantified data is followed by a hyphen, the highest value is in the next column^f The highest value of quantified data^g The sources are related to the original works for avoiding data duplications, and data from DARs (draft assessment report) are available in the cited EFSA reviews^h Clothianidin issuing from thiamethoxamⁱ Median values^j When data include the UF (1-methyl-3-(tetrahydro-3-furylmethyl)urea) derivative^k When data include the derivatives of imidacloprid (olefin, 5-OH, urea, desnitro olefin, desnitro HCl, and 6-CNA)^l When data include the derivatives of imidacloprid (5-OH, olefin, or 6-CNA)^m Data include some fipronil derivatives (sulfone-, sulfide-, or desulfynyl-fipronil)

Table 5 Residues (neonicotinoids and fipronil) in nectar or in nectar-derived matrices (nectar/honey)

Insecticide ^a	Detection rate ^b (%)	Range ^c (ng/g)	Mean ^d or magnitude ^{e,f} (ng/g)	Maximum ^f (ng/g)	Reference ^g
Acetamiprid	51	0.1–100 0.1–1,000	2.4	13.3 112.8	Sanchez-Bayo and Goka (2014); Pohorecka et al. (2012) Paradis et al. (2014)
Clothianidin	17	0.1–10 0.1–100	1.9 0.1 ^h to 4 ^h	10.1 12.2 ^h	Sanchez-Bayo and Goka (2014) Dively and Kamel (2012)
		1–10 0.1–10	2.3 0.9-	10.1 2.2	Pohorecka et al. (2012) Cutler and Scott-Dupree (2007); Johnson et al. (2010)
	100	0.1–1 10–1,000	1 ⁱ 89-	1 319	Pilling et al. (2014) Larson et al. (2013)
		0.1–100 0.1–10 0.1–10	5 1- 1.2-	16 3 8.6	Thompson et al. (2013) Wallner (2009) See Schmuck and Shöening (2000a) (DAR)
			0.3-	1	See Maus and Schöening (2002a) (DAR)
			2.8-	3	See Maus and Schöening (2001b) (DAR)
				5.4	See Maus and Schöening (2001c) (DAR)
		0.1–10 0.1–10	0.9- 0.32	3.7	Scott-Dupree and Spivak (2001) EFSA (2013a) (estimate)
		1–100	13.7	21.6	Sanchez-Bayo and Goka (2014)
	100	1–100	2.1+0.1 ^j to 9.2+4.1 ^j	10.8+10.8 ^j	Dively and kamel (2012)
Imidacloprid	21.4	1–100 10–100	6 13.37 to 72.81	72.8 95.2	Sanchez-Bayo and Goka (2014) Byrne et al. (2014)
		0.1–100	0.1 to 11.2+6.4 ^k	13.7+9.4 ^k	Dively and Kamel (2012)
	21.8	0.1–10 100–1,000 100–1,000	0.7 6.6+1.1+0.2 ^l	1.8 660 ^j 171	Chauzat et al. (2011) Paine et al. (2011) Larson et al. (2013)
		1–100	0.1 to 11.2+6.4 ^k	16+2.4+0.5 ^l	Krischik et al. (2007)
		0.1–100 1–100 1–10	0.1 to 11.2+6.4 ^k 10.3 3.45-	13.7+9.4 ^k 14 4.6	Dively and Kamel (2012) Stoner and Eitzer (2012) See Stork (1999) (DAR)
			1.59-	8.35	See Germany (2005) (DAR)
		29.7	0.7+1.2 ^l		Chauzat et al. (2009)
		0.1–10 0.1–10	1.9 0.6		Schmuck et al. (2001) Pohorecka et al. (2012)
		21	0.2 ^l -	3.9 ^l	Wiest et al. (2011)
		2.1	0.14 ^l 0.1–10 0.1–1 0.1–1	<3.9 ^l 0.8 0.5	Lambert et al. (2013) Scott-Dupree and Spivak (2001) Thompson et al. (2013)
			0.45		
Thiacloprid	64	1–1,000 1–100 1–100 1–100	6.5 1.8 11.6	208.8 36 33 11.6	Sanchez-Bayo and Goka (2014); Pohorecka et al. (2012) Schatz and Wallner (2009) Johnson et al. (2010) Paradis et al. (2014)
Thiamethoxam	65	0.1–100 0.1–100	6.4 0.1 to 9.5+4 ^h	17 12.2+6.4 ^h	Sanchez-Bayo and Goka (2014) Dively and Kamel (2012)
		0.1–100	4.2	12.9	Pohorecka et al. (2012)
	65	0.1–10 1–100 0.1–10 0.1–10	0.7 to 2.4 ⁱ +1 ⁱ 11 0.59	4.7+1 20 4	Pilling et al. (2014) Stoner and Eitzer (2012) EFSA (2013b); see Hecht-Rost (2007) (DAR)
		0.1–10		1.5 and 3.9	Thompson et al. (2013)

Table 5 (continued)

Insecticide ^a	Detection rate ^b (%)	Range ^c (ng/g)	Mean ^d or magnitude ^{e,f} (ng/g)	Maximum ^f (ng/g)	Reference ^g
		0.1–10	0.65	2.72	EFSA (2013e) (estimate)
		0.1–10		2	Paradis et al. (2014)
		0.1–10			In EFSA (2012a):
			1.0	2.1	See Shuld (2001a) (DAR)
				0.9	See Purdy (2000) (DAR)
				1	See Balluf (2001) (DAR)
6-CNA	17.6	0.1–10	1.2	10.2	Chauzat et al. (2011)
Fipronil	6.5	10–100	70	100	Pareja et al. (2011)
	0.3	10–100	28.5		Mullin et al. (2010)
		0.1–10			In EFSA (2013d):
			2.3	6.4	See Kerl (2005) (DAR)
				3.3	See Bocksch (2009) (DAR)

6-CNA (6-chloro-nicotinic acid)

^a Active ingredient^b Proportion of positive analyses (see text)^c Classified by decade^d Mean value from positive analyses^e The lowest value of quantified data is followed by a hyphen, the highest value is in the next column^f The highest value of quantified data^g The sources are related to the original works for avoiding data duplications, and data from DARs (draft assessment report) are available in the cited EFSA reviews^h Clothianidin issuing from thiamethoxamⁱ Median values^j When data include the UF (1-methyl-3-(tetrahydro-3-furylmethyl)urea) derivative^k When data include the derivatives of imidacloprid (olefin, 5-OH, urea, desnitro olefin, desnitro HCl, and 6-CNA)^l When data include the derivatives of imidacloprid (5-OH, olefin, or 6-CNA)

From these data, it appears that nectar/honey is significantly less contaminated than pollen/beebread, by a factor of 4 (clothianidin) to 12 (imidacloprid). Note that very recently, Paradis et al. (2014) reported a maximum of 112.8 ng/g in nectar for acetamiprid, Larson et al. (2013) reported 319 ng/g for clothianidin, Paine et al. (2011) reported 660 ng/g for imidacloprid, and Pareja et al. (2011) measured 100 ng/g for fipronil. The maximum level of fipronil in nectar/honey is three times higher than that in pollen/beebread, despite the fact that fipronil is less water soluble than the neonicotinoids. Obviously, these levels must be interpreted with respect to the corresponding toxicity data for each of these compounds. Another study by Kasiotis et al. (2014) measured a maximum residue level of imidacloprid of 73.9 ng/g, this value being similar to the 95.2 ng/g value detected by Byrne et al. (2014). The maximum for imidacloprid was found to be 41,273 ng/g by Kasiotis et al. (2014); however, it should be noted that some sampling was conducted directly by beekeepers after bee collapse incidents, so it is possible that external contamination may have occurred (data not included in Table 5). As with the residue levels in pollen and bee bread, these values

represent a worst case situation and do not give a general measure of contamination.

Table 5 shows that average residue levels in nectar/honey are significantly lower than the above maximums, again due to the data issuing from various types of application techniques (soil drench, injection, spray, seed dressing, etc.). Again, aerial treatments represent a significantly higher source of contamination in nectar/honey than when used as a seed dressing (Thompson 2012; EFSA 2012a). This explains the high variability of results when concentrations are ranked by decades, as observed for imidacloprid for instance. Similar to the case of pollen/beebread, imidacloprid used as seed dressing led to levels mainly in the range of 1–10 ng/g (sunflower, cotton, and canola; EFSA 2013c), but soil application on eucalyptus led to higher values by 2 orders of magnitude (Paine et al. 2011). That is why averaged data are also to be considered: dinotefuran (13.7 ng/g), thiacloprid (6.5 ng/g), thiamethoxam (6.4 ng/g), imidacloprid (6 ng/g), acetamiprid (2.4 ng/g), and clothianidin (1.9 ng/g). As with the maximum levels, it appears that nectar/honey is less contaminated than pollen/beebread by a factor of 1.2 (acetamiprid) to 11.5

(thiacloprid). This further confirms that the first matrix is less contaminated by neonicotinoids than the second one. In the particular case of the study by Kasiotis et al. (2014), mean levels were found to be 48.7 ng/g for imidacloprid and 3,285 ng/g for clothianidin. It is difficult to investigate the particular case of fipronil because data are still lacking and published data are rather heterogeneous. Higher levels of fipronil were measured in nectar/honey than in pollen/beebread.

Conclusions Pollen/beebread and nectar/honey appear to be very relevant routes of exposure to neonicotinoids and fipronil in terms of occurrence, average level, and maximum residue level. The few studies of fipronil provide very heterogeneous results. Pollen/beebread revealed average residue levels between 0.8 and 28.5 ng/g. Nectar/honey revealed average residue levels between 2.3 and 70 ng/g. For neonicotinoids, average residue levels from Sanchez-Bayo and Goka (2014) are in the range of 1.9–13.7 ng/g for nectar/honey, and in the range of 3–75.1 ng/g for pollen/beebread. However, higher values of average residue levels have been obtained in several studies (Tables 4 and 5). Maximum levels of these systemic insecticides were found in the range of 10.1–208.8 ng/g for nectar/honey, and in the range of 29–1,002 ng/g for pollen/beebread (Sanchez-Bayo and Goka 2014). In terms of maximum levels, the variability clearly shows that contamination of pollen and nectar is not predictable and controlled, and that very high residue levels can be found in both pollen and nectar. It is important to note that nontarget species are exposed to more than just one pesticide via pollen or nectar. This was recently exemplified by the detection of mixtures of three to four insecticides (from a pool of 22 insecticides analyzed) in the nectar collected by honey bees, including acetamiprid, thiacloprid, thiamethoxam, tau-fluvalinate, and deltamethin (Paradis et al. 2014). Note that for the latter study, the agricultural uses of fipronil in France had been suspended several years prior, as well as the uses of imidacloprid for sunflower and maize.

Finally, nontarget species are very likely to be exposed to multiple pesticides (insecticides, herbicides, and several fungicides) simultaneously or at different points in time, and via multiple routes including pollen and nectar. This is especially relevant for treated fruit trees. In the cases of neonicotinoids and fipronil, variability of exposure data remains high between and within studies, due to variability of (1) pesticide applications, (2) the crops considered, (3) the samples analyzed, and (4) measurement methods. Variability will be difficult to improve and assess because field trials demand robust protocols that are difficult to manage, and also the required sensitive analytical techniques are costly to utilize. Therefore, despite the large methodological progress that has been made in the last decade, the question of exposure inherently leads to heterogeneous results and remains the object of discussion.

Despite this variability, which does not imply inaccuracy of measurements in real situations, studies worldwide demonstrate the exposure of nontarget species to these pesticides. This exposure, specifically through nectar and pollen, has proved harmful for bees and other pollinators (Pisa et al. 2014, this issue).

Honeydew

Honeydew is produced mainly by aphids (Aphididae) and other heteropteran insects and consists of a sticky, sugary liquid. Among others, insects such as ants (Formicidae) feed directly on honeydew while insects such as honeybees (*A. mellifera*) and wasps collect honeydew. It may be argued that honeydew production on treated crops is negligible, as the aphids that produce it would not be present on such crops. Van der Sluijs et al. (2013) argue that given the longer life span of bees, concentrations in plant sap that are too low to kill aphids could eventually prove harmful to bees through repeated exposure. However, there is no data available to verify this hypothesis. EFSA (2013d) therefore concludes that honeydew should be taken into account as a potential exposure route for honeybees in the case of fipronil.

Conclusion

The chemical properties of neonicotinoids and fipronil mean that they have the potential to accumulate in the environment at field-realistic levels of use (Bonmatin et al. 2007). This combination of persistence (over months or years) and solubility in water leads to contamination of, and the potential for accumulation in, soils and sediments (ppb–ppm range), waterways (groundwater and surface water in the ppt–ppb range), and treated and nontreated vegetation (ppb–ppm range) (Goulson 2013).

Screening of these matrices for pesticides is very patchy, and even where it has been conducted, the toxic metabolites are often not included. However, where environmental samples have been screened they are commonly found to contain mixtures of neonicotinoids or fipronil, along with their toxic metabolites and other pesticides. In addition, measurements taken from water have been found to exceed ecotoxicological limits on a regular basis around the globe (e.g., Gonzalez-Pradas et al. 2002; Kreuger et al. 2010; Starner and Goh 2012; Masiá et al. 2013; Van Dijk et al. 2013).

The presence of these compounds in the environment suggests that all kinds of nontarget organisms will be exposed to them. The case of honeybees is very illustrative, as they are exposed from the sowing period until flowering. In spring, the use of seed-coating insecticides for crops poses a risk of acute intoxication for bees (and other pollinators) by direct exposure

of flying bees to dusts emitted by the drilling machine (Girolami et al. 2013). The use of spray also exposes nontarget organisms when foraging on flowers, especially on fruit trees. Regardless of the mode of application, bees bring contaminated pollen, nectar, and probably also contaminated water back to the hive. Analysis of residues in food stores of honeybee colonies from across the globe reveal exactly what we might predict, based on the physical and chemical properties of these compounds. These food stores routinely contain mixtures of neonicotinoids and fipronil, generally in the 1–100 ppb range, demonstrating chronic exposure of honeybees throughout their lives (Sanchez-Bayo and Goka 2014). Similar exposure can be expected for other less-studied pollinators and invertebrates. Such widespread contamination has an impact on both aquatic and terrestrial invertebrates (Pisa et al. 2014, this issue) and vertebrates (Gibbons et al. 2014, this issue) living in or near farmland, or in streams which may occur in proximity to farmed areas.

This environmental contamination will undoubtedly have impacts on the functioning of various ecosystems and their services (Chagnon et al. 2014, this issue) unless alternatives are developed (Furlan and Kreutzweiser 2014, this issue; Van der Sluijs et al. 2014, this issue).

Acknowledgments This manuscript benefited from the discussions in the International Task Force on Systemic Pesticides during its plenary meetings in Paris (2010), Bath (2011), Cambridge (2012), Padova (2012), Louvain-la-Neuve (2013), and Legnaro-Padova (2013). We thank V Amaral-Rogers, P. Mineau, C. Morrissey, and B. Sachienne for their help. The work has been funded by the Triodos Foundation's Support Fund for Independent Research on Bee Decline and Systemic Pesticides. This Support Fund has been created from donations by Adessium Foundation (The Netherlands), Act Beyond Trust (Japan), Utrecht University (Netherlands), Stichting Triodos Foundation (The Netherlands), Gesellschaft fuer Schmetterlingsschutz (Germany), M.A.O.C. Gravin van Bylandt Stichting (The Netherlands), Zukunft Stiftung Landwirtschaft (Germany), Study Association Storm (Student Association Environmental Sciences Utrecht University), and citizens. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Aajoud A, Raveton M, Azrou-Isghi D, Tissut M, Ravel P (2008) How can the fipronil insecticide access phloem? *J Agric Food Chem* 56: 3732–3737
- Ahmed N, Kanan H, Inanaga S, Ma Y, Sugimoto Y (2001) Impact of pesticide seed treatments on aphid control and yield of wheat in the Sudan. *Crop Prot* 20:929–934
- Alsayed H, Pascal-Lorber S, Nallanthigal C, Debrauwer L, Laurent F (2008) Transfer of the insecticide [¹⁴C] imidacloprid from soil to tomato plants. *Environ Chem Lett* 6:229–234
- Anon (2006) Draft assessment Report (DAR)—public version—initial risk assessment provided by the Rapporteur Member State Germany for the existing active substance imidacloprid. Volume 3, Annex B, B.8, February 2006. <http://dar.efsa.europa.eu/dar-web/provision>. Accessed 21 June 2014
- APENET (2011) Effects of coated maize seed on honey bees. Report based on results obtained from the third year (2011) activity of the APENET project. <http://www.reterurale.it/apenet>. Accessed 21 June 2014
- Armbrust KL, Peeler HB (2002) Effects of formulation on the run-off of imidacloprid from turf. *Pest Manag Sci* 58:702–706
- Bacey J (2003) Environmental fate of imidacloprid. California Department of Pesticide Regulation, California, p 8
- Baird S, Garrison A, Jones J, Avants J, Bringolf R, Black M (2013) Enantioselective toxicity and bioaccumulation of fipronil in fathead minnows (*Pimephales promelas*) following water and sediment exposures. *Environ Toxicol Chem* 32:222–227
- Balsari P, Manzone M, Marucco P, Tamagnone M (2013) Evaluation of seed dressing dust dispersion from maize sowing machines. *Crop Prot* 51:19–23
- Baskaran S, Kookana RS, Naidu R (1999) Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal application rates. *Pestic Sci* 55:1222–1228
- Baur P, Marzouk H, Schönherr J, Terence Grayson B (1997) Partition coefficients of active ingredients between plant cuticle and adjuvants as related to rates of foliar uptake. *J Agric Food Chem* 45:3659–3665
- Belzunces L, Tasei J-N (1997) Report on the effects of seed treatments in sunflower Gauch[®] (imidacloprid). Impacts on populations of bee colonies and the honey. Commission d'Etude de la Toxicité des Produits Antiparasitaires à Usage Agricole et des Produits assimilés, Ministère de l'Agriculture, Paris, 27pp
- Bernal J, Garrido-Bailon E, Nozal MJD, Gonzalez-Porto AV, Martin-Hernandez R, Diego JC, Jiménez JJ, Bernal JL, Higes M (2010) Overview of pesticide residues in stored pollen and their potential effect on bee colony (*Apis mellifera*) losses in Spain. *J Econ Entomol* 103:1964–1971
- Bijleveld van Lexmond M, Bonmatin JM, Goulson D, Noome DA (2014) Worldwide integrated assessment on systemic pesticides. Global collapse of the entomofauna: exploring the role of systemic insecticides. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3220-1
- Bobé A, Coste CM, Coope J (1997) Factors influencing the adsorption of fipronil on soils. *J Agric Food Chem* 45:4861–4865
- Bogdanov S (2006) Contaminants of bee products. *Apidologie* 37:1–18
- Blom G, Paulissen M, Vos C, Agricola H (2008) Effecten van klimaatverandering op landbouw en natuur – nationale knelpuntenkaart en adaptatiestrategieën. Report 182; plant research international B.V., pp. 1–3. (In Dutch) <http://edepot.wur.nl/7956>. Accessed 21 June 2014
- Bloomfield JP, Williams RJ, Goody DC, Cape JN, Guha P (2006) Impacts of climate change on the fate and behaviour of pesticides in surface and groundwater—a UK perspective. *Sci Total Environ* 369:163–177
- Bobé A, Coste CM, Cooper JF (1997) Factors influencing the adsorption of fipronil in soils. *J Agric Food Chem* 45:4861–4865
- Bonmatin J-M, Moineau I, Charvet R, Fleche C, Colin ME, Bengsch ER (2003) A LC/APCI MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. *Anal Chem* 75:2027–2033
- Bonmatin J-M, Moineau I, Charvet R, Colin ME, Fleche C, Bengsch ER (2005a) Behaviour of imidacloprid in fields. toxicity for honey bees. In: Lichtfouse E, Schwarzbauer J, Robert D (eds). *Environmental chemistry*. Springer, Berlin. pp. 483–494. http://link.springer.com/chapter/10.1007%2F3-540-26531-7_44. Accessed 21 June 2014
- Bonmatin J-M, Marchand PA, Charvet R, Moineau I, Bengsch ER, Colin ME (2005b) Quantification of imidacloprid uptake in maize crops. *J Agric Food Chem* 53:5336–5341

- Bonmatin J-M, Marchand PA, Cotte JF, Aajoud A, Casabianca H, Goutailler G, Courtiade M (2007) Bees and systemic insecticides (imidacloprid, fipronil) in pollen: subnano-quantification by HPLC/MS/MS and GC/MS. In: Del Re AAM, Capri E, Fragoulis, Trevisan M (eds) Environmental fate and ecological effects of pesticide. La Goliardica Pavese, Pavia, (It), pp. 827–824. <http://www.cabdirect.org/abstracts/20083103467.html;jsessionid=8EE58D309B91521CB0CFECD7D2568525>. Accessed 21 June 2014
- Briggs GG, Bromilow RH, Evans AA, Williams M (1983) Relationships between lipophilicity and the distribution of non-ionised chemicals in barley shoots following uptake by the roots. *Pestic Sci* 14:492–500
- Broznic D, Milin C (2012) Effects of temperature on sorption–desorption processes of imidacloprid in soils of Croatian coastal regions. *J Env Sci Health B Pestic Food Contam Agric Wastes* 47:779–794
- Broznic D, Marinic JTM, Juresic GC, Petkovic O, Milin C (2012) Hysteretic behavior of imidacloprid sorption–desorption in soils of Croatian coastal regions. *Soil Sediment Contam* 21:850–871
- Buchholz A, Nauen R (2002) Translocation and translaminar bioavailability of two neonicotinoid insecticides after foliar application to cabbage and cotton. *Pest Manag Sci* 58:10–16
- Bugbee B, Koerner G (2002) Yield comparisons and unique characteristics of the dwarf wheat cultivar ‘USU-Apogee’ research: super dwarf cultivar studies: ‘APOGEE’ WHEAT
- Burgerstein A (1887) Materialien zu einer monographie betreffend die erscheinungen der transpiration der pflanzen. *Zool-Bot Ges Wien* 37:691–782
- Byrne FJ, Visscher PK, Leimkuehler B, Fischer D, Grafton-Cardwell EE, Morse JG (2014) Determination of exposure levels of honey bees foraging on flowers of mature citrus trees previously treated with imidacloprid. *Pest Manag Sci* 70:470–482
- Carbo L, Dores EFGC, Ribeiro ML (2008) Determination of pesticides multiresidues in shallow groundwater in a cotton-growing region of Mato Grosso, Brazil. *J Braz Chem Soc* 00(00):1111–1117. <http://www.scielo.br/pdf/jbchs/v19n6/a09v19n6.pdf>. Accessed 21 June 2014
- Carvalho MA, Marques SC, Martos ET, Rigitano RLO, Dias ES (2014) Bioaccumulation of insecticide in *Agaricus subrufescens*. *Hortic Bras* 32:159–162
- CCME (2007) Canadian water quality guidelines; imidacloprid. Scientific supporting document. Canadian Council of Ministers of the Environment, Winnipeg. http://www.ccme.ca/assets/pdf/imidacloprid_ssd_1388.pdf. Accessed 21 June 2014
- Chamberlain P (1992) Sprayable agricultural compositions
- Chamberlain K, Tench AJ, Williams RH, Bromilow RH (1995) Phloem translocation of pyridinecarboxylic acids and related imidazolinone herbicides in *Ricinus communis*. *Pestic Sci* 45:69–75
- Chagnon M, Kreutzweiser DP, Mitchell EAD, Morrissey CA, Noome DA, van der Sluijs JP (2014) Risks of large scale use of systemic insecticides to ecosystem functioning and services. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3277-x
- Chauzat MP, Faucon JP, Martel AC, Lachaize J, Cougoule N, Aubert M (2006) A survey of pesticide residues in pollen loads collected by honey bees in France. *J Econ Entomol* 99:253–262
- Chauzat MP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon JP (2009) Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. *Environ Entomol* 38:514–523
- Chauzat MP, Martel AC, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, Carpentier P, Faucon JP (2011) An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France. *Environ Toxicol Chem* 30:103–111
- Chopra I, Chauhan R, Kumari B, Dahiya KK (2011) Fate of fipronil in cotton and soil under tropical climatic conditions. *Bull Environ Contam Toxicol* 86:242–245
- Cowles RS, Montgomery ME, Cheah CASJ (2006) Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. *J Econ Entomol* 99:1258–1267
- Cox L, Koskinen W, Yen P (1997) Sorption–desorption of imidacloprid and its metabolites in soils. *J Agric Food Chem* 45:1468–1472
- Cox L, Koskinen W, Celis R, Yen P, Hermosin M, Comejo J (1998a) Sorption of imidacloprid on soil clay mineral and organic components. *Soil Sci Soc Am J* 62:911–915
- Cox L, Koskinen W, Yen P (1998b) Changes in sorption of imidacloprid with incubation time. *Soil Sci Soc Am J* 62:342–347
- Cox L, Koskinen W, Yen P (1998c) Influence of soil properties on sorption–desorption of imidacloprid. *J Environ Sci Health B* 33:123–134
- Cox C (2001) Insecticide factsheet—imidacloprid. *J Pestic Reform* 21: 15–21
- Cresswell JE (2011) A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology* 20:149–157
- Curtis LC (1944) The influence of guttation fluid on pesticides. *Phytopathology* 34:196–205
- Cutler GC, Scott-Dupree CD (2007) Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. *J Econ Entomol* 100:765–772
- DeCant J (2010) Clothianidin registration of Prosper T400 seed treatment on mustard seed (oilseed and condiment) and Poncho/Votivo seed treatment on cotton. U.S. Environmental Protection Agency, Washington DC
- Demcheck DK, Tollett RW, Mize SV, Skrobialowski SC, Fendick Jr. RB, Swarzenski CM, Porter SD (2004) Water quality in the Acadian-Pontchartrain drainages, Louisiana and Mississippi, 1999–2001: Reston, VA, U.S. Geological Survey Circular 1232, p47
- Dieckmann Y, Christian F, Ishaque M, Kerl W, Köhle H et al. (2010a) Agrochemical formulations comprising co-polymers based on ethylenically unsaturated dicarboxylic mono and diesters. Patent No. US 2010/0063167 A1. 1–14
- Dieckmann Y, Görrh FC, Ishaque M, Kerl W, Köhle H, Kreuz K, Langewald J, Münster I, Picard L (2010b) Agrochemical formulations comprising 1-vinyl-2-pyrrolidinone co-polymers. USA. <http://www.google.com/patents/US20100075849>. Accessed 21 June 2014
- Dieckmann Y, Ishaque M, Muenster I, Picard L, Benz A et al. (2010c) Systemicity enhancers. Patent No. US 2010/0204045 A1, 1–21
- Dively GP, Kamel A (2012) Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. *J Agric Food Chem* 60:4449–4456
- Donnarumma L, Pulcini P, Pochi D, Rosati S, Lusco L, Conte E (2011) Preliminary study on persistence in soil and residues in maize of imidacloprid. *J Environ Sci Health B* 46:469–472
- Duby G, Boutry M (2009) The plant plasma membrane proton pump ATPase: a highly regulated P-type ATPase with multiple physiological roles. *Pflügers Arch* 457:645–655
- EFSA (2012a) Panel on Plant Protection Products and their Residues (PPR); Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA J 10(5):2668, 275 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014
- EFSA (2012b) Statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe. EFSA J 10(6):2752, 27 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014
- EFSA (2013a) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA J 11(1):3066, 58 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014
- EFSA (2013b) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance thiamethoxam. EFSA

- J 11(1):3067, 68 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014
- EFSA (2013c) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid. EFSA J 11(1):3068, 55 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014
- EFSA (2013d) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance fipronil. EFSA J 11(5):3158, 51 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014
- EFSA (2013e) Evaluation of the FERA study on bumble bees and consideration of its potential impact on the EFSA conclusions on neonicotinoids. EFSA J 11(6):3242, 20 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014
- EFSA (2013f) Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA J 11(7):3295, 266 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014
- Fantke P, Juraske R (2013) Variability of pesticide dissipation half-lives in plants. Environ Sci Technol 16:3548–3562
- Felsot AS, Cone W, Yu J, Ruppert JR (1998) Distribution of imidacloprid in soil following subsurface drip chemigation. Bull Environ Contam Toxicol 60:363–370
- Felsot AS, Ruppert JR (2002) Imidacloprid residues in Willapa bay (Washington state) water and sediment following application for control of burrowing shrimp. J Agric Food Chem 50:4417–4423
- Fernandez-Bayo JD, Nogales R, Romero E (2009) Effect of vermicomposts from wastes of the wine and alcohol industries in the persistence and distribution of imidacloprid and diuron on agricultural soils. J Agric Food Chem 57:5435–5442
- Flores-Céspedes F, González-Pradas E, Fernández-Pérez M, Villafranca-Sánchez M, Socías-Viciano M, Urea-Amate MD (2002) Effects of dissolved organic carbon on sorption and mobility of imidacloprid in soil. J Environ Qual 31:880–888
- Fossen M (2006) Environmental fate of imidacloprid. Environmental Monitoring Department of Pesticide Regulation. 1001 I Street Sacramento, CA, 95812–4015. <http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/Imidclprdfate2.pdf>. Accessed 21 June 2014
- Furlan L, Kreutzweiser DP (2014) Alternatives to neonicotinoid insecticides for pest control: case studies in agriculture and forestry. Environ Sci Pollut Res (this issue)
- Gerecke AC, Schärer M, Singer HP, Müller SR, Schwarzenbach RP, Sägesser M, Ochsenbein U, Popow G (2002) Sources of pesticides in surface waters in Switzerland: pesticide load through waste water treatment plants—current situation and reduction potential. Chemosphere 48:307–315
- Gibbons D, Morrissey C, Mineau P (2014) A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ Sci Pollut Res. doi:10.1007/s11356-014-3180-5
- Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Di Bernardo A, Greatti M, Giorio C, Tapparo A (2009) Translocation of neonicotinoid insecticides from coated seeds to seedling guttations: a novel way of intoxication for bees. J Econ Entomol 102:1808–1815
- Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A (2012) Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. J Appl Entomol 136:17–26
- Girolami V, Marzaro M, Vivan L, Mazzon L, Giorio C, Marton D, Tapparo A (2013) Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers. J Appl Entomol 137:35–44
- Giroux I (2003). (in French) Contamination de l'eau souterraine par les pesticides et les nitrates dans les régions en culture de pommes de terre. Campagne d'échantillonnage de 1999-2000-2001. Direction de suivi de l'état de l'environnement, Ministère de l'Environnement, Gouvernement du Québec, Québec. www.mddep.gouv.qc.ca/pesticides/pomme_terre/Pesticides_pomme_terre.pdf. Accessed 21 June 2014
- Goatley JL, Lewis RW (1966) Composition of guttation fluid from rye, wheat, and barley seedlings. Plant Physiol 41:373–375
- Gonçalves CM, Silva JC, Alpendurada MF (2007) Evaluation of the pesticide contamination of groundwater sampled over two years from a vulnerable zone in Portugal. J Agric Food Chem 55:6227–6235
- Gonzalez-Pradas E, Urena-Amate MD, Flores-Céspedes F, Fernandez-Perez M, Garratt J, Wilkins RJ (2002) Leaching of imidacloprid and procymidone in a greenhouse of southeast of Spain. Soil Sci Soc Am J 66:1821–1828
- Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. J Appl Ecol 50:977–987
- Greatti M, Sabatini AG, Barbattini R, Rossi S, Stravisi A (2003) Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results. Bull Insectol 56: 69–72
- Grosman DM, Upton WW (2006) Efficacy of systemic insecticides for protection of loblolly pine against southern pine engraver beetles (Coleoptera: Curculionidae: Scolytinae) and wood borers (Coleoptera: Cerambycidae). J Econ Entomol 99:94–101
- Gunasekara AS, Truong T, Goh KS, Spurlock F, Tjeerdema RS (2007) Environmental fate and toxicology of fipronil. J Pestic Sci 32:189–199
- Gupta S, Gajbhiye VT, Kalpana, Agnihotri NP (2002) Leaching behavior of imidacloprid formulations in soil. Bull Environ Contam Toxicol 68:502–508
- Gupta S, Gajbhiye VT (2007) Persistence of acetamiprid in soil. Bull Environ Contam Toxicol 78:349–352
- Gupta S, Gajbhiye VT, Gupta RK (2008a) Effect of light on the degradation of two neonicotinoids viz acetamiprid and thiacloprid in soil. Bull Environ Contam Toxicol 81:185–189
- Gupta S, Gajbhiye VT, Gupta RK (2008b) Soil dissipation and leaching behavior of a neonicotinoid insecticide thiamethoxam. Bull Environ Contam Toxicol 80:431–437
- Gustafson DI (1989) Ground water ubiquity score: a simple method for assessing pesticide leachability. Environ Toxicol Chem 8:339–357
- Guzsvány V, Csanádi J, Gaál F (2006) NMR study of the influence of pH on the persistence of some neonicotinoids in water. Acta Chim Slov 53:52–57
- Haith DA (2010) Ecological risk assessment of pesticide runoff from grass surfaces. Environ Sci Technol 44:6496–6502
- Halm MP, Rortais A, Arnold G, Taséi JN, Rault S (2006) New risk assessment approach for systemic insecticides: the case of honey bees and imidacloprid (Gaucho). Environ Sci Technol 40:2448–2454
- Hoffman EJ, Castle SJ (2012) Imidacloprid in melon guttation fluid: a potential mode of exposure for pest and beneficial organisms. J Econ Entomol 105:67–71
- Horwood MA (2007) Rapid degradation of termiticides under field conditions. Aust J Entomol 46:75–78
- Huseth AS, Groves RL (2013) Environmental fate of neonicotinoids: a potato case study. www.soils.wisc.edu/extension/wcmc/2013/pap/Huseth.pdf. Accessed 21 June 2014
- Huseth AS, Groves RL (2014) Environmental fate of soil applied neonicotinoid insecticides in an irrigated potato agroecosystem. PLoS One 9(5):1–11
- Ishaque M, Jung MR, Tuerk H, Schroeder-Grimonpont T, Reinhard K, Schnabel G, Klein CD, Holt TJ, Mascianica MP (2012) Polymers for increasing the soil mobility of low-solubility insecticides. <http://www.google.com/patents/US20120053221>. Accessed 21 June 2014
- Jeschke P, Nauen R (2008) Neonicotinoids—from zero to hero in insecticide chemistry. Pest Manag Sci 64:1084–1098

- Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* 59: 2897–2908
- Johnson RM, Ellis MD, Mullin CA, Frazier M (2010) Pesticides and honey bee toxicity—USA. *Apidologie* 41:312–331
- Juraske R, Castells F, Vijay A, Muñoz P, Antón A (2009) Uptake and persistence of pesticides in plants: measurements and model estimates for imidacloprid after foliar and soil application. *J Hazard Mater* 165:683–689
- Kapoor U, Srivastava MK, Srivastava AK, Patel DK, Garg V, Srivastava LP (2013) Analysis of imidacloprid residues in fruits, vegetables, cereals, fruit juices, and baby foods, and daily intake estimation in and around Lucknow. *India Environ Toxicol Chem* 32:723–727
- Kasiotis KM, Anagnostopoulos C, Anastasiadou P, Machera K (2014) Pesticide residues in honeybees, honey and bee pollen by LC-MS/MS screening: reported death incidents in honeybees. *Sci Total Environ* 485–486:633–642
- Katsuhara M, Hanba YT, Shiratake K, Maeshima M (2008) Expanding roles of plant aquaporins in plasma membranes and cell organelles. *Funct Plant Biol* 35:1–14
- Kattwinkel M, Kühne JV, Foit K, Liess M (2011) Climate change, agricultural insecticide exposure, and risk for freshwater communities. *Ecol Appl* 21:2068–2081
- Knipfer T, Besse M, Verdeil J-L, Fricke W (2011) Aquaporin-facilitated water uptake in barley (*Hordeum vulgare* L.) roots. *J Exp Bot* 62: 4115–4126
- Koch R, Burkness E, Hutchison W, Rabaey T (2005) Efficacy of systemic insecticide seed treatments for protection of early-growth-stage snap beans from bean leaf beetle (Coleoptera: Chrysomelidae) foliar feeding. *Crop Prot* 24:734–742
- Koltzenburg S, Dombo P, Oetter G, Bratz M (2010) Comb polymers and use thereof for the production of active or effective ingredient formulations. USA. <http://www.google.com/patents/US20100048655>. Accessed 21 June 2014
- Koulman A, Lane GA, Christensen MJ, Fraser K, Tapper BA (2007) Peramine and other fungal alkaloids are exuded in the guttation fluid of endophyte-infected grasses. *Phytochemistry* 68:355–360
- Kreuger J, Graaf S, Pating J, Adielsson S (2010) Pesticides in surface water in areas with open ground and greenhouse horticultural crops in Sweden 2008. pp. 49. http://www-mv.slu.se/webfiles/vv/CKB/Ekohydrologi_117_ENG.pdf. Accessed 21 June 2014
- Kreutzweiser DP, Good K, Chartrand D, Scarr T, Thompson D (2007) Non-target effects on aquatic decomposer organisms of imidacloprid as a systemic insecticide to control emerald ash borer in riparian trees. *Ecotoxicol Environ Saf* 68:315–325
- Kreutzweiser DP, Thompson DG, Scarr TA (2009) Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates. *Ecotoxicol Environ Saf* 72:1053–1057
- Krischik VA, Landmark AL, Heimpel GE (2007) Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae). *Environ Entomol* 36:1238–1245
- Krohn J, Hellpointner E (2002) Environmental fate of imidacloprid. *Pflanzenschutz- Nachrichten Bayer* 55:1–25
- Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One* 7:e29268
- Kühnholz S, Seeley TD (1997) The control of water collection in honey bee colonies. *Behav Ecol Sociobiol* 41:407–422
- Kurwadkar ST, Dewinne D, Wheat R, McGahan DG, Mitchell FL (2013) Time dependent sorption behavior of dinotefuran, imidacloprid and thiamethoxam. *J Environ Sci Health B* 48:237–242
- Lambert O, Piroux M, Puyo S, Thorin C, L'Hostis M, Wiest L, Buleté A, Delbac F, Pouliquen H (2013) Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of western France. *PLoS One* 8:e67007
- Larson JL, Redmond CT, Potter DA (2013) Assessing insecticide hazard to bumble bees foraging on flowering weeds in treated lawns. *PLoS One* 8:e66375
- Laurent FM, Rathahao E (2003) Distribution of [(14)C]imidacloprid in sunflowers (*Helianthus annuus* L.) following seed treatment. *J Agric Food Chem* 51:8005–8010
- Le Faouder J, Bichon E, Brunschwig P, Landelle R, Andre F, Le Bizet B (2007) Transfer assessment of fipronil residues from feed to cow milk. *Talanta* 73:710–717
- Magalhaes LC, Hunt TE, Siegfried BD (2009) Efficacy of neonicotinoid seed treatments to reduce soybean aphid populations under field and controlled conditions in Nebraska. *J Econ Entomol* 102:187–195
- Maiefisch P, Angst M, Brandl F, Fischer W, Hofer D, Kayser H, Kobel W et al (2001) Chemistry and biology of thiamethoxam: a second generation neonicotinoid. *Pest Manag Sci* 57:906–913
- Main AR, Headley JV, Peru KM, Michel NL, Cessna AJ et al (2014) Widespread use and frequent detection of neonicotinoid insecticides in wetlands of Canada's prairie Pothole region. *PLoS One* 9(3): e92821
- Marzaro M, Vivan L, Targa A, Mazzon L, Mori N, Greatti M, Petrucco Toffolo E, Di Bernardo A, Giorio C, Marton D, Tapparo A, Girolami V (2011) Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat. *Bull Insectol* 64:119–126
- Masiá A, Campo J, Vázquez-Roig P, Blasco C, Picó Y (2013) Screening of currently used pesticides in water, sediments and biota of the Guadalquivir river basin (Spain). *J Hazard Mater* 263(Pt 1):95–104
- Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Sattelle DB (2005) Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trend Pharmacol Sci* 22:573–580
- Maxim L, van der Sluijs JP (2007) Uncertainty: cause or effect of stakeholders' debates? Analysis of a case study: the risk for honeybees of the insecticide Gaucho. *Sci Total Environ* 376:1–17
- Mesnager R, Defarge N, Spiroux de Vendômois J, Séralini GE (2014) Major pesticides are more toxic to human cells than their declared active principles. *Biomed Res Int* 2014:179691
- Misra A, Tyler G (1999) Influence of soil moisture on soil solution chemistry and concentrations of minerals in the calcicoles *Phleum phleoides* and *Veronica spicata* grown on a limestone soil. *Ann Bot* 84:401–410
- Mize SV, Porter SD, Demcheck DK (2008) Influence of fipronil compounds and rice-cultivation land-use intensity on macroinvertebrate communities in streams of southwestern Louisiana, USA. *Environ Pollut* 152:491–503
- Mohapatra S, Deepa M, Jagdish GK, Rashmi N, Kumar S, Prakash GS (2010) Fate of fipronil and its metabolites in/on grape leaves, berries and soil under semi arid tropical climatic conditions. *Bull Environ Contam Toxicol* 84:587–591
- Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, Vanengelsdorp D, Pettis JS (2010) High levels of miticides and agrochemicals in north American apiaries: implications for honeybee health. *PLoS One* 5(3):e9754
- Mullins JW (1993) Imidacloprid. A new nitroguanidine insecticide. *Am Chem Soc Symp Ser* 524:183–198
- Nauen R, Ebbinghaus-Kintscher U, Schmuck R (2001) Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). *Pest Manag Sci* 57:577–586
- Nauen R, Ebbinghaus-Kintscher U, Salgado VL, Kaussmann M (2003) Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pestic Biochem Physiol* 76:55–69
- Nauen R, Reckmann U, Thomzik J, Thielertand W, Baur P (2008) Biological profile of spirotetramat (Movento®)—a new two-way systemic (ambimobile) insecticide against sucking pest species. *Bayer Crop Sci J* 61:245–278

- Nikolakakis A, Chapple A, Friessleben R, Neumann P, Schad T, Schmuck R, Schnier H, Schnorrbach H, Schoning R, Maus C (2009) An effective risk management approach to prevent bee damage due to the emission of abraded seed treatment particles during sowing of seeds treated with bee toxic insecticides. *Julius Kühn Archiv* 423:132–148
- Nuytens D, Devarrewaere W, Verboven P, Foqué D (2013) Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review. *Pest Manag Sci* 69:564–575
- Overmyer JP, Mason BN, Armbrust KL (2005) Acute toxicity of imidacloprid and fipronil to a nontarget aquatic insect, *Simulium vittatum* Zetterstedt cytospecies IS-7. *Bull Environ Contam Toxicol* 74:872–879
- Paine TD, Hanlon CC, Byrne FJ (2011) Potential risks of systemic imidacloprid to parasitoid natural enemies of a cerambycid attacking eucalyptus. *Biol Control* 56:175–178
- Paradis D, Bérail G, Bonmatin JM, Belzunces LP (2014) Sensitive analytical methods for 22 relevant insecticides of 3 chemical families in honey by GC-MS/MS and LC-MS/MS. *Anal Bioanal Chem* 406:621–633
- Pareja L, Colazzo M, Pérez-Parada A, Niell S, Carrasco-Letelier L, Besil N, Cesio MV et al (2011) Detection of pesticides in active and depopulated beehives in Uruguay. *Int J Environ Res Public Health* 8:3844–3858
- Peña A, Rodríguez-Liébaná JA, Mingorance MD (2011) Persistence of two neonicotinoid insecticides in wastewater, and in aqueous solutions of surfactants and dissolved organic matter. *Chemosphere* 84:464–470
- PMRA; Pest Management Regulatory Authority (2013) Action to protect bees from exposure to neonicotinoid pesticides. Published by Health Canada in Ottawa, ON. 4 pp. http://www.hc-sc.gc.ca/cps-spc/alt_formats/pdf/pest/part/consultations/_noi2013-01/doi2013-01-eng.pdf. Accessed 21 June 2014
- PPDB (2012) Pesticide properties database. <http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm>. Accessed 21 June 2014
- Pierobon M, Bouillo N, Lange RFM, Meyer K, Kolter K (2008) Use of amphiphilic copolymers as solubilising agents. <http://www.google.com/patents/US20080153925>. Accessed 21 June 2014
- Pilling E, Campbell P, Coulson M, Ruddie N, Tornier I (2014) A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. *PLoS One* 8:e77193
- Pisa L, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs C, Goulson D, Kreutzweiser D, Krupke C, Liess M, McField M, Morrissey C, Noome DA, Settele J, Simon-Delso N, Stark J, van der Sluijs, van Dyck H, Wiemers M (2014) Effects of neonicotinoids and fipronil on non-target invertebrates. *Environ Sci Pollut Res* (this issue)
- Pistorius J, Bischoff G, Heimbach U, Stähler M (2009) Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. *Julius Kühn Archiv* 423:118–126
- Pistorius J, Brobyn T, Campbell P, Forster R, Lortsch JA, Marolleau F, Maus C, Luckmann J, Suzuki H, Wallner K, Becker R (2012) Assessment of risks to honey bees posed by guttation. *Julius-Kühn-Archiv* 437:199–208
- Pochi D, Biocca M, Fanigliulo R, Pulcini P, Conte E (2012) Potential exposure of bees, *Apis mellifera* L., to particulate matter and pesticides derived from seed dressing during maize sowing. *Bull Environ Contam Toxicol* 89:354–361
- Pohorecka K, Skubida P, Miszczak A, Semkiw P, Sikorski P, Zagibajlo K, Teper D, Koltowski Z, Zdańska D, Skubida M, Bober A (2012) Residues of neonicotinoid insecticides in bee collected plant materials from oilseed rape crops and their effect on bee colonies. *J Apic Sci* 56:115–134
- Poland TM, Haack RA, Petrice TR, Miller DH, Bauer LS (2006) Laboratory evaluation of the toxicity of systemic insecticides for control of *Anoplophora glabripennis* and *Plectrodera scalator* (Coleoptera: Cerambycidae). *J Econ Entomol* 99:85–93
- Reetz JE, Zühlke S, Spiteller M, Wallner K (2011) Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees? *Apidologie* 42:596–606
- Rennich K, Pettis J, van Engelsdorp D, Bozarth R, Eversole H, Roccacaccia K, Smith M, Stitzinger J, Andree M, Snyder R, Rice N, Evans J, Levi V, Lopez D, Rose R (2012) 2011–2012 National honey bee pests and diseases survey report. USDA. 17 p. http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/2011_National_Survey_Report.pdf. Accessed 21 June 2014
- Rigitano RLO, Bromilow RH, Briggs GG, Chamberlain K (1987) Phloem translocation of weak acids in *Ricinus communis*. *Pestic Sci* 19:113–133
- Rortais A, Arnold G, Halm MP, Touffet-Briens F (2005) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36:71–83
- Rouchaud J, Gustin F, Wauters A (1994) Soil biodegradation and leaf transfer of insecticide imidacloprid applied in seed dressing in sugar beet crops. *Bull Environ Contam Toxicol* 53:344–350
- Sanchez-Bayo F, Goka K (2006) Ecological effects of the insecticide imidacloprid and a pollutant from antidandruff shampoo in experimental rice fields. *Environ Toxicol Chem* 25:1677–1687
- Sanchez-Bayo F, Goka K (2014) Pesticide residues and bees—a risk assessment. *PLoS One* 9(4):e94482
- Sarkar MA, Biswas PK, Roy S, Kole RK, Chowdhury A (1999) Effect of pH and type of formulation on the persistence of imidacloprid in water. *Bull Environ Contam Toxicol* 63:604–609
- Sarkar MA, Roy S, Kole RK, Chowdhury A (2001) Persistence and metabolism of imidacloprid in different soils of West Bengal. *Pest Manag Sci* 57:598–602
- Schatz F, Wallner K (2009) Pflanzenschutzmittelapplikation in blühenden raps (*Brassica napus*) und deren auswirkungen auf die rückstandssituation in honig, nektar und pollen der honigbiene (*Apis mellifera* L.). Thesis (Diploma), Universität Hohenheim, Hohenheim, Germany
- Schmuck R, Schöning R, Stork A, Schramel O (2001) Risk posed to honeybees (*Apis mellifera* L., Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Manag Sci* 57:225–238
- Schnier HF, Wenig G, Laubert F, Simon V, Schmuck R (2003) Honey bee safety of imidacloprid corn seed treatment. *Bull Insectol* 56:73–76
- Scorza RP, Smelt JH, Boesten JJTI, Hendriks RFA, Van der Zee SEATM (2004) Vadose zone processes and chemical transport: preferential flow of bromide, bentazon, and imidacloprid in a Dutch clay soil. *J Environ Qual* 33:1473–1486
- Scott-Dupree CD, Spivak M (2001) The impact of Gaucho and TI-435 seed-treated canola on honey bees, *Apis mellifera* L., Université de Guelph, Ontario Canada Université
- Selim HM, Jeong CY, Elbana TA (2010) Transport of Imidacloprid in soils: miscible displacement experiments. *Soil Sci* 175:375–381
- Shawki MAA, Titera D, Kazda J, Kohoutkova J, Taborsky V (2005) Toxicity to honeybees of water guttation and dew collected from winter rape treated with Nurelle D®. *Plant Protect Sci* 42:9–14
- Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, van Dyck H, van Praagh J, van der Sluijs JP, Whitehorn PR, Wiemers M (2014) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites *Environ Sci Pollut Res* (this issue)

- Singh S, Singh TN (2013) Guttation 1: chemistry, crop husbandry and molecular farming. *Phytochem Rev* 12:147–172
- Singh S, Singh TN, Chauhan JS (2009a) Guttation in rice: occurrence, regulation, and significance in varietal improvement. *J Crop Improv* 23:351–365
- Singh S, Singh TN, Chauhan JS (2009b) Water transport in crop plants with special reference to rice: key to crop production under global water crisis. *J Crop Improv* 23:194–212
- Skerl MIS, Bolta SV, Cesnik HB, Gregorc A (2009) Residues of pesticides in honeybee (*Apis mellifera carnica*), bee bread and in pollen loads from treated apple orchards. *Bull Environ Contam Toxicol* 83: 374–377
- Skrobialowski SC, Mize SV, Demcheck DK (2004) Environmental setting, water quality, and ecological indicators of surface water quality in the Mermentau river basin, southwestern Louisiana, 1998–2001. US Geological survey—resources investigations report 03-4185. <http://water.usgs.gov/nawqa/bib/pubs.php?state=LA>. Accessed 21 June 2014
- Spiteller M (1993) Aerobic metabolism of imidacloprid, 14C-NTN 33893, in an aquatic model ecosystem. Bayer AG, Report No. PF3950
- Stamer K, Goh KS (2012) Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010–2011. *Bull Environ Contam Toxicol* 88: 316–321
- Stoner KA, Eitzer BD (2012) Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*). *PLoS One* 7(6):e39114
- Stoner KA, Eitzer BD (2013) Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in Connecticut. *PLoS One* 8:e77550
- Suchail S, Guez D, Belzunces LP (2001) Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ Toxicol Chem SETAC* 20:2482–2486
- Sungur S, Tunur C (2012) Investigation of pesticide residues in vegetables and fruits grown in various regions of Hatay, Turkey. *Food Addit Contam B Surveill* 5:265–267
- Sur R, Stork A (2003) Uptake, translocation and metabolism of imidacloprid in plants. *Bull Insectol* 56:35–40
- Tanner G (2010) Development of a method for the analysis of neonicotinoid insecticide residues in honey using LC-MS/MS and investigations of neonicotinoid insecticides in matrices of importance in apiculture. Universität Wien, Vienna. <http://othes.univie.ac.at/9119/>. Accessed 21 June 2014
- Tapparo A, Giorio C, Marzaro M, Marton D, Soldà L, Girolami V (2011) Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds. *J Environ Monit* 13: 1564–1568
- Tapparo A, Marton D, Giorio C, Zanella A, Soldà L, Marzaro M, Vivan L, Girolami V (2012) Assessment of the environmental exposure of honeybees to particulate matter containing neonicotinoid insecticides coming from corn coated seeds. *Environ Sci Technol* 46: 2592–2599
- Tattar TA, Dotson JA, Ruizzo MS, Steward VB (1998) Translocation of imidacloprid in three tree species when trunk- and soil-injected. *J Arboric* 24:54–56
- Thompson HM (2012) Interaction between pesticides and other factors in effects on bees. Supporting Publications 2012: EN-340. 204 pp. <http://www.efsa.europa.eu/fr/publications.htm?text=thompson+2012+bee&p=10>. Accessed 21 June 2014
- Thompson H, Harrington P, Wilkins S, Pietravalle S, Sweet D, Jones A (2013) Effects of neonicotinoid seed treatments on bumble bee colonies under field conditions. The Food and Environment Research Agency, Sand Hutton, York, UK. March 2013. <http://fera.co.uk/ccss/documents/defraBumbleBeeReportPS2371V4a.pdf>. Accessed 21 June 2014
- Thuyet DQ, Jorgenson BC, Wissel-Tyson C, Watanabe H, Young TM (2012) Wash off of imidacloprid and fipronil from turf and concrete surfaces using simulated rainfall. *Sci Total Environ* 414:515–524
- Thuyet DQ, Watanabe H, Ok J (2013) Effect of pH on the degradation of imidacloprid and fipronil in paddy water. *J Pestic Sci* 38:223–227
- Tingle CCD, Rother JA, Dewhurst CF, Lauer S, King WJ (2003) Fipronil: environmental fate, ecotoxicology and human health concerns. *Rev Environ Contam* 176:1–66
- Tišler T, Jemec A, Mozetic B, Trebse P (2009) Hazard identification of imidacloprid to aquatic environment. *Chemosphere* 76:907–914
- Tomizawa M, Casida JE (2003) Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annu Rev Entomol* 48:339–364
- Tomizawa M, Casida JE (2005) Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annu Rev Pharmacol Toxicol* 45: 247–268
- Trapp S (2004) Plant uptake and transport models for neutral and ionic chemicals. *Environ Sci Pollut Res* 11:33–39
- US EPA (1993) EFGWB review of imidacloprid, environmental fate and groundwater branch. United States Environmental Protection Agency, Washington, DC
- US EPA (1993b) Comparison of the leaching potential of imidacloprid (NTN) to other turf insecticides considered in the preliminary turf cluster assessment. Memo from J. Wolf, soil scientist, to H. Jacoby, chief. Washington, DC. June 15
- US EPA (1996) Fipronil pesticide fact sheet. EPA-737-F-96-005. EPA, Washington DC
- US EPA (2003) Office of pesticide programs, factsheet clothianidin. EPA Publ 7501C
- US EPA (2005) Pesticide fate database. Environmental fate and effects division of the office of pesticide programs
- US EPA (2008) Imidacloprid summary document registration review: Initial docket December 2008. Docket Number: EPA-HQ-OPP-2008-0844
- US EPA (2010) Environmental fate and ecological risk assessment for the registration of clothianidin for use as a seed treatment on mustard seed (oilseed and condiment) and cotton
- US EPA (2013) Pollinator risk assessment framework. <http://www.epa.gov/scipoly/sap/meetings/2012/september/091112transcript.pdf>. Accessed 21 June 2014
- Vaknin Y, Gan-Mor S, Bechar A, Ronen B, Eisikowitch B (2000) The role of electrostatic forces in pollination. *Plant Syst Evol* 222:133–142
- Van der Geest B (2012) Bee poisoning incidents in the Pomurje region of eastern Slovenia in 2011. *Julius Kühn Archiv* 437:124. <http://pub.jki.bund.de/index.php/JKA/article/download/1962/2338>. Accessed 21 June 2014
- Van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin J-M, Belzunces LP (2013) Neonicotinoids, bee disorders and the sustainability of pollinator services. *Curr Opin Environ Sustain* 5:1–13
- Van der Sluijs JP, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Simon-Delso N, Stark JD, Tapparo A, van Dyck H, van Praagh J, Whitehorn PR, Wiemers M (2014) Conclusions of the worldwide integrated assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3229-5
- Van der Werf HMG (1996) Assessing the impact of pesticides on the environment. *Agric Ecosyst Environ* 60:81–96
- Van Dijk TC, Van Staalduinen MA, Van der Sluijs JP (2013) Macro-invertebrate decline in surface water polluted with imidacloprid. *PLoS ONE* 8(5):e62374
- Visscher PK, Crailsheim K, Sherman G (1996) How do honey bees (*Apis mellifera*) fuel their water foraging flights? *J Insect Physiol* 42: 1089–1094

- Vollner L, Klotz D (1997) Leaching and degradation of pesticides in groundwater layers. In: Agency IAE (ed). Environmental behaviour of crop protection chemicals. pp. 187–203. Vienna, Austria
- Wallner K (2009) Sprayed and seed dressed pesticides in pollen, nectar and honey of oilseed rape. *Julius Kuhn archive* 423:152–153
- Westwood F, Bean KM, Dewar AM, Bromilow RH, Chamberlain K (1998) Movement and persistence of [¹⁴C]imidacloprid in sugar-beet plants following application to pelleted sugar-beet seed. *Pestic Sci* 52:97–103
- Whitehorn PR, O'Connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336:351–352
- Wiest L, Buleté A, Giroud B, Fratta C, Amic S, Lambert O, Pouliquen H, Arnaudguilhem C (2011) Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection. *J Chromatogr A* 1218:5743–5756
- Winterlin W, Walker G, Luce A (1973) Carbaryl residues in bees, honey, and bee bread following exposure to carbaryl via the food supply. *Arch Environ Contam Toxicol* 1:362–374
- Ying G-G, Kookana RS (2006) Persistence and movement of fipronil termiticide with under-slab and trenching treatments. *Environ Toxicol Chem SETAC* 25:2045–2050
- Zheng W, Liu W (1999) Kinetics and mechanism of the hydrolysis of imidacloprid. *Pest Manag Sci* 55:482–485
- Zywitz D, Anastassiades M, Scherbaim E (2004) Analysis of neonicotinoid insecticides in fruits and vegetables using LC-MS (MS). EPRW 2004. http://www.rfb.it/bastaveleni/documenti/poster_neonicotinoid_insecticides_eprw. Accessed 21 June 2014

Authors are in alphabetic order with the exception of the corresponding author. They declare no competing conflict of interest. All authors are working for public agencies or academic institutions, except NSD working both for the Utrecht University and the Technical Center CARI (mainly supported by public funds) and DN whose independent work for the TFSP is financed by the Triodos Foundation.

Effects of neonicotinoids and fipronil on non-target invertebrates

L. W. Pisa · V. Amaral-Rogers · L. P. Belzunces · J. M. Bonmatin · C. A. Downs ·
D. Goulson · D. P. Kreutzweiser · C. Krupke · M. Liess · M. McField · C. A. Morrissey ·
D. A. Noome · J. Settele · N. Simon-Delso · J. D. Stark · J. P. Van der Sluijs · H. Van Dyck ·
M. Wiemers

Received: 8 May 2014 / Accepted: 15 August 2014 / Published online: 17 September 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract We assessed the state of knowledge regarding the effects of large-scale pollution with neonicotinoid insecticides and fipronil on non-target invertebrate species of terrestrial, freshwater and marine environments. A large section of the assessment is dedicated to the state of knowledge on sublethal effects on honeybees (*Apis mellifera*) because this important pollinator is the most studied non-target invertebrate species.

Lepidoptera (butterflies and moths), Lumbricidae (earthworms), Apoidae sensu lato (bumblebees, solitary bees) and the section “other invertebrates” review available studies on the other terrestrial species. The sections on freshwater and marine species are rather short as little is known so far about the impact of neonicotinoid insecticides and fipronil on the diverse invertebrate fauna of these widely exposed habitats.

Responsible editor: Philippe Garrigues

L. W. Pisa (✉) · N. Simon-Delso · J. P. Van der Sluijs
Environmental Sciences, Copernicus Institute, Utrecht University,
Heidelberglaan 2, 3584 CS Utrecht, The Netherlands
e-mail: l.w.pisa@uu.nl

V. Amaral-Rogers
Buglife, Bug House, Ham Lane, Orton Waterville, Peterborough PE2
5UU, UK

L. P. Belzunces
Laboratoire de Toxicologie Environnementale, INRA, UR 406
Abeilles & Environnement, Site Agroparc, 84000 Avignon, France

J. M. Bonmatin
Centre de Biophysique Moléculaire, UPR 4301 CNRS, affiliated to
Orléans University and to INSERM, 45071 Orléans cedex 02, France

C. A. Downs
Haereticus Environmental Laboratory, P.O. Box 92, Clifford,
VA 24533, USA

D. Goulson
School of Life Sciences, University of Sussex, Sussex BN1 9RH, UK

D. P. Kreutzweiser
Canadian Forest Service, Natural Resources Canada, 1219 Queen
Street East, Sault Ste Marie, ON P6A 2E5, Canada

C. Krupke
Department of Entomology, Purdue University, West Lafayette, IN,
USA

M. Liess
Department System-Ecotoxicology, Helmholtz Centre for
Environmental Research, UFZ, Permoserstrasse 15, 04318 Leipzig,
Germany

M. McField
Healthy Reefs for Healthy People Initiative, Smithsonian Institution,
Belize City, Belize

C. A. Morrissey
Department of Biology and School of Environment and
Sustainability, University of Saskatchewan, 112 Science Place,
Saskatoon, SK S7N 5E2, Canada

D. A. Noome
Task Force on Systemic Pesticides, 46, Pertuis-du-Sault,
2000 Neuchâtel, Switzerland

D. A. Noome
Kijani, Kasungu National Park, Private Bag 151, Lilongwe, Malawi

J. Settele · M. Wiemers
Department of Community Ecology, Helmholtz-Centre for
Environmental Research, UFZ, Theodor-Lieser-Str. 4, 06120 Halle,
Germany

J. Settele
German Centre for Integrative Biodiversity Research (iDiv),
Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

For terrestrial and aquatic invertebrate species, the known effects of neonicotinoid pesticides and fipronil are described ranging from organismal toxicology and behavioural effects to population-level effects. For earthworms, freshwater and marine species, the relation of findings to regulatory risk assessment is described. Neonicotinoid insecticides exhibit very high toxicity to a wide range of invertebrates, particularly insects, and field-realistic exposure is likely to result in both lethal and a broad range of important sublethal impacts. There is a major knowledge gap regarding impacts on the grand majority of invertebrates, many of which perform essential roles enabling healthy ecosystem functioning. The data on the few non-target species on which field tests have been performed are limited by major flaws in the outdated test protocols. Despite large knowledge gaps and uncertainties, enough knowledge exists to conclude that existing levels of pollution with neonicotinoids and fipronil resulting from presently authorized uses frequently exceed the lowest observed adverse effect concentrations and are thus likely to have large-scale and wide ranging negative biological and ecological impacts on a wide range of non-target invertebrates in terrestrial, aquatic, marine and benthic habitats.

Keywords Pesticides · Neonicotinoids · Fipronil · Non-target species · Invertebrates · Honeybee · Earthworms · Butterflies · Freshwater habitat · Marine habitat

Introduction

Neonicotinoids and fipronil are relatively new, widely used, systemic compounds designed as plant protection products to kill insects which cause damage to crops. They are also used in veterinary medicine to control parasites such as fleas, ticks and worms on domesticated animals and as pesticides to control non-agricultural pests. Other papers in this special issue have shown that neonicotinoid insecticides and fipronil

are presently used on a very large scale (e.g. Simon-Delso et al. 2014, this issue), are highly persistent in soils, tend to accumulate in soils and sediments, have a high runoff and leaching potential to surface and groundwater and have been detected frequently in the global environment (Bonmatin et al. 2014, this issue). Effects of exposure to the large-scale pollution with these neurotoxic chemicals on non-target insects and possibly other invertebrates can be expected as identified for other insecticides. However, for the majority of insect and other invertebrate species that are likely to be exposed to neonicotinoids and fipronil in agricultural or (semi)natural ecosystems, no or very little information is available about the impact of these pesticides on their biology. Here we assess the present state of knowledge on effects on terrestrial and aquatic invertebrates.

Terrestrial invertebrates

Honeybees

Many studies have focused on investigating the effects of neonicotinoids and fipronil on honeybees (*Apis mellifera*). Apart from its cultural and honey production value, the honeybee is the most tractable pollinator species and critical for the production of many of the world's most important crops (Klein et al. 2007; Breeze et al. 2011). Losses of honeybees are generally measured as winter loss on national to regional level, and indications are that honeybee populations undergo high losses in many parts of the world (Oldroyd 2007; Stokstad 2007; van Engelsdorp and Meixner 2010; Van der Zee et al. 2012a, b).

No single cause for high losses has been identified, and high losses are associated with multiple factors including pesticides, habitat loss, pathogens, parasites and environmental factors (Decourtye et al. 2010; Mani et al. 2010; Neumann and Carreck 2010; Kluser et al. 2011). Apart from direct biotic and abiotic factors, changes in honeybee populations also depend on the economic value of honeybees and thus on human effort (Aizen and Harder 2009; Mani et al. 2010). Neonicotinoids are among the most used insecticides worldwide and are thus prime targets for investigating possible relationships with high honeybee losses.

Acute and chronic lethal toxicity to honeybees

Neonicotinoids and fipronil show high acute toxicity to honeybees (Table 1). The neonicotinoid family includes imidacloprid, clothianidin and thiamethoxam (the latter is metabolized to clothianidin in the plant and in the insect). Imidacloprid, clothianidin and thiamethoxam belong to the nitro-containing neonicotinoids, a group that is generally more toxic than the cyano-containing neonicotinoids, which includes acetamiprid and thiacloprid. Although neonicotinoids

N. Simon-Delso
Beekeeping Research and Information Centre (CARI), Place Croix
du Sud 4, 1348 Louvain-la-Neuve, Belgium

J. D. Stark
Puyallup Research and Extension Centre, Washington State
University, Puyallup, WA 98371, USA

J. P. Van der Sluijs
Centre for the Study of the Sciences and the Humanities, University
of Bergen, Postboks 7805, 5020 Bergen, Norway

H. Van Dyck
Behavioural Ecology and Conservation Group, Biodiversity
Research Centre, Earth and Life Institute, Université Catholique de
Louvain (UCL), Croix du Sud 4-5, bte L7.07.04,
1348 Louvain-la-Neuve, Belgium

Table 1 Toxicity of insecticides to honeybees, compared to DDT. Dose used is given in gram per hectare, median lethal dose (LD₅₀) is given in nanogram per bee. The final column expresses toxicity relative to DDT (DDT is 1). Source: Bonmatin (2011)

Pesticide	®Example	Main use	Typical dose (g/ha)	Acute LD ₅₀ (ng/bee)	Ratio of LD ₅₀ as compared to DDT
DDT	Dinocide	Insecticide	200–600	27,000	1
Thiacloprid	Proteus	Insecticide	62.5	12,600	2.1
Amitraz	Apivar	Acaricide	–	12,000	2.3
Acetamiprid	Supreme	Insecticide	30–150	7,100	3.8
Coumaphos	Perizin	Acaricide	–	3,000	9
Methiocarb	Mesuroi	Insecticide	150–2,200	230	117
Tau-fluvalinate	Apistan	Acaricide	–	200	135
Carbofuran	Curater	Insecticide	600	160	169
λ-cyhalotrin	Karate	Insecticide	150	38	711
Thiametoxam	Cruiser	Insecticide	69	5	5,400
Fipronil	Regent	Insecticide	50	4.2	6,475
Imidacloprid	Gaucho	Insecticide	75	3.7	7,297
Clothianidin	Poncho	Insecticide	50	2.5	10,800
Deltamethrin	Decis	Insecticide	7.5	2.5	10,800

are applied as foliar insecticides with possible direct exposure risks to honeybees, a large part of neonicotinoid use consists of seed coating or root drench application. Fipronil belongs to the phenylpyrazole family of pesticides and, like the neonicotinoids, has systemic properties (Simon-Delso et al. 2014).

Given that neonicotinoids and fipronil act systemically in plants, oral lethal doses for honeybees have been extensively studied for these compounds. Unlike many older classes of insecticides, neonicotinoids may be more toxic when ingested (Suchail et al. 2001; Iwasa et al. 2004). The level of neonicotinoids and fipronil that honeybees are exposed to in the nectar and pollen of treated plants varies greatly, although there are trends based upon application method. Generally, soil drenches and foliar application result in higher concentrations of the active compounds in plants than seed treatments, with the latter application used in large, annual cropping systems like grain crops, cotton and oilseed crops.

In practice, the honeybee lethal dose 50 (LD₅₀) for these pesticides varies for a wide range of biotic and abiotic conditions. The LD₅₀ of imidacloprid, for example, has shown values between 3.7 and 40.9, 40 and 60, 49 and 102 and 490 ng/bee (Nauen et al. 2001; Schmuck et al. 2001; Suchail et al. 2001; DEFRA 2007, 2009). This variation, of a factor 100 (5–500 ng/bee), has been observed not only between colonies but also among bees taken from a single colony. A major component of this observed variation likely stems from the discrepancy in the contact and oral toxicities of these compounds, with contact lethal doses generally being higher than oral lethal doses. However, contact with the floral parts is frequent when the bees visit flowers, and this

is different from the topical application used in laboratory conditions.

Other sources of variability may be attributed to differences in environmental conditions during testing as well as any inherent differences in the condition of the tested bees themselves. For example, data have shown that measured LD₅₀ values for bees vary with temperature (Medrzycki et al. 2011), the age of bees (Schmuck 2004; Medrzycki et al. 2011), the honeybee subspecies tested (Suchail et al. 2000), the pattern of exposure (Illarionov 1991; Belzunces 2006) and prior exposure of bees to pesticides (Belzunces 2006). Given the large variability of honeybee toxicity data, it has been suggested that LD₅₀ values should only be used to compare levels of toxicity among pesticides rather than drawing conclusions about the risk of mortality posed to honeybees via environmental exposure to pesticides (Belzunces 2006).

Oral subchronic exposure to imidacloprid and six of its metabolites induced a high toxicity at concentrations of 0.1, 1 and 10 ppb (part per billion) or ng/g, whereas the metabolites olefin-imidacloprid and 5-OH-imidacloprid were toxic in acute exposure. The main feature of subchronic toxicity is the absence of a clear dose–effect relationship that can account for a maximum effect of the lowest concentration due to the existence of multiple molecular targets, as has been demonstrated in the honeybee (Déglise et al. 2002; Thany et al. 2003; Thany and Gauthier 2005; Barbara et al. 2008; Gauthier 2010; Dupuis et al. 2011; Bordereau-Dubois et al. 2012). The absence of clear dose–effect relationships has also been observed in other studies, at higher concentrations (Schmuck 2004).

Existence of non-monotonic dose–response relations implies that some chemicals, including neonicotinoids,

have unexpected and potent effects at (very) low doses. These non-linear and often non-intuitive patterns are due to the complex interplay of receptor binding and gene reprogramming effects of such substances and can generate unexpected dose–response relationships, many of which are still being mapped out (Fagin 2012; Charpentier et al. 2014). This poses major challenges to risk assessment based on the classical log-probit model.

As previously reviewed by van der Sluijs et al. (2013), there are no standardised protocols for measuring chronic lethal effects. In traditional risk assessment of pesticides, they are usually expressed in three ways: LD₅₀: the dose at which 50 % of the exposed honeybees die (usually within a 10 day time span); no observed effect concentration (NOEC): the highest concentration of a pesticide producing no observed effect; and lowest observed effect concentration (LOEC): the lowest concentration of a pesticide producing an observed effect.

For imidacloprid, including its neurotoxic metabolites, lethal toxicity can increase up to 100,000 times compared to acute toxicity when the exposure is extended in time (Suchail et al. 2001). There has been some controversy on the findings of that study, which are discussed in detail by Maxim and Van der Sluijs (2007, 2013). However, the key finding that exposure time amplifies the toxicity of imidacloprid is consistent with later findings, implying that the standard 10 day chronic toxicity test for bees is far too short for testing neonicotinoids and fipronil, given their persistence and hence the likely chronic exposure of bees under field conditions. Indeed, honeybees fed with 10⁻¹ of the LC₅₀ of thiamethoxam showed a 41.2 % reduction of life span (Oliveira et al. 2013). Recent studies have shown that chronic toxicity of neonicotinoids can more adequately be expressed by time to 50 % mortality instead of by the 10 day LD₅₀ (Sánchez-Bayo 2009; Maus and Nauen 2010; Tennekes 2010; Tennekes 2011; Tennekes and Sánchez-Bayo 2012; Mason et al. 2013; Rondeau et al. 2014). There is a linear relation between the logarithm of the daily dose and the logarithm of the time to 50 % mortality (Tennekes 2010, 2011; Tennekes and Sánchez-Bayo 2012; Tennekes and Sánchez-Bayo 2013; Rondeau et al. 2014). Sanchez-Bayo and Goka (2014) demonstrated that field-realistic residues of neonicotinoid insecticides in pollen pose high risk to honeybees and bumblebees, whilst in the field synergisms with ergosterol inhibiting fungicides will further amplify these risks. They found that imidacloprid poses the highest risk to bumblebees (31.8–49 % probability to reach the median lethal cumulative dose after 2 days feeding on field-realistic dose in pollen) and thiamethoxam the highest risk to honeybees (3.7–29.6 % probability to reach median lethal cumulative dose). In experiments with honeybee colonies, similar, long-term chronic effects have been found with typical times of 80–120 days for 1 ppm dinotefuran and 400 ppb clothianidin (Yamada et al. 2012). Note that these

studies used concentrations that are on the uppermost limit of the currently reported ranges of concentrations found in pollen and nectar in the field. However, such data are sparse and limited to a few crops only, so it cannot yet be concluded whether such concentrations are rare or common in the field—the question of “field-relevant dose” is not yet fully resolved, and it is likely that there is a wide range in these values over space and time (Van der Sluijs et al. 2013).

Field and laboratory studies attempting to test field-realistic lethal doses have shown variable, often conflicting, results. In one study, chronic oral and contact exposure during 10–11 days to 1 µg/bee of acetamiprid and 1,000 µg/bee of thiamethoxam caused no significant worker mortality (Aliouane et al. 2009). Conversely, laboratory studies using imidacloprid showed high worker mortality when honeybees consumed contaminated pollen (40 ppb) (Decourtye et al. 2003, 2005) and contaminated sugar syrup (0.1, 1.0 and 10 ppb) (Suchail et al. 2001). These results were contrary to those of field studies performed by Schmuck et al. (2001), who reported no increased worker mortality when colonies were exposed to sunflower nectar contaminated with imidacloprid at rates from 2.0 to 20 µg/kg. Faucon et al. (2005) also found no worker mortality in a field study of honeybees fed imidacloprid in sugar syrup. A meta-analysis by Cresswell (2011) concluded that oral exposure to imidacloprid at realistic field concentrations did not result in worker mortality, although a subsequent study by Yamada et al. (2012) feeding a range of dinotefuran (1–10 ppm) and clothianidin (0.4–4 ppm) concentrations demonstrated colony failure within 104 days in each case, suggesting that detection of colony-level effects may require longer post-exposure observation.

Field studies to investigate the exposure of bees to pesticides face major difficulties. For the analysis of very low concentrations of compounds present in pollen, nectar, bees or other matrices, appropriate methods that meet validity criteria of quantitative analysis have to be developed. Pilling et al. (2013) exposed bees to thiamethoxam-treated maize and oilseed rape but were not able to quantify concentrations lower than 1 ppb, although this may be a result of the authors using a lower seed treatment application than is used in normal agricultural practice. Even though both treatment and control colonies experienced relatively high losses (mostly queens laying only drone brood) and the authors were unable to undertake any statistical analysis due to a lack of replication, they wrongly concluded that there is a low risk to honeybees from exposure to treated maize and oilseed rape.

Also, in terms of activity and feeding behaviour, bees might not be foraging on treated crops in (exactly) the same way as they would do on untreated crops (Colin et al. 2004). Furthermore, comparison of treated and control areas can be totally flawed because control fields might not be “clean” but treated with other pesticides,

including insecticides. The recent study of Pilling and co-workers on thiamethoxam (Pilling et al. 2013) is illustrative for this case as it did not provide information about the treatment status of the control plots.

For mass-dying of bees in spring near corn fields during sowing of neonicotinoid-treated seeds, there now is a one to one proven causal link. Acute intoxication occurs through exposure to the dust cloud around the pneumatic sowing machines during foraging flights to adjacent forests (providing honeydew) or nearby flowering fields (Apenet 2010; Girolami et al. 2012; Tapparo et al. 2012; Krupke et al. 2012; Pochi et al. 2012; Tapparo et al. 2012). In these cases, dead bees have typically been found to have high levels of seed treatment neonicotinoids on, or in, their bodies. Such mass colony losses during corn sowing have been documented in Italy, Germany, Austria, Slovenia, the USA and Canada (Gross 2008; Krupke et al. 2012; Sgolastra et al. 2012; Tapparo et al. 2012). In response to the incidents, the adherence of the seed coating has been improved owing to better regulations, and an improved sowing technique has recently become compulsory throughout Europe (European Commission 2010). However, despite the deployment of air deflectors in the drilling machines and improved seed coating techniques, emissions are still substantial and the dust cloud remains acutely toxic to bees (Biocca et al. 2011; Marzaro et al. 2011; Girolami et al. 2012; Tapparo et al. 2012; Sgolastra et al. 2012).

Acute lethal effects of neonicotinoids dispersed as particles in the air seem to be promoted by high environmental humidity (Girolami et al. 2012). Honeybees also transport toxic dust particles on their bodies into the hive (Girolami et al. 2012). Sunny and warm days also seem to favour the dispersal of active substances (Greatti et al. 2003).

Sublethal effects on honeybees

Effects on activity, locomotion, metabolism and ontogenetic development Imidacloprid, thiamethoxam and clothianidin have been shown to rapidly induce flight muscle paralysis in honeybees exposed to guttation drops containing these substances, resulting in the cessation of wing movements (Girolami et al. 2009). Imidacloprid further impairs the mobility of bees, as reflected by decreases in running and walking and increases in the time that exposed bees remain stationary (Medrzycki et al. 2003). However, when exposed to subchronic doses of neonicotinoids, decreases in locomotion were not observed in honeybees and bumblebees by Cresswell et al. (2012b).

Ontogenetic development is a crucial period that determines the physiological and functional integrity of adult individuals. Thus, in addition to the effects on adults, neonicotinoids may act on larval development with consequences for the adult stage. Adult honeybees exposed to

imidacloprid during the larval stage exhibit impairment of olfactory associative behaviour (Yang et al. 2012). This could be due to altered neural development. Impairments in mushroom body development in the bee brain and the walking behaviour of honeybee workers have been observed in individuals exposed to imidacloprid during the larval period (Tomé et al. 2012). Effects on adult bees exposed during the larval stage could also be attributed to the induction of cell death by imidacloprid in larvae (Gregorc and Ellis 2011). In the early stages of adult life, after emergence, imidacloprid can disrupt the development of hypopharyngeal glands by decreasing the size of the acini and by increasing the expression of hsp70 and hsp90 (Smodis Skerl et al. 2009; Hatjina et al. 2013). Derecka et al. (2013) provided beehives in the field for 15 days with syrup tainted with 2 µg/l imidacloprid. They found that these levels of imidacloprid, at the low end of the field-realistic range, significantly impact energy metabolism in worker bee larvae.

Impacts of pesticides on metabolism may affect the detoxifying, intermediary and energetic metabolism pathways. Imidacloprid impairs brain metabolism in the honeybee which results in an increase of cytochrome oxidase in mushroom bodies (Decourtye et al. 2004a, b).

Effects on behaviour, learning and memory Optimal function of the honeybee nervous system is critical to individual and colony functioning (Desneux et al. 2007; Thompson and Maus 2007). Increasing levels of research effort have been devoted to developing an improved understanding of how sublethal exposure to neonicotinoids and fipronil may affect the honeybee nervous system. There is evidence that sublethal exposure can affect learning, memory and orientation in honeybees.

Laboratory experiments administering a single dose of imidacloprid demonstrated that learning was altered (Guez et al. 2001; Lambin et al. 2001), and exposure to chronic sublethal doses has demonstrated that learning and foraging are impaired by imidacloprid and fipronil (Decourtye et al. 2003). Furthermore, thiamethoxam has been shown to decrease memory capacity (Aliouane et al. 2009). The methodologies and doses varied in these laboratory tests, but all used concentrations above 20 ppb; this is towards the upper end of concentrations found in most field situations. These concentrations would not be expected to be found in pollen or nectar following seed treatment applications, but have been found in cucurbit flowers following soil drench applications (Dively and Hooks 2010). Field experiments offer the potential for powerful tests; however, results have been mixed, and many studies focus on honeybee orientation to and from a feeding source. A study that trained honeybee foragers to a sugar syrup reward in a complex maze demonstrated that 38 % of bees found the food source following ingestion of 3 ng/bee of

thiamethoxam, compared with 61 % in an unexposed control group (Decourtye and Devillers 2010). A series of studies training foragers to orient to a sugar feeder found that foragers were unable to return to the hive after ingesting imidacloprid at concentrations ranging from 100 to 1,000 ppb (Bortolotti et al. 2003; Ramirez-Romero et al. 2005; Yang et al. 2008). In contrast, other semi-field studies have shown no effects upon foraging or survivorship following exposure to canola, maize and sunflowers grown from neonicotinoid-treated seeds (Schmuck et al. 2001; Cutler and Scott-Dupree 2007; Nguyen et al. 2009). Possible explanations for these conflicting results may be that when given a range of foraging opportunities, honeybees may reduce foraging visits to food sources containing pesticides (Mayer and Lunden 1997; Colin et al. 2004), or that neonicotinoids do not have effects on colonies in the exposure regimes tested here.

Recently, Henry et al. (2012a, b) described the results of innovative field experiments using radio frequency identification (RFID) tags to determine the colony-level effects of orientation impairment upon foragers fed a sublethal dose of imidacloprid (1.42 ng in 20 μ l of sucrose syrup). In two separate experiments, treated foragers failed to return to the colony at rates of 10.2 and 31.6 %, relative to untreated foragers feeding upon the same flowering plants. A higher risk of not returning was associated with the more difficult orientation tasks. Using these forager loss rates, the researchers modelled the colony-level effects and found significant, largely consistent deviations from normal colony growth rates, in some cases to levels that may put the colony at risk of collapse. A subsequent suggestion by Cresswell and Thompson (2012) to alter the simulation slightly to reflect the period when seed-treated crops are flowering demonstrated that the risk of collapse was no longer evident. However, a follow-up calculation by Henry et al. (2012a) using a larger dataset that incorporated a range of empirically derived colony growth estimates revealed even higher deviations from normal than the original work: a more serious negative outcome for colonies. The variable outcomes based upon model assumptions reflect uncertainties that have plagued honeybee researchers and further underscore the importance of ensuring that models are robust and represent a range of scenarios. The key contribution of this work was the demonstration that sublethal doses can impose a stressor (i.e. non-returning foragers) that can have significant negative outcomes on a colony level.

Learning and memory represent fundamental functions involved in the interaction of individuals with their environment and are critical in enabling bees to respond to the requirements of the colony throughout their life. Imidacloprid impairs learning and olfactory performance via both acute and chronic exposure pathways, and summer bees appear more sensitive than winter bees (Decourtye et al. 2003). These effects are observed not only in the laboratory but also in

semi-field conditions, and bees do not recover after exposure ceases. Results obtained with acetamiprid and thiamethoxam showed that the action of neonicotinoids depends on the level/degree of exposure and cannot be generalized to structurally related compounds. Unlike contact exposure, oral exposure of acetamiprid resulted in an impairing of long-term retention of olfactory learning (El Hassani et al. 2008). Conversely, for thiamethoxam, subchronic exposure, but not acute exposure, elicited a decrease of olfactory memory and an impairment of learning performance (El Hassani et al. 2008; Aliouane et al. 2009).

Neonicotinoids have specific routes of metabolism in insects, particularly in the honeybee, that lead to complex influences on learning and memory processes. Imidacloprid and thiamethoxam are metabolized into toxic metabolites that may potentially bind to different honeybee nicotinic acetylcholine receptors (Nauen et al. 2001; Suchail et al. 2001, 2004a; Nauen et al. 2003; Ford and Casida 2006; Benzidane et al. 2010; Casida 2011). The metabolism of acetamiprid results in the appearance of different metabolites in the honeybee, among which 6-chloronicotinic acid is toxic in chronic exposure but not in acute exposure and remains stable for at least 72 h, especially in the head and the thorax (Suchail et al. 2001, 2004a; Brunet et al. 2005). Considering the presence of multiple active metabolites over time, it is very difficult to ascertain what steps of the memory process (acquisition, consolidation or retrieval) are affected by imidacloprid, acetamiprid, thiamethoxam or their metabolites.

Habituation may be defined as “a form of learning that consists in the gradual and relatively prolonged decrease of the intensity or the frequency of a response following the repeated or prolonged stimulus responsible for eliciting such a response” (Braun and Bicker 1992; Epstein et al. 2011a, b; Belzunces et al. 2012). Habituation can be regarded as an important adaptive behaviour because it allows individuals to minimize their response and, therefore, their energy investment, towards unimportant stimuli. The neonicotinoid imidacloprid alters patterns of habituation in honeybees following contact exposure to a sublethal dose (Guez et al. 2001; Lambin et al. 2001). Imidacloprid-induced changes in habituation appear to vary depending on the age of bees and time after exposure. Furthermore, these changes in habituation may be due to factors such as differential sensitivity of different nicotinic acetylcholine receptors (nAChRs) to imidacloprid (Déglise et al. 2002; Thany et al. 2003; Thany and Gauthier 2005; Barbara et al. 2008; Gauthier 2010; Dupuis et al. 2011; Bordereau-Dubois et al. 2012; Farooqui 2013), or the accumulation of imidacloprid metabolites like olefin and 5-hydroxy-imidacloprid, which can delay or accelerate habituation, respectively (Guez et al. 2001, 2003).

Olfaction and taste are very important physiological senses for honeybees (Detzel and Wink 1993; Giurfa 1993; Balderrama et al. 1996; Goulson et al. 2001; Reinhard et al.

2004; Gawleta et al. 2005; Couvillon et al. 2010; Maisonnasse et al. 2010; Kather et al. 2011). The effects of neonicotinoids on gustation can be explored by studying the modulation of the gustatory threshold, which can be defined as the lowest concentration of a sucrose solution applied to the antenna that triggers a feeding response. Different active compounds have been shown to induce dissimilar effects on gustation in honeybees. For example, fipronil increases the gustatory threshold of bees subjected to contact exposure (El Hassani et al. 2005). Whilst similar results were found for imidacloprid, acetamiprid decreases the threshold of bees that are exposed orally, but not topically (El Hassani et al. 2009). Thiamethoxam elicits a decrease in honeybee responsiveness to sucrose, and exposure to acetamiprid increases the responsiveness of honeybees to water regardless of exposure route (El Hassani et al. 2008; Aliouane et al. 2009).

The discrepancy in the effects observed could be explained in part by neonicotinoid metabolism that induced the appearance of toxic metabolites (Suchail et al. 2004a, b; Brunet et al. 2005) and by the existence of different nAChRs that are either sensitive and resistant to particular neonicotinoids (Déglise et al. 2002; Thany et al. 2003; Thany and Gauthier 2005; Barbara et al. 2008; Gauthier 2010; Dupuis et al. 2011; Bordereau-Dubois et al. 2012). Although it has been demonstrated in pollinating flies and in beetles, the repellent effect of imidacloprid and other neonicotinoids has not been investigated in the honeybee (Easton and Goulson 2013).

Accurate navigation is essential for efficient foraging and, hence, for colony health and survival. Neonicotinoids and fipronil may impair navigation in different ways. Sublethal exposure of honeybees to clothianidin and imidacloprid elicits a decrease in foraging activity and induces longer foraging flights (Schneider et al. 2012). Thiamethoxam induces high mortality by causing failure in the homing behaviour of foraging bees, leading to large losses of foragers from the colony (Henry et al. 2012a, b). Although this effect has been demonstrated for the pyrethroid deltamethrin for almost 20 years (Vandame et al. 1995), impacts on the homing behaviour of foraging bees continue to be left out of the assessment process for pesticide registration.

Proper foraging behaviour is essential for both individual bees and the colony as a whole because it determines the availability of food (stores) and, consequently, the survival of the colony. Exposure to imidacloprid, clothianidin and fipronil can lead to reductions in the proportion of active bees in the hive and, furthermore, initiate behaviours that can reduce the efficiency of foraging flights. For example, exposed individuals may spend longer periods of time at a food source, decrease the frequency of visits, increase the time between foraging trips, engage in longer foraging flights, reduce foraging distances, exhibit problems revisiting the same feeding site or exhibit reductions in visual learning capacities (Nielsen et al. 2000; Morandin and Winston 2003;

Colin et al. 2004; Ramirez-Romero et al. 2005; Yang et al. 2008; Han et al. 2010; Schneider et al. 2012; Teeters et al. 2012). Fischer et al. (2014) exposed adult honeybees to sublethal doses of imidacloprid (7.5 and 11.25 ng/bee), clothianidin (2.5 ng/bee) and thiacloprid (1.25 µg/bee) and subsequently tracked the flight paths of individual bees with harmonic radar. The rate of successful return was significantly lower in treated bees, the probability of a correct turn at a salient landscape structure was reduced and less directed flights during homing flights were performed. These findings show that sublethal doses of these three neonicotinoids either block the retrieval of exploratory navigation memory or alter this form of navigation memory. Reproduction and colony development may be regarded as integrative endpoints for assessing the final impacts of pesticides on bees as both are a compulsory condition of social insect physiology.

Neonicotinoids such as thiacloprid, thiamethoxam and imidacloprid decrease brood production, larval eclosion, colony growth rate and the number of queens reared in bumblebees (Tasei et al. 2000; Mommaerts et al. 2010; Whitehorn et al. 2012). Studies suggest that the reduction in brood production may be associated with a reduction in pollen and sugar consumption by adult bees (Laycock et al. 2012a, b). The rearing of honeybees on brood comb containing high levels of pesticide residues results in delayed larval development and emergence and shortened adult longevity (Wu et al. 2011). Since the brood combs in the latter study contained five neonicotinoids at relatively high concentrations, it is difficult to ascribe the observed effects to any one pesticide, or pesticide class. An epidemiological study involving Hill's criteria (minimal conditions that prove evidence of a causal relationship) revealed conflicting results on the involvement of dietary traces of neonicotinoids in the decline of honeybee populations (Cresswell et al. 2012a) and could not establish a causal link between observations of bee decline and neonicotinoid use rates.

Interaction with pathogens

Detrimental effects of pesticides might be increased in combination with other environmental stress agents (Mason et al. 2013). Specific pathogens and parasites are ancestral companions of (some) honeybee populations, and accidental movement of parasites and pathogens by man has exposed both honeybees and wild bees to non-native enemies to which they may have reduced resistance (e.g. Goulson 2003; Graystock et al. 2013a, b). Imidacloprid can act synergistically with the pathogen *Nosema* spp. by increasing *Nosema*-induced mortality (Alaux et al. 2010). It affects social immunity and so increases the number of *Nosema* spores in the guts of bees from imidacloprid-exposed colonies exposed in cage studies (Pettis et al. 2012). Sequential exposure to *Nosema ceranae* can sensitize bees to thiacloprid by eliciting potentiation that

leads to high mortality rates, a feature shared with fipronil (Vidau et al. 2011; Aufauvre et al. 2012). Similarly, other experiments with fipronil and *N. ceranae* have demonstrated reciprocal sensitization (Aufauvre et al. 2012). Furthermore, exposure to pesticides during embryonic and post-embryonic development may alter the susceptibility of adult bees to pathogens. For example, adult honeybees reared in brood combs containing high levels of pesticide residues exhibit higher levels of infection by *N. ceranae* and higher levels of *Nosema* spores (Wu et al. 2012).

Di Prisco et al. (2013) demonstrated that clothianidin negatively modulates nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B, a protein involved in DNA transcription) immune signaling in insects and adversely affects honeybee antiviral defences controlled by this transcription factor. They identified a negative modulator of NF- κ B activation specific for insects. Exposure to clothianidin, by enhancing the transcription of the gene encoding this inhibitor, reduces immune defences and promotes the replication of the deformed wing virus present in honeybees. Similar immunosuppression was found to be induced by imidacloprid. The occurrence of this insecticide-induced viral proliferation at sublethal doses that are well within field-realistic concentrations suggests that the studied neonicotinoids are likely to have a negative effect under field conditions.

Synergistic effects with other pesticides

In agricultural ecosystems, honeybees are seldom exposed to only a single pesticide. Combined exposures could be of high concern because they can elicit synergies and potentiations. For example, thiacloprid acts synergistically with ergosterol biosynthesis inhibitor (EBI) fungicides in bees exposed in laboratory conditions but not in tunnel conditions (Schmuck et al. 2003).

Analyses of honeybees and colony contents indicate that honeybees are indeed frequently exposed to multiple pesticides simultaneously (Mullin et al. 2010; Krupke et al. 2012; Paradis et al. 2013). However, the study of pesticide mixtures can be challenging (Lydy et al. 2004), and there is a paucity of information in the literature regarding the mixtures encountered by honeybees. Triazole fungicides have been found in pollen collected from colonies (Krupke et al. 2012) and have been shown to synergize toxicity of some neonicotinoids (thiacloprid and acetamiprid) up to 559-fold in the laboratory, although the same results have not been shown in semi-field studies (Schmuck et al. 2003). Piperonyl butoxide also has been found in pollen and has been shown to synergize toxicity of some neonicotinoids (thiacloprid and acetamiprid) up to 244-fold in the laboratory (Iwasa et al. 2004). Despite the challenges associated with this type of research, this is a clear research gap that should be addressed in the future, given that

honeybees rarely encounter only a single pesticide during foraging and/or in the hive.

Toxicity to bumblebees and solitary bees

Bumblebees (genus *Bombus*) are primitive social bees. Colonies start from overwintering queens, build up to a few hundred adult workers and break down when new queens and males are produced. A small number of bumblebee species are commercially reared for pollination, but many of the non-managed bumblebees also contribute substantially to crop pollination (Chagnon et al. 1993; Bosch and Kemp 2006; Greenleaf and Kremen 2006; Goulson 2010). Solitary bees that are also commonly managed in agricultural settings include the alfalfa leafcutter bee (*Megachile rotundata*), alkali bees (*Nomia melanderi*), blue orchard bees (*Osmia lignaria*) and Japanese horn-faced bees (*Osmia cornifrons*). *M. rotundata* is the major pollinator of alfalfa, which is grown as a high value livestock feed in North America. It is often considered a domesticated species, although populations frequently occur naturally. This species contributed US\$5.26 billion to the value of alfalfa hay in 2009 (Calderone 2012). In addition to managed bees, there are more than 20,000 species of wild bees in the world, many of which contribute to crop pollination, and all of them contribute to pollination of wild flowers.

There are few long-term population-level studies involving bumblebees and other bee species, and in many cases, the impacts of pesticide exposure and dosage are unclear. These species differ from honeybees in that they generally exhibit smaller foraging ranges and often prefer to nest in the ground. Therefore, populations located near agricultural operations and associated pesticide applications may have fewer alternative options for food and habitat resources. Furthermore, ground-nesting species may face additional exposure risks (i.e. pesticide-contaminated soil) that are not encountered by honeybees, but which remain to be evaluated. Finally, whilst bumblebees tend to be bigger, solitary bees are often smaller than honeybees; thus, these species likely receive a different dose relative to their body weight than honeybees do.

Likely levels of exposure of wild bee species are poorly understood. Whilst neonicotinoid levels have been quantified in the nectar and pollen of various crop plant species (Cresswell 2011; Anon 2012), the degree to which wild bees utilize these resources has not been measured, and furthermore, basic values of toxicity, such as LD₅₀ and LC₅₀, are completely lacking for the vast majority of these species. The few studies that do exist have employed a range of methods with conflicting results so that drawing general conclusions is difficult at this stage. Moreover, these studies are criticised for low sample size, which limits power to detect effects and/or highly unnatural laboratory conditions.

It is clear that neonicotinoids and fipronil are highly toxic to all bee species tested so far, which in addition to honeybees

includes various *Bombus* species, several social stingless bee species and the solitary species *O. lignaria* and *M. rotundata* (Scott-Dupree et al. 2009; Valdovinos-Núñez et al. 2009; Gradish et al. 2010; Mommaerts et al. 2010; Tomé et al. 2012). Cresswell et al. (2012a, b) demonstrated that bumblebees exhibit sublethal responses to imidacloprid at 10 ppb, whilst honeybees were unaffected at this concentration. Scott-Dupree et al. (2009) found that *O. lignaria* is more sensitive to both clothianidin and imidacloprid than *Bombus impatiens*, with *M. rotundata* more sensitive still. Stark et al. (1995) found no difference in the 24 h contact LD₅₀ of imidacloprid between honeybees and the solitary bee species *M. rotundata* and *N. melanderi*. Scott-Dupree et al. (2009) demonstrated that *B. impatiens* individuals were more tolerant of thiamethoxam and clothianidin than *O. lignaria* and *M. rotundata*. However, the orchard bee *O. lignaria* exhibits delayed hatching and development when fed imidacloprid at rates from 30 to 300 µg/kg (Abbott et al. 2008). Arena and Sgolastra (2014) compared the acute toxicity of numerous pesticides and found that *Scaptotrigona postica* and *M. rotundata* were more sensitive than honeybees to fipronil, whilst *N. melanderi* was more tolerant. Together, these results suggest that “other” bees may be at least as sensitive, if not more sensitive, to neonicotinoids than honeybees, although more work is clearly needed.

A number of studies have used queenless micro-colonies of bumblebees (containing only workers) to examine the sublethal effects of cumulative neonicotinoid exposure to low, field-realistic doses. Several have found no detectable effects; for example, Tasei et al. (2000) exposed *Bombus terrestris* micro-colonies to 6–25 ppb of imidacloprid and found no significant response. Similarly, Franklin et al. (2004) exposed *B. impatiens* to concentrations of up to 36 ppb of clothianidin without detecting an impact (see also Morandin and Winston 2003). Most recently, Laycock et al. (2012a, b) exposed micro-colonies of *B. terrestris* to a range of concentrations of imidacloprid (0–125 µg/l) and detected a 30 % reduction in fecundity at doses as low as 1 ppb. In the only comparable work on other bee species, Abbott et al. (2008) injected concentrations of up to 300 ppb of neonicotinoids into pollen stores of *O. lignaria* and *M. rotundata* with no measurable impact on larval development.

Interestingly, negative effects seem to be detected more frequently and at lower concentrations when bees have to forage at a distance, even when the distances are tiny. Mommaerts et al. (2010) found no impact of imidacloprid exposure on micro-colonies of *B. terrestris* at field-realistic concentrations when food was provided in the nest, but when workers had to walk just 20 cm down a tube to gather food they found significant sublethal effects on foraging activity, with a median sublethal effect concentration (EC₅₀) of just 3.7 ppb. The same researchers also studied queenright colonies foraging in a glasshouse where food was 3 m from their nest and found that ingestion of 20 ppb of imidacloprid caused

significant worker mortality, including bees dying at the feeder. Significant mortality was also observed at 10 ppb, but not at 2 ppb. This may explain why some lab studies have failed to find effects.

With impacts more pronounced when bees have to leave the colony, one might predict more marked effects when bees are foraging naturally, travelling kilometres across the landscape (Knight et al. 2005; Osborne et al. 2008). Only four studies have examined impacts of exposure to neonicotinoids on non-*Apis* bees when free-flying in the landscape. Tasei et al. (2001) placed *Bombus lucorum* colonies in the field for 9 days, either adjacent to an imidacloprid-treated field or a control field of sunflowers. During this time, 54 % more of the foragers from the ten imidacloprid-exposed colonies failed to return compared to the ten control colonies; however, this difference was not statistically significant as sample sizes were very small. After 9 days, the colonies were returned to the lab and fed ad libitum. Treated colonies grew more slowly but the difference was not significant. Gill et al. (2012) provided *B. terrestris* colonies with feeders containing 10 ppb of imidacloprid in sugared water whilst simultaneously allowing bees freedom to forage outside the nest. Bees exposed to imidacloprid brought back pollen less often and tended to bring back smaller loads, compared to control bees. Feltham et al. (2014) simulated exposure of queenright *B. terrestris* colonies to a crop of flowering oilseed rape, providing them with sugared water and pollen containing 0.7 and 6 ppb of imidacloprid, respectively, for 2 weeks. They found a 57 % reduction in the mass of pollen brought back to colonies, which persisted for at least 4 weeks after treatment ceased. Only one study to date has attempted to examine the effects of exposure to neonicotinoids on colony-level development of bumblebees under field conditions; Whitehorn et al. (2012) used the same field-realistic doses as Feltham et al. (2014) and then allowed colonies to develop naturally in the field. They recorded significantly reduced nest growth and an 85 % decrease in queen production in imidacloprid-exposed colonies compared to control colonies. This reduction in colony performance is likely due to a combination of factors such as reduced pollen input (as demonstrated by Gill et al. 2012 and Feltham et al. 2014) and perhaps impaired fecundity of queens (following Laycock et al. 2012a, b). In an 11 week greenhouse study, caged queenright colonies of *B. impatiens* were fed treatments of 0, 10, 20, 50 and 100 ppb of imidacloprid, respectively, and clothianidin in sugar syrup (50%) (Scholer and Krischik 2014). At 6 weeks, queen mortality was significantly higher in 50 and 100 ppb and by 11 weeks in 20–100 ppb neonicotinyl-treated colonies. Starting at 20 ppb, there is a statistically significant reduction in queen survival (37 % for imidacloprid, 56 %

for clothianidin), worker movement, colony consumption and colony weight compared to 0 ppb treatments. At 10 ppb imidacloprid and 50 ppb clothianidin, fewer males were produced (Scholer and Krischik 2014).

Bryden et al. (2013) conceived a model to simulate bumblebee colony development to assess the colony-level impacts of well-known sublethal effects on individuals. Their study shows that bumblebee colonies fail when exposed to sustained sublethal levels of pesticide. This is explained by impairment of colony function. Social bee colonies have a positive density dependence, and they are subject to an Allee effect. There is a critical stress level for the success of a colony such that a small increase in the level of stress can make the difference between failure and success.

It seems likely that intoxicated bees are fully able to gather food when it is presented to them within the nest, but when bees have to navigate over realistic distances to extract nectar and pollen from complex, patchily distributed flowers, the effects of intoxication become evident. Studies have focused mainly on behavioural effects in adult bees shortly after exposure to neonicotinoids, but there is evidence from both honeybees (Yang et al. 2012) and stingless bees (Tomé et al. 2012) that exposure during larval stages can impair development of the central nervous system and, hence, result in reduced adult performance several weeks after colony exposure. Therefore, the implications for risk assessment are clear; lab trials, and even trials where colonies are placed immediately adjacent to treated crops, are not appropriate for detecting these impacts. Similarly, experiments need to run for many weeks to examine the long-term effects of exposure on bee health.

The existing toxicological data suggests that impacts on diverse bee taxa are broadly similar at the level of the individual bee, with some evidence that bumblebees and solitary bees may be more susceptible than honeybees. It is clear that field-realistic doses of neonicotinoids can have a range of significant detrimental effects on larval development, adult fecundity, adult foraging behaviour and colony performance in social species. However, the effects of neonicotinoids on the vast majority of bee species have not been examined, and caution is necessary when extrapolating from social to solitary species. No studies have evaluated the impacts of neonicotinoids on solitary species under field conditions. It might plausibly be argued that the large colony size exhibited by honeybees and some stingless bees could buffer these species against reductions in foraging performance, as well as any navigational errors on the part of workers; however, this is unlikely to be the case for either bumblebee colonies, which have just a few hundred workers at most, or solitary bees, where a single female has sole responsibility for provisioning of offspring. Thus, impacts at the population level may be inversely related to levels of sociality. This possibility awaits experimental investigation.

Butterflies and moths (Lepidoptera)

Among agricultural practices, pesticide use is known to impact butterflies and moths; however, based on observational field data, it is difficult to distinguish the impacts of pesticides from other agricultural customs, such as fertilizer application or landscape simplification, e.g. by removal of hedgerows (Geiger et al. 2010). In the case of butterflies or moths that inhabit structures adjacent to areas where pesticides are applied via aerial spraying, indirect effects of drift from spraying may pose risks both during and after applications (Sinha et al. 1990). In the 1980s for example, helicopter application of pesticides in vineyards of the Mosel Valley in Germany nearly led to the extinction of an isolated population of the Apollo butterfly (*Parnassius apollo*) which was restricted to adjacent rocky slopes (Kinkler et al. 1987; Richarz et al. 1989; Schmidt 1997). In Northern Italy, butterfly communities in natural grasslands have suffered drastic declines downwind of intensively sprayed orchards, leading to the disappearance of all but the most generalist species (Tarmann 2009). Furthermore, spray applications of pesticides may alter soil quality (Freemark and Boutin 1995) and thereby indirectly affect the larvae and pupae of moth species residing in the upper layers of the soil surface during the spring.

Contrary to other non-target species (e.g. bees, birds, spiders, ground beetles), very few comparative pesticide sensitivity tests have been carried out for butterflies and moths. This is surprising given the significant role these insects play for conservation programs. One such study conducted by Brittain et al. (2010b) evaluated the impact of pesticides on various groups of pollinators. When comparing intensively managed systems (high pesticide application rates) with less intensively managed systems (fewer pesticide applications), the authors demonstrated that fewer bumblebee and butterfly species were observed in intensively managed habitat patches. The study also demonstrated that wild bees have higher pesticide-related risks than butterflies (Brittain et al. 2010b).

Moreover, studies by Feber et al. (1997) and Rundlöf et al. (2008) have demonstrated negative impacts of pesticides on butterflies. Both studies evaluated the impacts of organic versus conventional agriculture on butterfly populations. In each case, organic farms were found to host greater numbers and species of butterflies. This response was likely due in part to reduced applications of herbicides in organic systems, as herbicides reduce the abundance of host and nectar plants that are crucial for the survival of larvae as well as adults (Boggs 2003). In contrast, similar studies comparing Lepidopteran communities between organic and conventional agriculture systems found no differences in the number or species richness of butterflies (Weibull et al. 2000 and Brittain et al. 2010a). In the case of these studies, characteristics of the surrounding landscape such as the absence of specific vegetation

elements (e.g. hedgerows or floral nectar sources) at both the local and regional scales, or the broad scale application of pesticides, may have influenced the outcome of the findings.

In contrast to the few ecological and ecotoxicological studies on the direct and indirect impacts of pesticides on non-target Lepidoptera, numerous results are available on the impacts of pesticides on the butterfly and moth species that are regarded as agricultural pests during the larval stage (Haynes 1988; Davis et al. 1991a, b, 1993; Liang et al. 2003). The impacts of systemic pesticides on Lepidoptera have been investigated for some 32 pest species of moths in nine different families (Table 2). This represents a tiny fraction of the estimated 200,000 Lepidoptera species. The results demonstrate considerable variation in the impact of pesticides on different species of Lepidoptera. For example, Doffou et al. (2011a, b) noted that the susceptibility of two cotton pests, *Pectinophora gossypiella* (Gelechiidae) and *Cryptophlebia leucotreta* (Tortricidae), to acetamiprid differs almost 3-fold (LD_{50} =11,049 and 3,798 ppm, respectively). First instar *Cydia pomonella* caterpillars (Tortricidae) are more than 100 times as sensitive as final fifth instar caterpillars, with an LC_{50}/LC_{90} of 0.84/1.83 and 114.78/462.11 ppm, respectively (Stara and Kocourek 2007a, b).

Not surprisingly, different neonicotinoid compounds vary in toxicity. Thiacloprid and acetamiprid for example are recorded to have stronger effects on the survival of *Phyllonorycter ringoniella* than all other neonicotinoid substances (Funayama and Ohsumi 2007a, b). Acetamiprid has been shown to be more toxic than thiacloprid in several studies, but the degree of difference varies greatly. For example, a study by Cichon et al. (2013) found thiacloprid to be two times as toxic to *C. pomonella* as acetamiprid (LC_{99}/LC_{50} =1.55/0.17 vs 0.71/0.08 ppm, respectively), whilst Magalhaes and Walgenbach (2011) recorded a 60-fold difference in the sensitivity of the same species to these compounds (LC_{50} =1.06 and 65.63 ppm, respectively).

Many studies have documented systemic pesticide resistance in Lepidoptera; for example, *Phthorimaea operculella* has been found to be resistant to fipronil (Doğramaci and Tingey 2007), *Spodoptera litura* to both fipronil and imidacloprid (Huang et al. 2006a, b; Ahmad et al. 2008; Abbas et al. 2012), *C. pomonella* to acetamiprid and thiacloprid (Cichon et al. 2013; Knight 2010; Stara and Kocourek 2007a, b), and *Plutella xylostella* to acetamiprid (Ninsin et al. 2000a, b). In the latter field study from Japan, an almost 10-fold higher dosage was required to reach the same lethal concentration ($LC_{50/95}$ =315/2,020 compared to 35.1/137 ppm in susceptible laboratory colonies). Applications of such high concentrations may further increase negative impacts on non-target species of insects. Even low sublethal doses can have severe impacts on Lepidoptera populations. In a study on *Helicoverpa armigera* by Ahmad et al. (2013), a

16th of the LC_{50} of imidacloprid (5.38 ppm) increased the next generation survival rate by a factor of 4 (i.e. equivalent to LC_{10}) compared to a treatment with the LC_{50} dose. Sublethal effects included a significant reduction in the survival and fecundity as well as increased mortality in the first and subsequent generations. Asaro and Creighton (2011a, b) noted that loblolly pines appeared to be protected from the Nantucket pine tip moth (*Rhyacionia frustrana*) even 1 year after treatment, and the treatment effect apparently was not confined to the target pest species, but extended to further non-target insect species.

There is a clear need for studies on the impact of pesticides on butterflies and moths and in particular those species that are not agricultural pests, but which commonly inhabit managed landscapes. Extensive studies on the direct and indirect effects of pesticides on these non-target groups are urgently needed on different geographical scales and across long time periods (Aebischer 1990) and which include all developmental stages of butterflies and moths (i.e. egg, larva, pupa, adult). It is of paramount importance to include varying intensities of pesticide applications, their persistence and their interplay with biotic and abiotic factors (Longley and Sotherton 1997; Brittain et al. 2010b).

Other invertebrates

This section will review the studies on neonicotinoids and non-target organisms, in particular the predatory invertebrates of natural pest species. Biological pest control plays an important role in integrated pest management (Byrne and Toscano 2007; Peck and Olmstead 2010; Prabhaker et al. 2011; Khani et al. 2012) with studies suggesting that predators may contribute to the similarity in crop yields between non-treated and pesticide-treated fields (Albajes et al. 2003; Seagraves and Lundgren 2012).

Routes of exposure

Non-target organisms can be exposed to neonicotinoid pesticides in a variety of ways. Predatory invertebrates may become contaminated by consuming pests such as leafhoppers or aphids that feed on treated crops (Albajes et al. 2003; Papachristos and Milonas 2008; Moser and Obrycki 2009; Prabhaker et al. 2011; Khani et al. 2012). Direct contamination through the diet can also be a problem for other beneficial plant-feeding invertebrates (Dilling et al. 2009; Girolami et al. 2009; Moser and Obrycki 2009; Prabhaker et al. 2011; Khani et al. 2012). For example, several species of hoverfly and parasitoid wasps attack agricultural pests, but also subsidise their diet with nectar. Therefore, these insects can be affected by neonicotinoids, which are translocated into the nectar and pollen of treated crop plants (Stapel et al. 2000; Kruschik et al. 2007).

Table 2 Studies on the effects of systemic pesticides in Lepidoptera

Family	Species	Host	Imidacloprid	Thiamethoxam	Clothianidin	Acetamiprid	Thiacloprid	Dinotefuran	Fipronil
Gelechiidae	<i>Pectinophora gossypiella</i>	Cotton				Doffou et al. (2011a, b)			
Gelechiidae	<i>Phthorimaea operculella</i>	Potato	Symington (2003)				Saour (2008)		Dogramaci and Tingey (2008)
Gracillariidae	<i>Camieraria ohridella</i>	Horse chestnut tree	Stygar et al. (2013)						
Gracillariidae	<i>Phyllocnistis citrella</i>	Citrus	Villanueva-Jimenez and Hoy (1998), Setamou et al. (2010)						
Gracillariidae	<i>Phyllonorycter ringoniella</i>	Apple	Funayama and Ohsumi (2007a, b)	Funayama and Ohsumi (2007a, b)	Funayama and Ohsumi (2007a, b)	Funayama and Ohsumi (2007a, b)	Funayama and Ohsumi (2007a, b)	Funayama and Ohsumi (2007a, b)	
Lyoniidae	<i>Leucopetra coffeella</i>	Coffee		Diez-Rodriguez et al. (2006)					
Noctuidae	<i>Agrotis ipsilon</i>	Corn and various crops			Kullik et al. (2011a)				
Noctuidae	<i>Helicoverpa armigera</i>	Various crops	Ahmad et al. (2013)						
Noctuidae	<i>Helicoverpa zea</i>	Cotton	Kilpatrick et al. (2005)	Kilpatrick et al. (2005)		Kilpatrick et al. (2005)			Pedibhotla et al. (1999)
Noctuidae	<i>Heliothis virescens</i>	Tobacco							
Noctuidae	<i>Lacanobia subijuncta</i>	Apple and various fruits		Brunner et al. (2005)	Brunner et al. (2005)	Brunner et al. (2005)			Fang et al. (2008)
Noctuidae	<i>Sesamia inferens</i>	Rice							
Noctuidae	<i>Spilargia obliqua</i>	Polyphagous	Ansari et al. (2012)						
Noctuidae	<i>Spodoptera litura</i>	Polyphagous	Abbas et al. (2012)						Ahmad et al. (2008), Huang et al. (2006a, b)
Psychidae	<i>Thyridopharyx ephemeruformis</i>	Thuja and other ornamental plants			Rhainds and Sadof (2009)			Rhainds and Sadof (2009)	
Pyrilidae	<i>Acrobasis vaccinii</i>	Blueberry							Mann et al. (2009)
Pyrilidae	<i>Cactoblastis cactorum</i>	Opuntia	Bloem et al. (2005)			Wise et al. (2010)	Wise et al. (2010)		Fang et al. (2008), He et al. (2013), Chen and Klein (2012), Cheng et al. (2010), He et al. (2007, 2008), Li et al. (2007)
Pyrilidae	<i>Chilo infuscatellus</i>	Sugarcane							
Pyrilidae	<i>Chilo suppressalis</i>	Rice	Yu et al. (2007a, b)						
Pyrilidae	<i>Ostrinia nubilalis</i>	Stored grain	Yue et al. (2003)	Yu et al. (2007a, b)					Durham et al. (2001, 2002), Siegfried et al. (1999)
Pyrilidae	<i>Plodia interpunctella</i>	Stored grain	Yue et al. (2003)	Yue et al. (2003)					
Pyrilidae	<i>Tryporyza incertulas</i>	Rice	Wang et al. (2005)						
Sesiidae	<i>Pennisetia marginata</i>	Raspberry	McKern et al. (2007)						

Table 2 (continued)

Family	Species	Host	Imidacloprid	Thiamethoxam	Clothianidin	Acetamiprid	Thiacloprid	Dinotefuran	Fipronil
Tortricidae	<i>Choristoneura rosaceana</i>	Apple		Brunner et al. (2005)	Brunner et al. (2005)	Brunner et al. (2005), Dunley et al. (2006)			
Tortricidae	<i>Cryptophlebia leucoreta</i>	Cotton				Dolfou et al. (2011a, b)			
Tortricidae	<i>Cydia pomonella</i>	Apple		Brunner et al. (2005)	Brunner et al. (2005)	Brunner et al. (2005), Cichon et al. (2013), Magalhaes and Walgenbach (2011), Knight (2010), Magalhaes and Walgenbach (2011), Mota-Sanchez et al. (2008)	Cichon et al. (2013), Magalhaes and Walgenbach (2011), Stara and Kocourek (2007), Voudouris et al. (2011), Reyes et al. (2007)		
Tortricidae	<i>Epiphyas postvittana</i>	Trees	Taverner et al. (2012)				Taverner et al. (2011, 2012)		
Tortricidae	<i>Grapholita lobarzewskii</i>	Apples	Charmillot et al. (2007)				Charmillot et al. (2007)		
Tortricidae	<i>Grapholita molesta</i>	Apple		Jones et al. (2012)		Magalhaes and Walgenbach (2011), Jones et al. (2010)	Magalhaes and Walgenbach (2011)		
Tortricidae	<i>Pandemis pyrusana</i>	Apple		Brunner et al. (2005)	Brunner et al. (2005)	Brunner et al. (2005), Dunley et al. (2006)			
Tortricidae	<i>Rhyacionia frustrana</i>	Pine trees	Asaro and Creighton (2011a, b)						Asaro and Creighton (2011)
Yponomeutidae	<i>Plutella xylostella</i>	Cabbage	Hill and Foster (2000)			Ninsin et al. (2000a, b), Sayyed and Crickmore (2007), Ninsin and Tanaka (2005), Ninsin (2004a, b), Ninsin and Miyata (2003)			Li et al. (2006), Sayyed and Wright (2004), Shi et al. (2004), Zhou et al. (2011)

Other routes of exposure include contact with treated surfaces, exposure to sprays or consumption of guttation droplets (Papachristos and Milonas 2008; Prabhaker et al. 2011; Khani et al. 2012). For example, neonicotinoid soil drenches or injections have been found to adversely affect the development of Lepidoptera larvae pupating within the soil (Dilling et al. 2009), whilst soil drenches have been found to significantly lower the overall abundance of insect species and species richness. In one study, imidacloprid was used on eastern hemlock (*Tsuga canadensis*) to effectively control the hemlock woolly adelgid (*Adelges tsugae*); however, the abundance of non-target detritivorous, fungivorous and phytophagous invertebrates was significantly lower in soil drench and injection treatments, when compared to untreated plots (Dilling et al. 2009).

Parasitoid wasps such as *Gonatocerus ashmeadi* can come into contact with neonicotinoids when emerging from the eggs of its host. One such host, the glassy-winged sharpshooter (*Homalodisca itripennis*), a common agricultural pest of many different crops, lays its eggs on the underside of leaves, beneath the epidermal layer. If eggs are laid on neonicotinoid-treated plants, *G. ashmeadi* nymphs may be exposed to toxins when they emerge from the egg and chew through the leaf to get to the surface (Byrne and Toscano 2007).

A 3 year study by Peck (2009) found that when imidacloprid was used as a lawn treatment to target neonate white grubs (Coleoptera: Scarabaeidae), it exhibited cumulative detrimental effects on the abundance of Hexapods, Collembola, Thysanoptera and Coleoptera adults, which were suppressed by 54–62 % overall throughout the course of the study. Population numbers of non-target organisms can also be indirectly affected by a reduction in prey or host species (Byrne and Toscano 2007; Dilling et al. 2009).

Diptera

Of the Diptera, the genus *Drosophila* provides well-known and convenient model species for toxicity testing. Mechanisms of resistance to imidacloprid and its metabolism have been studied in *Drosophila melanogaster*. Particularly, cytochrome P450 monooxygenases (CYPs) are involved, as is the case in mosquitoes (Riaz et al. 2013). According to Kalajdzic et al. (2012), three P450 genes (Cyp4p2, Cyp6a2 and Cyp6g1)

located on the 2R chromosome were highly up-regulated in imidacloprid-resistant flies. However, the same authors did not find that imidacloprid induced expression of Cyp6g1 and Cyp6a2 (Kalajdzic et al. 2013). More recently, it has been shown that imidacloprid was metabolized to eight derivatives in *D. melanogaster*. In this process, only the P450 Cyp6g1 was involved in the enhanced metabolism in vivo (Hoi et al. 2014). Direct toxicity (LC₅₀) has been determined for various *D. melanogaster* strains. For instance, the toxicity of several neonicotinoids and butene-fipronil was evaluated (Arain et al. 2014) with neonicotinoids being less toxic than butene-fipronil. It was suggested that differences exist between adults and larvae. Acute LC₅₀ values can be compared to LC₅₀ measured after chronic exposure, within two studies. With a mutant strain, Frantzios et al. (2008) found a decrease by a factor of 2 for adult flies (acute vs chronic) and a factor of 3 for larvae. Very recently, Charpentier and co-workers have distinguished between male and female flies, from a field strain (Charpentier et al. 2014). Here, the chronic LC₅₀ was 29 times lower than the acute LC₅₀ for males; it was 172 times lower for females and 52 times lower for larvae. Additionally, this study demonstrated that a significant increase of mortality (27–28 %), with a V-shape, was occurring at concentrations 1,100 and 4,600 times lower than the chronic LC₅₀ for males and females, respectively. Other parameters that are crucial for reproduction were tested (mating and fecundity). The LOEC was determined at a concentration that is 3,300,000 and more than 7,900,000 times lower than the acute LC₅₀ for females and males, respectively. These data can be linked to data concerning mortalities observed by chronic exposure of bees at very low concentrations.

Hymenoptera (excluding bees)

A few studies have investigated the effect of neonicotinoid pesticides on parasitic wasps used as biological control agents. Stapel et al. (2000) found that the parasitoid wasp *Microplitis croceipes* had significantly reduced foraging ability and longevity after feeding on extrafloral nectar of cotton (*Gossypium hirsutum*) treated with imidacloprid. Prabhaker et al. (2007) give acute toxicity for two different exposure times for the parasitic wasp species *Eretmocerus eremicus*, *Encarsia formosa*, *Aphytis melinus* and *G. ashmeadi* (Table 3).

Table 3 Acute neonicotinoid toxicity for different Hymenoptera species (Prabhaker et al. 2007)

Species	48 h exposure time mg (AI)/ml		24 h exposure time mg (AI)/ml
	Acetempiprid	Thiamethoxam	Imidacloprid
<i>Eretmocerus eremicus</i>	108.27	1.01	1.93
<i>Encarsia formosa</i>	12.02	0.397	0.980
<i>Gonatocerus ashmeadi</i>	0.134	1.44	2.63
<i>Aphytis melinus</i>	0.005	0.105 (24 h exposure time)	0.246

In another study, *Anagyrus pseudococci* (a nectar-feeding wasp) was fed using buckwheat (*Fagopyrum esculentum*) flowers that had been exposed to imidacloprid as a soil treatment, applied at the label rate. Only 38 % of the wasps survived after 1 day, compared to 98 % fed on untreated flowers. This decreased to 0 % survivorship after 7 days for treated flowers, compared to 57 % on the untreated flowers (Krischik et al. 2007).

As stated in the section on exposure routes, exposure to imidacloprid did not affect mortality of *G. ashmeadi* (a parasitoid wasp) during development within its host, and the adults were sensitive during emergence from the host egg. When mortality was assessed within 48 h of emergence, the LC₅₀ for the parasitoid was 66 ng of imidacloprid per cm² leaf (Byrne and Toscano 2007).

Neonicotinoids are commonly used in household products as highly concentrated bait formulations to control ants (Rust et al. 2004; Jeschke et al. 2010); however, the use of agrochemical products at less concentrated doses is an issue for non-target ants. For the leafcutter ant *Acromyrmex subterraneus subterraneus*, Galvanho et al. (2013) found that sublethal doses of imidacloprid reduced grooming behaviour. Grooming behaviour in this ant is a defence against pathogenic fungi like *Beauveria* species. Barbieri et al. (2013) recently discovered that interactions between different ant species may be negatively affected using sublethal doses of neonicotinoids. In interspecific interactions, individuals of a native ant species (*Monomorium antarcticum*) lowered their aggression towards an invasive ant species (*Linepithema humile*) although survival was not affected. Exposed individuals of *L. humile* displayed an increase in aggression with the outcome that the probability of survival was reduced.

Hemiptera

Whilst many Hemiptera are acknowledged as being problematic agricultural pests, a number are important predators of these pests, although they do also feed on some plant tissues, which would be contaminated by neonicotinoids (Prabhaker

et al. 2011). Table 4 shows LC₅₀ rates for different Hemiptera species.

Neuroptera

It is not only the agricultural use of neonicotinoids that affects beneficial invertebrates. In one study, Marathon 1 % G, a product for amateur use on flowers containing imidacloprid, had been found to affect lacewings (*Chrysopa* spp.) when used at the label rate. Survival rates on untreated flowers were found to be 79 % for adults, compared to 14 % on treated flowers over a 10 day period (Rogers et al. 2007).

Coleoptera

A number of studies have looked into the effects of neonicotinoids on various taxa of Coleoptera such as Histeridae (Hister beetles) (Kunkel et al. 1999), Carabidae (ground beetles) (Kunkel et al. 2001; Mullin et al. 2010) and Coccinellidae (ladybird beetles) (Smith and Krischick 1999; Youn et al. 2003; Lucas et al. 2004; Papachristos and Milonas 2008; Moser and Obrycki 2009; Eisenback et al. 2010; Khani et al. 2012).

Some Coleoptera, notably in the carabid and staphyliniid families, are voracious predators and are a vital aspect of integrated pest management. For example, although the provision of beetle banks as nesting habitat takes land out of crop production, in wheat (*Triticum aestivum*) fields, any losses have been found to be more than offset by savings from a reduced need for aphid-controlling pesticides (Landis et al. 2000).

Many of these beetle groups are undergoing rapid declines. In the UK, three quarters of carabid species have reduced in numbers, half of which have been undergoing population declines of more than 30 %, although the reason for these considerable declines are unknown (Brooks et al. 2012). These groups have been particularly useful as bioindicators, due to their sensitivity to habitat changes especially in agricultural environments (Kromp 1999; Lee et al. 2001). In the EU Draft Assessment Report for imidacloprid, acute toxicity tests were

Table 4 LC₅₀ rates for different Hemiptera species

Species	Chemical	LC ₅₀ residual contact (mg AI/l)		Reference
		Nymphs	Adults	
<i>Orius Laevigatus</i>	Imidacloprid	0.04	0.3	Delbeke et al. (1997)
<i>Hyaliodes vitripennis</i>	Thiacloprid	1.5	0.3	Bostanian et al. (2005)
<i>Hyaliodes vitripennis</i>	Thiamethoxam	1.43	0.5	Bostanian et al. (2005)
<i>Geocoris punctipes</i>	Imidacloprid		5,180	Prabhaker et al. (2011)
	Thiamethoxam		2,170	
<i>Orius insidiosus</i>	Imidacloprid		2,780	
	Thiamethoxam		1,670	

undertaken on the carabid beetle *Poecilus cupreus*, finding the larvae to be highly sensitive. Despite the rapporteur Member State deeming that the concentrations tested were too high for it to conclude no risk to carabids for use on sugar beet, there was no indication of further research required (EFSA 2006).

When exposed to turf plots treated with imidacloprid, the carabid beetle *Harpalus pennsylvanicus* displayed a range of neurotoxic problems including paralysis, impaired walking and excessive grooming. These abnormal behaviours then rendered the individuals vulnerable to predation from ants (Kunkel et al. 2001). A study by Mullin et al. (2010) exposed 18 different carabid species to corn seedlings treated to field-relevant doses of either imidacloprid, thiamethoxam or clothianidin. Nearly 100 % mortality was observed for all species over 4 days.

Coccinellids predators are well known for their ability to control common pests, both in agricultural and domestic environments. In soil treatments of imidacloprid, reduced mobility and delayed reproduction have been found in pollen-feeding species such as *Coleomegilla maculata* (Smith and Krischick 1999), whilst egg production and oviposition periods of the Mealybug destroyer (*Cryptolaemus montrouzieri*) (Khani et al. 2012) and *Hippodamia undecimnotata* (Papachristos and Milonas 2008) were significantly reduced. Table 5 shows available acute toxicity for some coccinellid species.

Harmonia axyridis (harlequin ladybird) larvae were exposed to corn seedlings grown from seeds treated with the label recommended doses of either thiamethoxam or clothianidin. Seventy-two percent of the larvae exhibited neurotoxic symptoms such as trembling, paralysis and loss of coordination, with only 7 % recovery from the poisoning (Moser and Obrycki 2009).

Arachnida

In addition to crop protection, applications of neonicotinoid insecticides in veterinary medicine have expanded. Imidacloprid is applied to domestic pets as a

spot-on formulation against ear mites (*Otodectes cynotis*) (Jeschke et al. 2010). However, studies on mites have found a positive effect on population numbers. Zeng and Wang (2010) found that sublethal doses of imidacloprid (determined for the green peach aphid (*Myzus persicae*)) significantly increased the hatch rate of eggs and pre-adult survivorship of the carmine spider mite (*Tetranychus cinnabarinus*). James and Price (2002) also found that imidacloprid increased egg production by 23–26 % in two-spotted spider mites (*Tetranychus urticae*) in the laboratory. Another study found that fecundity of this species was slightly elevated when treated with thiamethoxam (Smith et al. 2013).

Szczepaniec et al. (2013) discovered that the application of neonicotinoids suppressed expression of plant defence genes when applied to cotton and tomato plants. These genes alter the levels of phytohormones and decrease the plant's resistance to spider mites (*T. urticae*). When mites were added to the crops, population growth increased from 30 to over 100 % on neonicotinoid-treated plants in the greenhouse and up to 200 % in the field experiment. This study was prompted after the same author had investigated an outbreak of *T. urticae* in New York City, USA. In an attempt to eradicate the emerald ash borer beetle (*Agrillus planipennis*) from Central Park, imidacloprid was applied to trees as a soil drench and trunk injections. This resulted in an outbreak of *T. urticae* on elms due to the natural predators being poisoned through ingestion of prey exposed to imidacloprid, combined with fecundity elevation in the mites themselves (Szczepaniec et al. 2011).

Another study found that thiamethoxam and imidacloprid treatments significantly increased two-spotted spider mite (*T. urticae*) densities on cotton plants when compared to the untreated controls (Smith et al. 2013). This study suggested that the increased usage of neonicotinoids could explain the recent infestation increases of two-spotted spider mite occurring in various crops across the mid-south of the USA.

Table 5 Acute neonicotinoid toxicity for different Coccinellid species

Species	Chemical	LD ₅₀ (ng AI per beetle)	LC ₅₀ (µg AI/ml)	Reference
<i>Sasajiscymnus tsugae</i>	Imidacloprid	0.71		Eisenback et al. (2010)
<i>Harmonia axyridis</i>	Imidacloprid		364	Youn et al. (2003)
<i>Harmonia variegata</i>	Thiamethoxam		788.55	Rahmani et al. (2013)
<i>Cryptolaemus montrouzieri</i>	Imidacloprid		17.25–23.9	Khani et al. (2012)
<i>Coccinella undecimpunctata</i>	Imidacloprid		34.2	Ahmad et al. (2011)
<i>Coccinella undecimpunctata</i>	Acetamiprid		93.5	Ahmad et al. (2011)
<i>Coleomegilla maculata</i> —adult	Imidacloprid	0.074		Lucas et al. (2004)
<i>Coleomegilla maculata</i> —larvae	Imidacloprid	0.034		Lucas et al. (2004)

Earthworms (Lumbricidae)

Earthworms are vitally important members of the soil fauna, especially in agricultural soils where they can constitute up to 80 % of total soil animal biomass (Luo et al. 1999). They play critical roles in the development and maintenance of soil physical, chemical and biological properties (Lee 1985). Their activities improve soil structure by increasing porosity and aeration, facilitating the formation of aggregates and reducing compaction (Edwards and Bohlen 1996; Mostert et al. 2000). Soil fertility is enhanced by earthworm effects on biogeochemical cycling (Coleman and Ingham 1988; Bartlett et al. 2010), the modification of microbial biomass and activity (Sheehan et al. 2008), breakdown of plant litter (Knollengberg et al. 1985) and the mixing of litter with soil (Wang et al. 2012a).

Neonicotinoid and other systemic insecticides can pose a risk of harm to earthworm survival and behaviour, potentially disrupting soil development and maintenance processes. The same neural pathways that allow neonicotinoids to act against invertebrate pests (Elbert et al. 1991) are also present in earthworms (Volkov et al. 2007). Thus, when neonicotinoids are applied for the protection of agricultural and horticultural crops, earthworms can be exposed by direct contact with the applied granules or seeds, or with contaminated soil or water. Moreover, their feeding activities may result in ingestion of contaminated soil and organic particles (e.g. Wang et al. 2012b). Foliar residues in plant litter after systemic uptake from soils or from direct plant injections also pose a risk to litter-feeding earthworms that consume the contaminated plant litter (e.g. Kreutzweiser et al. 2009).

Neonicotinoids can persist and move in soils thereby increasing the likelihood that earthworms will be exposed for extended periods of time. Laboratory and field trials with neonicotinoids have demonstrated that their half-life in soils varies depending on soil conditions but can range from several weeks to several years (Cox et al. 1997; Sarkar et al. 2001; Cox et al. 2004; Bonmatin et al. 2005; Fossen 2006; Gupta and Gajbhiye 2007; Goulson 2003). Imidacloprid is the most widely used neonicotinoid, and its adsorption to soils is increased by moisture and organic matter content (Broznic et al. 2012), resulting in increased imidacloprid concentrations in organic-rich soils compared to low-organic soils (Knoepp et al. 2012). Earthworms generally prefer moist, organic-rich soils. When soil organic carbon content is low, the high solubility of imidacloprid renders it mobile and it is readily moved through soils (Broznic et al. 2012; Knoepp et al. 2012; Kurwadkar et al. 2013), thereby increasing the likelihood that earthworms could be exposed to the pesticide in soils outside the direct area of application.

Effects on survival

Neonicotinoids can be highly toxic to earthworms. However, reported median lethal concentrations (LC₅₀) were variable depending on the particular insecticide, test conditions, route of exposure and duration (Table 6). In 13 separate studies, the reported LC₅₀ ranged from 1.5 to 25.5 ppm, with a mean of 5.8 and median of 3.7 ppm. In seven studies that reported lowest concentrations at which effects on survival were measureable, those lowest effective concentrations ranged from 0.7 to 25 ppm, with a mean of 4.7 and median of 1.0 ppm. *Eisenia fetida* was the most common test species in these survival studies and represented the range of reported lethal concentrations, giving little indication from among these studies that other species were more sensitive than *E. fetida*.

When compared to other common insecticides, neonicotinoids tend to be among the most toxic to earthworms. Wang et al. (2012a) tested the acute toxicities of 24 insecticides to *E. fetida* and found that the neonicotinoids were the most toxic in soil bioassays and that acetamiprid and imidacloprid in particular were the two most toxic insecticides overall. They also reported that a contact toxicity bioassay demonstrated that the neonicotinoids were extremely toxic by a contact route of exposure (LC₅₀ of 0.0088 to 0.45 µg cm⁻²), although the units of contact toxicity concentration were difficult to compare to standard lethal concentrations. Across a broader range of 45 pesticides, Wang et al. (2012b) found that in soil bioassays, the neonicotinoid insecticide, clothianidin, was the most toxic pesticide to *E. fetida*. Alves et al. (2013) compared three insecticides used for seed treatment and reported that imidacloprid was the most toxic to earthworms. In soil bioassays with five different insecticides, Mostert et al. (2002) found that imidacloprid was the second most toxic (behind carbaryl) to earthworms. We found only two studies that reported the toxicity of fipronil, another common, agricultural systemic insecticide, and both found it to be substantially (at least 100 times) less lethal to earthworms than the neonicotinoids (Mostert et al. 2002; Alves et al. 2013).

Effects on reproduction

Only a few studies tested sublethal effects of neonicotinoids on earthworm reproduction, but it is apparent that reductions in fecundity can occur at low concentrations (Table 6). Baylay et al. (2012) reported EC₅₀s for imidacloprid and thiacloprid against cocoon production by *Lumbricus rubellus* of 1.5 and 1.3 ppm, respectively, whilst Gomez-Eyles et al. (2009) found similar EC₅₀s for the same two insecticides at 1.4 and 0.9 ppm for *E. fetida*. The latter study also reported measurable reductions in cocoon production at 0.3 ppm of thiacloprid. Alves et al. (2013) reported an EC₅₀ for reproduction effects of imidacloprid on *Eisenia andrei* of 4 ppm with measureable

Table 6 Impacts of neonicotinoids and fipronil on earthworms. The impact rating scale is as follows: —, large decrease; –, moderate decrease; 0, little or no measurable effect (where little is either a small or a brief change); +, moderate increase; and ++, large increase. Endpoints are listed together, separated by a semi-colon, for studies that examined multiple measurement endpoints. Lowest effective concentration is the lowest concentration at which a significant effect was reported, not necessarily the mathematically modelled lowest effective concentration

Taxa	Insecticides	Location	Measurement endpoint	Impact	LC/EC ₅₀	Lowest effective concentration	Reference
<i>Eisenia fetida</i>	Imidacloprid	China	Contact toxicity survival; soil toxicity survival	–; –	LC ₅₀ =0.027 µg cm ⁻² ; LC ₅₀ =2.82 ppm		Wang et al. (2012a)
<i>Eisenia fetida</i>	Imidacloprid	France	Survival; biochemical (hsp70); avoidance	–; –; ++		0.66; 0.66; 0.2 ppm	Ditthbrenner et al. (2012)
<i>Eisenia fetida</i>	Imidacloprid	France	Survival; body mass	–; –		0.66; 0.2 ppm	Ditthbrenner et al. (2011a)
<i>Eisenia fetida</i>	Imidacloprid	UK	Cocoon production; weight change	–; –	EC ₅₀ =1.41; EC ₅₀ =2.77 ppm		Gomez-Eyles et al. (2009)
<i>Eisenia fetida</i>	Imidacloprid	China	Survival	–	LC ₅₀ =2.30 ppm	1 ppm	Zang et al. (2000)
<i>Eisenia fetida</i>	Imidacloprid	China	Survival	–	LC ₅₀ =2.30 ppm		Luo et al. (1999)
<i>Eisenia fetida</i>	Imidacloprid	Canada	Survival; weight loss	–; –		25; 14 ppm	Kreutzweiser et al. (2008b)
<i>Eisenia fetida</i>	Fipronil	Brazil	Survival; reproduction; avoidance	0; –; +		>1,000; 62; >10 ppm	Alves et al. (2013)
<i>Eisenia fetida</i>	Clothianidin	China	Contact toxicity survival; soil toxicity survival	–; –	LC ₅₀ =0.28 µg cm ⁻² ; LC ₅₀ =6.06 ppm		Wang et al. (2012b)
<i>Eisenia fetida</i>	Thiacloprid	China	Contact toxicity survival; soil toxicity survival	–; –	LC ₅₀ =0.45 µg cm ⁻² ; LC ₅₀ =10.96 ppm		Wang et al. (2012a)
<i>Eisenia fetida</i>	Thiacloprid	UK	Cocoon production; weight change	–; –	EC ₅₀ =0.968; EC ₅₀ =19.0 ppm	0.291; 1.91 ppm	Gomez-Eyles et al. (2009)
<i>Eisenia fetida</i>	Acetamiprid	China	Contact toxicity survival; soil toxicity survival	–; –	LC ₅₀ =0.0088 µg cm ⁻² ; LC ₅₀ =1.52 ppm		Wang et al. (2012a)
<i>Eisenia fetida</i>	Nitenpyram	China	Contact toxicity survival; soil toxicity survival	–; –	LC ₅₀ =0.22 µg cm ⁻² ; LC ₅₀ =3.91 ppm		Wang et al. (2012a)
<i>Lumbricus terrestris</i>	Imidacloprid	France	Survival; biochemical (hsp70); avoidance	0; +; 0		4 ppm	Ditthbrenner et al. (2012)
<i>Lumbricus terrestris</i>	Imidacloprid	France	Survival; body mass	0; –		2 ppm	Ditthbrenner et al. (2011b)
<i>Lumbricus terrestris</i>	Imidacloprid	USA	Feeding activity; abundance	–; –		43 mg m ⁻²	Tu et al. (2011)
<i>Lumbricus terrestris</i>	Imidacloprid	France	Burrowing	–		2 ppm	Ditthbrenner et al. (2011b)
<i>Lumbricus terrestris</i>	Imidacloprid	France	Body mass change; cast production	–; –	NA; EC ₅₀ =0.84 ppm	0.66; 0.66 ppm	Ditthbrenner et al. (2010)
<i>Lumbricus terrestris</i>	Imidacloprid	France	Cast production; body mass change	–; –	LC ₅₀ =10.7 ppm	1.89; 0.189 ppm	Capowiez et al. (2010)
<i>Lumbricus terrestris</i>	Imidacloprid and thiacloprid mixture	UK	Survival; weight change; cocoon production; metabolism	0; –; –; 0	EC ₅₀ imidacloprid=1.46 and EC ₅₀ thiacloprid=1.28 ppm		Baylay et al. (2012)
<i>Aporrectodea caliginosa</i>	Imidacloprid	France	Survival; biochemical (hsp70); avoidance	0; –; ++		2; 2 ppm	Ditthbrenner et al. (2012)
<i>Aporrectodea caliginosa</i>	Imidacloprid	France	Survival; body mass	–; –		2; 0.66 ppm	Ditthbrenner et al. (2011a)
<i>Aporrectodea caliginosa</i>	Imidacloprid	France	Burrowing	–		0.2 ppm	Ditthbrenner et al. (2011b)
<i>Aporrectodea caliginosa</i>	Imidacloprid	France	Body mass change; cast production	–; –	NA; EC ₅₀ =0.76 ppm	0.66; 0.66 ppm	Ditthbrenner et al. (2010)
<i>Aporrectodea nocturna</i>	Imidacloprid	France	Weight loss; avoidance; burrowing	–; +; –		0.5; 0.1; 0.05 ppm	Capowiez and Berard (2006)
<i>Aporrectodea nocturna</i>	Imidacloprid	France	Burrowing	–		0.1 ppm	Capowiez et al. (2006)
<i>Aporrectodea nocturna</i>	Imidacloprid	France	Survival; weight loss	–; –	LC ₅₀ =3.74 ppm	0.1 ppm	Capowiez et al. (2005)
<i>Aporrectodea nocturna</i>	Imidacloprid	France	Burrowing	–		0.01 ppm	Capowiez et al. (2003)
<i>Allolobophora icterica</i>	Imidacloprid	France	Weight loss; avoidance; burrowing	–; +; –		0.5; 0.01; 0.05 ppm	Capowiez and Berard (2006)
<i>Allolobophora icterica</i>	Imidacloprid	France	Burrowing	–		0.1 ppm	Capowiez et al. (2006)
<i>Allolobophora icterica</i>	Imidacloprid	France	Survival; weight loss	–; –	LC ₅₀ =2.81 ppm	0.1 ppm	Capowiez et al. (2005)
<i>Allolobophora icterica</i>	Imidacloprid	France	Burrowing	–		0.01 ppm	Capowiez et al. (2003)
<i>Dendrobaena octaedra</i>	Imidacloprid	Canada	Survival; leaf decomposition	0; –		31 ppm	Kreutzweiser et al. (2009)
<i>Dendrobaena octaedra</i>	Imidacloprid	Canada	Survival; weight loss; reproduction; leaf decomposition	–; –; –; –	LC ₅₀ =5.7 ppm	3; 3; 7; 7 ppm	Kreutzweiser et al. (2008b)

Table 6 (continued)

Taxa	Insecticides	Location	Measurement endpoint	Impact	LC/EC ₅₀	Lowest effective concentration	Reference
<i>Dendrobaena octaedra</i>	Imidacloprid	Canada	Survival; weight loss; reproduction; leaf decomposition	0; –; 0; –		11; 3.2 ppm	Kreutzweiser et al. (2008a)
<i>Eisenia andrei</i>	Imidacloprid	Brazil	Survival; reproduction; avoidance	–; –; ++	LC ₅₀ =25.53; EC ₅₀ =4.07; EC ₅₀ =0.11 mg/kg	25; 0.75; 0.13 ppm	Alves et al. (2013)
Pheretima group	Imidacloprid	South Africa	Survival	–	LC ₅₀ =3.0 ppm		Mostert et al. (2002)
Pheretima group	Fipronil	South Africa	Survival	0		>300 ppm	Mostert et al. (2002)
<i>Apporectodea</i> spp.	Clothianidin	USA	Abundance; biomass; cast production	–; –; –	NA, field applications		Larson et al. (2012)

adverse effects at 0.7 ppm. Kreutzweiser et al. (2008b) tested the effects of imidacloprid in forest litter on the litter-dwelling earthworm *Dendrobaena octaedra* and reported significant reductions in cocoon production among surviving earthworms at 7 ppm.

Effects on behaviour

A number of studies focused on behavioural endpoints under the premise that effects on behaviour are often ultimately linked to population or community effects (Little 1990; Dittbrenner et al. 2012). The behavioural attributes considered here are avoidance behaviour, burrowing, cast production and weight change (as an indicator of feeding behaviour). Among the 31 reported values for behavioural effects, weight change was the most common, followed by burrowing, avoidance behaviour and cast production (Table 6). Only a few studies gave median effective concentrations (EC₅₀), and they ranged from 0.1 (avoidance) to 19 (weight change) ppm, with a mean EC₅₀ of 3.7 and median of 1.3 ppm. These behavioural EC₅₀s were about 1.5 to 2.8 times lower than the mean and median lethal concentrations of 5.8 and 3.7 ppm.

However, many more studies reported lowest concentrations at which behavioural effects were detected, and those ranged from 0.01 to 14 ppm with a mean of 1.2 and median of 0.5 ppm. Thus, measurable behavioural effects were more sensitive endpoints than measurable survival effects. Measurable behavioural effects occurred at concentrations of about two to four times lower than the mean and median lowest effective concentrations on survival of 4.7 and 1.0 ppm. Burrowing (smaller, shorter, more narrow burrows) was the most sensitive behavioural endpoint with effects detected at mean and median concentrations of 0.3 and 0.07 ppm (range 0.01 to 2, $n=8$). Avoidance behaviour was the next most sensitive endpoint with effects detected at mean and median concentrations of 0.5 and 0.13 ppm ($n=5$), followed by cast production (mean 1.1, median 0.7 ppm, $n=3$) and weight change (mean 2.1, median 0.7 ppm, $n=13$). All of these indicate that measurable adverse effects on earthworm behaviour would be expected at neonicotinoid concentrations below 1 ppm in soil.

Risks to earthworms

The actual risk of harmful effects on earthworm populations posed by neonicotinoid insecticides will depend on exposure concentration, exposure duration, route of exposure, rate of uptake and inherent species sensitivity. From the toxicity studies reviewed here, it appears that individual earthworms across all common species are at risk of mortality if they consume soil or organic particles with neonicotinoid insecticide concentrations of about 1 ppm or higher for several days. Higher numbers (up to 50 %) of earthworms would be

expected to be at risk of mortality when concentrations reach about 3 ppm and higher. Although it was difficult to compare the exposure concentrations to standard bioassays, it appears that the risk of mortality from surface contact exposure can be ten times or more higher than the risk of mortality from consumption of contaminated soils (Wang et al. 2012a). On the other hand, the route of exposure can affect the likelihood of lethal effects on earthworms. When earthworms were exposed to foliar residues in leaf litter from imidacloprid-injected trees, a significant feeding inhibition effect was detected that reduced leaf consumption but did not cause earthworm mortality, even at concentrations of about 10 ppm (Kreutzweiser et al. 2008a).

The risk of sublethal effects on some important behavioural attributes is higher than the risk of mortality to individuals. Insecticide effects on burrowing and avoidance behaviours would be expected at concentrations of about 0.1 to 0.5 ppm and higher. Whilst alterations in burrowing behaviour, especially reductions in burrowing depths, have implications for the transfer properties of soils (Capowiez et al. 2006; Dittbrenner et al. 2011b), the consequences in real-world field conditions are not clear. Fewer, smaller and shorter burrows could reduce air, water and solute transport through soils affecting overall soil ecology, but none of the studies we found actually tested these implications in experimental or field settings.

The concentrations that pose risk of mortality (assuming high toxicity by contact exposure) and sublethal effects on earthworms fall within the range of reported field concentrations, albeit at the upper end of that range of concentrations. Dittbrenner et al. (2011b) indicate that predicted environmental concentrations for imidacloprid in agricultural soils would be about 0.3 to 0.7 ppm, suggesting risks of at least sublethal effects on earthworms could be quite high. Bonmatin et al. (2005) reported that imidacloprid in soils can reach several hundred parts per billion shortly after sowing of treated seeds. Soil samples from a tea plantation treated with clothianidin had average concentrations of up to 0.45 ppm shortly after application (Chowdhury et al. 2012). Donnarumma et al. (2011) found concentrations of imidacloprid in soils at about 0.6 to 0.8 ppm by 2 weeks after application of treated seeds. Ramasubramanian (2013) reported clothianidin concentrations in soils of 0.27 to 0.44 ppm up to 3 days after single applications and 0.51 to 0.88 ppm by 3 days after double applications of water-soluble granules. Collectively, these studies show that operational applications of neonicotinoids can result in soil concentrations that are likely to pose a high risk of sublethal effects and potential risk of lethal effects (especially by contact toxicity) to earthworms.

At least two issues related to the assessment of risk to earthworms from exposure to neonicotinoids have not been adequately addressed in the published literature. The first is the length of exposure periods in toxicity testing compared to

the length of exposure to persistent concentrations in natural soils. Most toxicity tests are short term, in the order of days to weeks. On the other hand, neonicotinoid residues can persist in soils for months to years (Bonmatin et al. 2014, this issue). For most pesticides, lethal or effective concentrations become lower as exposure periods increase, and this is likely the case for neonicotinoids (Tennekes 2010; Tennekes and Sánchez-Bayo 2012, 2013; Rondeau et al. 2014). It is plausible that long-term low-level concentrations of neonicotinoids in soils may pose higher risk to earthworms than what can be inferred from the published toxicity tests. The second issue pertains to the heterogeneous distribution of neonicotinoid residues in natural soils. When residues enter the soil at the surface from spray or granule deposition or from litter fall, concentrations in soils are likely to be higher on or near the surface than in deeper soils. Residues entering soils from planted seed or from contaminated water are likely to be higher at or near the source of contamination than elsewhere. Both situations would result in concentration “hot spots” near the points of entry. Conversely, most toxicity tests prepare test concentrations as parts per million (or equivalent) and assume complete mixing. Therefore, levels of exposure to earthworms at or near those hot spots in natural soils will consequently be higher than would be predicted from residue analyses of bulk samples from laboratory or field test systems.

Mortality or behavioural effects on individual earthworms do not necessarily translate to population effects with ecological consequences. Populations of organisms with short generation times (e.g. several generations per year as is the case for most earthworm species) and/or high dispersal capacity have a higher likelihood of recovery from pesticide-induced population declines than those with longer regeneration periods and limited dispersal capacity (Kreutzweiser and Sibley 2013). However, the tendency for neonicotinoids to persist in organic soils reduces the likelihood of this recovery pathway because subsequent generations may be exposed to concentrations similar to those to which the parent generation was exposed. Life history strategies and their influences on community responses and recovery from pesticide effects have been demonstrated by population modelling of other non-target organisms (Wang and Grimm 2010), and similar principles may apply to assessing risks to overall earthworm populations and communities. Population models that account for differential demographics and population growth rates within communities have been shown to provide more accurate assessments of potential pesticide impacts on populations and communities than conventional lethal concentration estimates can provide (Stark and Banks 2003). The use of ecological models to incorporate a suite of factors including seasonal variations, community assemblage mechanisms and lethal and sublethal insecticide effects and their influences on the risks to organisms, populations or communities can provide useful insights into receptor/pesticide interactions and

can thereby improve risk assessments (Bartlett et al. 2010). Ecological and population modelling combined with pesticide exposure modelling and case-based reasoning (drawing on past experience or information from similar chemical exposures) can provide further refinements and improve risk assessment for earthworm communities and their ecological function (van den Brink et al. 2002). Empirical field studies of earthworm population responses to realistic field concentrations of neonicotinoids are lacking and would greatly improve risk assessment efforts.

Aquatic invertebrates

Freshwater invertebrates

Aquatic invertebrates are extremely important components of aquatic ecosystems. They play roles as decomposers, grazers, sediment feeders, parasites and predators. They also provide much of the food that vertebrates associated with these systems feed upon. Pesticides, including neonicotinoids, reach surface waters through various routes, but in particular through atmospheric deposition (drift) after application by various sprayers, by surface runoff and by seepage of contaminated groundwater. Aquatic invertebrates are particularly susceptible to pesticides. Unlike terrestrial organisms, aquatic organisms generally cannot avoid exposure easily by moving to uncontaminated areas, particularly when pesticides are water soluble. Uptake of pesticides in aquatic invertebrates occurs through respiration (gills and trachea), feeding and through the epidermis, be it cuticle or skin.

Neonicotinoids have been used for a comparatively shorter period of time than other insecticides. However, they are found in freshwater systems more and more frequently. For example, surface water monitoring for pesticides in California has revealed that imidacloprid has frequently exceeded water quality guidelines of 1 ppb (Starner and Goh 2012). In the Washington State, USA, the State Department of Ecology and the State Department of Agriculture have been monitoring salmon-bearing rivers and streams for pesticides, including imidacloprid for a number of years and this insecticide is frequently found (<http://agr.wa.gov/PestFert/natresources/SWM/>).

However, even though imidacloprid and other neonicotinoids are present in freshwater systems, the question remains to what extent such concentrations affect aquatic organisms in the field. Here we discuss a number of studies dealing with neonicotinoid toxicity to aquatic invertebrates and make some observations about their potential impact on aquatic ecosystems.

Laboratory studies

Crustacea and Amphipoda Several laboratory studies have been published on the toxicity of the neonicotinoid imidacloprid on a range of aquatic invertebrates (Table 7). Stark and Banks (2003) developed acute toxicity data and population-level toxicity data for the water flea *Daphnia pulex* exposed to thiamethoxam (Actara). Thiamethoxam was the least toxic insecticide evaluated in this study of seven insecticides, and its LC₅₀ of 41 ppm was well above any anticipated concentration expected to be found in surface water systems.

Chen et al. (2010) estimated the acute toxicity of imidacloprid to the water flea, *Ceriodaphnia dubia* (LC₅₀=2.1 ppb), and the chronic toxicity to *C. dubia* populations. The effects of the adjuvant, R-11 alone and in combination with imidacloprid were also assessed. In the population study, exposure of *C. dubia* to imidacloprid concentrations of 0.3 ppb reduced population size to 19 % of the control population. This concentration is well below the U.S. EPA's expected environmental concentration of 17.4 ppb, indicating that imidacloprid may cause damage to aquatic invertebrates in the field.

The acute and chronic effects of imidacloprid on the amphipod *Gammarus pulex* were studied by Nyman et al. (2013). Feeding by *G. pulex* and body lipid content were significantly reduced after exposure to a constant imidacloprid concentration of 15 ppb. Furthermore, *G. pulex* individuals were unable to move and feed after 14 days of constant exposure resulting in a high level of mortality.

Interestingly, the standard test organism *Daphnia magna* is especially insensitive to neonicotinoids (Beketov and Liess 2008). An acute LC₅₀ of around 7,000 ppb is several orders of magnitude above effective concentrations found for several other invertebrates. This implies that *D. magna* cannot be used as a sensitive test organism protective for many species.

Insecta Acute toxicity estimates of neonicotinoids on aquatic insects have also been published. LC₅₀ estimates for aquatic insects range from 3 to 13 ppb. Imidacloprid LC₅₀ estimates for the mayfly *Baetis rhodani*, the black fly *Simulium latigonium* (Beketov and Liess 2008) and the mosquito *Aedes taeniorhynchus* (Song et al. 1997) are 8.5, 3.7 and 13 ppb, respectively. LC₅₀ estimates for *B. rhodani* and *S. latigonium* exposed to thiacloprid were 4.6 and 3.7 ppb, respectively (Beketov and Liess 2008). A chronic LC₅₀ of 0.91 ppb was reported for the midge *Chironomus tentans* for imidacloprid (Stoughton et al. 2008). A study on the effects of imidacloprid as a mixture with the organophosphate insecticides dimethoate and chlorpyrifos on the midge *Chironomus dilutus* found that imidacloprid acted synergistically with chlorpyrifos and antagonistically with dimethoate (LeBlanc et al. 2012).

Table 7 Selection of studies on the effects of imidacloprid on freshwater macrophuna

	Compound	Experimental design	Effect	LC ₅₀ /EC ₅₀	LOAEL	Reference
Aquatic taxa						
Oligochaeta	Imidacloprid	10 day exposure to contaminated sediment	Survival, growth, behaviour, avoidance		<0.05 mg/kg	Sardo and Soares (2010)
<i>Chironomus tentans</i> and <i>Hyalella azteca</i>	Imidacloprid	Standard toxicity test	Survival	0.91 µg/l (28 days)		Stoughton et al. (2008)
Mesocosm communities	Neonics and other insecticides		Drift response			Berghahn et al. (2012)
<i>Daphnia</i> , <i>Gammarus pulex</i>	Imidacloprid		Survival			Ashauer et al. (2011)
Mayflies	Imidacloprid		Nymph abundance emergence patterns and adult body size			Alexander et al. (2008)
<i>Ceriodaphnia dubia</i>	Imidacloprid	Lab toxicity tests	Mortality	2.1 ppb		Chen et al. (2010)
<i>D. magna</i>	Imidacloprid	Lab toxicity tests	Population growth rate			Song et al. (1997)
<i>Aedes aegypti</i>	Imidacloprid	Lab toxicity tests	Mortality	10.4 mg/l		Song et al. (1997)
<i>Aedes taeniorhynchus</i>	Imidacloprid	Lab toxicity tests	Mortality	44 ppb		Song et al. (1997)
Mayflies, Oligochaetes	Imidacloprid	Lab toxicity tests	Mortality	13 ppb		Alexander et al. (2008)
Odonata, Libellulidae	Imidacloprid, fipronil	Field	Feeding inhibition			Jinguji et al. (2013)
Macro-invertebrate community	Imidacloprid	Stream mesocosm	Larval and adult survival, emergence			Pestana et al. (2009)
Crustacean: <i>Asellus aquaticus</i> , <i>Gammarus fossarum</i>	Imidacloprid and atrazine	Standard toxicity test	Community diversity, leaf litter breakdown			Lukancic et al. (2010)
Caddisfly: <i>Sericoostoma</i> , <i>Chironomus riparius</i>	Imidacloprid	Standard toxicity test	Survival, respiration, electron transport system			Pestana et al. (2009)
Ostracoda, <i>Daphnia magna</i>	Imidacloprid	Lab toxicity test	Burrowing behaviour; antipredator behaviour			Sánchez-Bayo (2006)
<i>Chironomus dilutus</i>	Imidacloprid+mixtures (chlorpyrifos, dimethoate)	Lab toxicity test	Survival			LeBlanc et al. (2012)
Terrestrial taxa						
Aphidius ervi	Imidacloprid+cadmium	Lab toxicity tests	Survival			Kramarz and Stark (2003)
	Imidacloprid+cadmium	Lab toxicity tests	Population growth rate			

Oligochaetes Sardo and Soares (2010) investigated the effects of imidacloprid on the aquatic oligochaete *Lumbriculus variegatus*. They exposed this worm species to imidacloprid concentrations ranging from 0.05 to 5.0 mg/kg sediment. Mortality was fairly low (35 % in the highest concentration), but *L. variegatus* avoided imidacloprid-contaminated sediment. Furthermore, individual growth (biomass) was inhibited at all concentrations tested compared to the control.

Mesocosm studies Alexander et al. (2008) examined the effect of imidacloprid as a 12 day pulse or 20 day continuous exposure on the mayflies *Epeorus* spp. and *Baetis* spp. Nymph densities were reduced after both types of exposures. Sublethal effects were observed as well. Adults were smaller and had smaller head and thorax size after exposure to imidacloprid concentrations as low as 0.1 ppb. However, these effects were only found in males.

Within community test systems, neonicotinoids had strong effects especially on insects (Hayasaka et al. 2012). However, to our knowledge, all experiments investigating a dose–response relationship observed effects at the lowest concentrations evaluated. Hence, it is difficult to establish a NOEC. Within outdoor mesocosm studies, a LOEC of 1.63 ppb was estimated for imidacloprid. Adverse effects on benthic communities with 5 % reductions in the abundance of invertebrates were observed by Pestana et al. (2009). For thiacloprid, strong effects on sensitive long living insects were observed at pulsed exposure to 0.1 ppb (Liess and Beketov 2011), the lowest effective concentration observed so far in communities.

Berghahn et al. (2012) conducted stream mesocosm studies whereby 12 h pulses of imidacloprid (12 ppb) were introduced three times at weekly intervals. Results showed that drift of insects and the amphipod *Gammarus roeseli* increased after exposure to pulses of imidacloprid. These results indicated that imidacloprid was having a negative effect on *G. roeseli*.

In another stream mesocosm study, Böttger et al. (2013) evaluated pulses of imidacloprid on *G. roeseli*. The number of brood carrying females was reduced in the imidacloprid treatments compared to the control groups in the last 3 weeks of the study.

The populations of an aquatic invertebrate, the common mosquito *Culex pipiens*, exposed over several generations to repeated pulses of low concentrations of the neonicotinoid thiacloprid, continuously declined and did not recover in the presence of a less sensitive competing species, the water flea *D. magna*. By contrast, in the absence of a competitor, insecticide effects on the more sensitive species were only observed at concentrations one order of magnitude higher, and the species recovered more rapidly after a contamination event. The authors conclude that repeated toxicant pulse of populations that are challenged with interspecific competition may result

in a multigenerational culmination of low-dose effects (Liess et al. 2013).

Risk to aquatic ecosystems A species sensitivity distribution (SSD) of acute toxicity data for a wider range of species, including ostracods, cladocerans and other aquatic organisms, predicts a hazardous concentration for 5 % of aquatic species (HC5) for imidacloprid in water in the range 1.04–2.54 ppb (Sanchez-Bayo and Kouchi 2012).

Van Dijk et al. (2013) developed a regression analysis for abundance of aquatic macro-invertebrate species and nearby imidacloprid concentrations in Dutch surface waters. Data from 8 years of nationwide monitoring covering 7,380 different locations of macro-invertebrate samples and 801 different locations of imidacloprid samples were pooled. Next, the biological samples (macro-invertebrate abundance counts) were combined with nearby (in space and time) chemical samples (imidacloprid concentrations), and next, a statistical analysis was done on the complete pooled combined dataset. They found that macro-invertebrate abundance consistently declines along the gradient of increasing median nearby imidacloprid concentration in the pooled dataset. This pattern turned out to be robust: it is independent of year and location. Overall, a significant negative relationship ($P < 0.001$) was found between abundance of all macro-invertebrate species pooled and nearby imidacloprid concentration. A significant negative relationship was also found for abundance of each of the pooled orders Amphipoda, Basommatophora, Diptera, Ephemeroptera and Isopoda, and for several species separately. The order Odonata had a negative relationship very close to the significance threshold of 0.05 ($P = 0.051$). In accordance with previous research, a positive relationship between abundance and nearby imidacloprid pollution was found for the order Actiniedida. However, other pesticides were not included into the analyses by Van Dijk et al. (2013). Therefore, possible co-linearity or synergisms between neonicotinoids and other pollutants still need to be further explored (Vijver and Van den Brink 2014).

Pesticide exposure was identified to strongly reduce the amount and abundance of vulnerable invertebrate species in streams using the SPEAR approach (Liess and von der Ohe 2005). The approach was extended from German streams to Australian, Danish, French and Finnish streams revealing the same effects of pesticide exposure on vulnerable invertebrate species (Rasmussen et al. 2013; Liess et al. 2008; Schäfer et al. 2012). Beketov et al. (2013) analysed the effect of pesticide presence on invertebrate species richness in European (Germany and France) and Australian streams. They found an overall reduction of 42 % for Europe and 27 % for Australia in species richness between uncontaminated and heavily contaminated streams. The limitation of these studies in the context of assessment of neonicotinoid impact is that toxicity was mainly due to insecticides, other than neonicotinoids, as general usage of the latter only increased recently.

The results of laboratory and semi-field (mesocosm) studies indicate that aquatic invertebrates are very sensitive to the neonicotinoid insecticides. However, most of the studies we found in the literature were conducted with imidacloprid. For pesticide risk assessment, the published results to date indicate that it may be difficult to predict community-level effects using the tiered aquatic effect assessment scheme and acute and chronic toxicity data. When extrapolating from acute and chronic single species test systems, the assessment factors identified by the uniform principle of the relevant EU legislation (1107/2009) do not predict safe concentrations in multi-species outdoor mesocosms. For example, acute laboratory effects of thiacloprid on sensitive insect species show that effects occur after exposure to the range of 3–13 ppb. Accordingly, an assessment factor of 100 would indicate a safe concentration of 0.03 to 0.13 ppb for thiacloprid. However, outdoor mesocosm results employing a pulsed exposure show a LOEC below 0.1 ppb for thiacloprid (Liess and Beketov 2011). Lower concentrations were not investigated. Obviously, an assessment factor higher than 100 is needed to identify safe concentrations on the basis of acute test results. For the HC5 calculated on acute lethal concentrations, an assessment factor of larger than 10 is necessary (Liess and Beketov 2012). Additionally, in a laboratory study, chronic effects of sensitive insect species were exhibited after exposure to 0.91 ppb imidacloprid. Employing an assessment factor of 10 would indicate a safe concentration of approximately 0.1 ppb imidacloprid. However, this concentration is not safe according to the results obtained in complex community investigations. Unfortunately, to the best of our knowledge, no community-level investigation with imidacloprid evaluating a range of concentrations below 0.1 ppb has been published. This type of study would help with determining a NOEC for imidacloprid. Overall, the results of the published literature indicate that certain neonicotinoids have the potential to cause significant damage to aquatic ecosystems by causing negative effects in individuals and populations of aquatic invertebrates at very low concentrations. Protective concentrations for these products in aquatic systems still need to be determined.

Marine and coastal invertebrates

There is very limited information regarding the assessment of the environmental toxicology and contamination of neonicotinoids in marine ecosystems. Standardised environmental toxicological characterization focuses on only a few species models and rarely examines species that represent keystone organisms in marine or coastal ecosystems (CCME 2007). Monitoring and surveillance of neonicotinoid pollution in marine coastal habitats are non-existent.

Toxicology The earliest published marine ecotoxicological studies of neonicotinoids were with opossum shrimps

(*Mysidopsis bahia*) which are distributed in marine coastal waters (Ward 1990, 1991; Lintott 1992). Median LC₅₀ (96 h) for the technical grade of imidacloprid was 34.1 ppb with a mortality-NOEC of 13.3 ppb (Ward 1990). Exposure to a commercial formulation (ADMIRE) of imidacloprid resulted in a 96 h mortality-NOEC of 21 ppb. Maximum acceptable toxicant concentrations for *M. bahia* to imidacloprid were 23 parts per trillion (ppt) for growth effects and 643 ppt for reproductive effects (Ward 1991).

Toxicology for other marine arthropods includes *Artemia* spp. and a brackish water mosquito (*Aedes taeniorhynchus*). The 48 h LC₅₀ for *Artemia* was 361 ppm, whilst *Aedes* exhibited a 72 h LC₅₀ of 21 ppb, and a 48 h LC₅₀ of 13 ppb for an early instar stage of development (Song et al. 1997; Song and Brown 1998). Osterberg et al. (2012) demonstrated that in the blue crab (*Callinectes sapidus*), megalopae were an order of magnitude more sensitive to lethal effects of imidacloprid (24 h-LC₅₀=10 ppb for megalopae vs 24 h-LC₅₀=1.1 ppb for juveniles).

There are no known published OECD/EPA parameter-based studies on non-arthropod marine invertebrates. For the marine mussel, *Mytilus galloprovincialis*, a transcriptomic and proteomic survey was conducted as a response to imidacloprid and thiacloprid exposures (Dondero et al. 2010). This study concluded that the two neonicotinoids induced distinct toxicodynamic responses and that caution should be heeded when conducting ecological risk assessments for chemical mixtures that target the same receptor. Rodrick (2008) demonstrated that imidacloprid had an effect on oyster hemocyte immunocompetence and that there was an additive effect when oysters were exposed to a compound stress of salinity and exposure to imidacloprid. Tomizawa et al. (2008) used the gastropod *Aplysia californica* as a model to characterize imidacloprid and thiacloprid as agonists of the acetylcholine-binding protein, indicating that neonicotinoids could also affect marine gastropods.

Environmental pollution There are no published works regarding the marine environmental contamination of neonicotinoids. Until recently, there has been little public concern of neonicotinoid non-point source pollution of marine environments from land runoff. At least within the USA, this attitude is beginning to change. In the State of Washington 2013, the Willapa-Grays Harbor Oyster Growers Association received a conditional registration from the U.S. Environmental Protection Agency to use imidacloprid to control native burrowing shrimp in Willapa Bay, Washington that may threaten commercial shellfish beds (EPA Reg. no. 88867–1). In Hawaii, there have been public protests and scrutiny over the use of neonicotinoid pesticides in their industrial agricultural practices and their likely negative impacts on coral reefs and sea grass beds (Sergio 2013). For both Hawaii and the U.S. Virgin Islands, there is concern that the use of

neonicotinoids as a method for termite control may be polluting and impacting coastal resources.

Conclusion

At field-realistic levels of pollution, neonicotinoids and fipronil generally have negative effects on physiology and survival for a wide range of non-target invertebrates in terrestrial, aquatic, marine and benthic habitats. Effects are most often found by in vitro testing, using a limited number of test species. This basically means that there is a deficit of information for the grand majority of other invertebrates. In vitro testing to establish safe environmental concentration thresholds is hindered by the fact that most test protocols are based on older methodology, validated for pesticides with very different chemical and toxicological characteristics. New and improved methodologies are needed to specifically address the unique toxicology of these neurotoxic chemicals, including their non-lethal effects and synergistic effects for a variety of terrestrial, aquatic and marine organisms.

The amount of published in vivo field tests is small and experimental setups often suffer from inability to control for variation in (semi)natural circumstances or have insufficient statistical power due to the high financial costs of large robust field experiments. Given the clear body of evidence presented in this paper showing that existing levels of pollution with neonicotinoids and fipronil resulting from presently authorized uses frequently exceed lowest observed adverse effect concentrations and are thus likely to have large-scale and wide ranging negative biological and ecological impacts, the authors strongly suggest that regulatory agencies apply more precautionary principles and tighten regulations on neonicotinoids and fipronil.

Acknowledgments This manuscript benefited from the discussions in the International Task Force on Systemic Pesticides during its plenary meetings in Paris (2010), Bath (2011), Cambridge (2012), Padua (2012), Louvain-la-Neuve (2013) and Legnaro (2013). The authors are organised in alphabetic order, except the first who is also the corresponding author. All authors work for public agencies, except V. Amaral-Rogers who is employed by Buglife, a UK charity devoted to the conservation of invertebrates, and D.A. Noome, whose independent work for the TFSP is financed by the Stichting Triodos Foundation, and N. Simon-Delso working for CARI (association supported by the Belgium government). Contributions of J. Settele and M. Wiemers were part of www.legato-project.net (funded by the BMBF, German Ministry for Education and Research). The work has been funded by the Triodos Foundation's Support Fund for Independent Research on Bee Decline and Systemic Pesticides. This Support Fund has been created from donations by Adessium Foundation (The Netherlands), Act Beyond Trust (Japan), Utrecht University (Netherlands), Stichting Triodos Foundation (The Netherlands), Gesellschaft fuer Schmetterlingsschutz (Germany), M.A. O.C. Gravin van Bylandt Stichting (The Netherlands), Zukunft Stiftung Landwirtschaft (Germany), Study Association Storm (Student Association Environmental Sciences Utrecht University) and citizens. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest The authors declare no conflict of interest.

References

- Abbas N, Shad SA, Razaq M (2012) Fitness cost, cross resistance and realized heritability of resistance to imidacloprid in *Spodoptera litura* (Lepidoptera: Noctuidae). *Pestic Biochem Physiol* 103:181–188
- Abbott VA, Nadeau JL, Higo HA, Winston ML (2008) Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae). *J Econ Entomol* 101:784–796
- Aebischer NJ (1990) Assessing pesticide effects on non-target invertebrates using long-term monitoring and time-series modelling. *Funct Ecol* 4:369–373
- Ahmad M, Sayyed AH, Saleem MA, Ahmad M (2008) Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. *Crop Prot* 27:1367–1372
- Ahmad M, Rafiq M, Arif MI, Sayyed AH (2011) Toxicity of some commonly used insecticides against *Coccinella undecimpunctata* (Coleoptera: Coccinellidae). *Pak J Zool* 43:1161–1165
- Ahmad S, Ansari MS, Ahmad N (2013) Acute toxicity and sublethal effects of the neonicotinoid imidacloprid on the fitness of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Int J Trop Insect Sci* 33:264–275
- Aizen, Harder (2009) The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr Biol* 19:915–918
- Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP, Le Conte Y (2010) Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environ Microbiol* 12:774–782
- Albajes R, López C, Pons X (2003) Predatory fauna in cornfields and response to imidacloprid seed treatment. *J Econ Entomol* 96:1805–1813
- Alexander AC, Heard K, Culp JM (2008) Emergent body size of mayfly survivors. *Freshw Biol* 53:171–180
- Aliouane Y, El Hassani AK, Gary V, Armengaud C, Lambin M, Gauthier M (2009) Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior. *Environ Toxicol Chem* 28:113–122
- Alves PRL, Cardoso EJBN, Martines AM, Sousa JP, Pasini A (2013) Earthworm ecotoxicological assessments of pesticides used to treat seeds under tropical conditions. *Chemosphere* 90:2674–2682
- Anon (2012) EFSA Statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe. *EFSA Journal* 10: 2752
- Ansari MS, Ali H, Shafqat S (2012) Insecticidal effect on a population of *Spilarctia obliqua* (Lepidoptera: Arctiidae). *Entomol Res* 42:330–338
- Apenet, (2010) <http://www.reterurale.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/3280>
- Araín MS, Hu XX, Li GQ (2014) Assessment of toxicity and potential risk of butene-fipronil using *Drosophila melanogaster*, in comparison to nine conventional insecticides. *Bull Environ Contam Toxicol* 92:190–195
- Arena, M., and Sgolastra, F (2014) A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicol*, 1–11
- Asaro C, Creighton J (2011) Use of systemic fipronil and imidacloprid to control regeneration pests of loblolly pine. *J Econ Entomol* 104: 1272–1279
- Ashauer R, Hintermeister A, Potthoff E, Esche BI (2011) Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity

- of *Daphnia magna* across most modes of action. *Aquat Toxicol* 103: 38–45
- Aufauvre J, Biron DG, Vidau C, Fontbonne R, Roudel M, Diogon M, Vigues B, Belzunces LP, Delbac F, Blot N (2012) Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee. *Scientific reports* 2, srep00326-srep00326
- Balderrama N, Nunez J, Giurfa M, Torrealba J, Dealbornoz EG, Almeida LO (1996) A deterrent response in honeybee (*Apis mellifera*) foragers: dependence on disturbance and season. *J Insect Physiol* 42: 463–470
- Barbara G, Grünwald B, Pauté S, Gauthier M, Raymond-Delpech V (2008) Study of nicotinic acetylcholine receptors on cultured antennal lobe neurones from adult honeybee brains. *Invert Neurosci* 8:19–29
- Barbieri RF, Lester PJ, Miller AS, Ryan KG (2013) A neurotoxic pesticide changes the outcome of aggressive interactions between native and invasive ants. *Proc R Soc B Biol Sci* 28:20132157
- Bartlett MD, Briones MJI, Neilson R, Schmidt O, Spurgeon D, Creamer RE (2010) A critical review of current methods in earthworm ecology: from individuals to populations. *Eur J Soil Biol* 46:67–73
- Baylay AJ, Spurgeon DJ, Svendsen C, Griffin JL, Swain SC, Sturzenbaum SR, Jones OAH (2012) A metabolomics based test of independent action and concentration addition using the earthworm *Lumbricus rubellus*. *Ecotoxicology* 21:1436–1447
- Beketov MA, Liess M (2008) Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. *Arch Environ Contam Toxicol* 55:247–253
- Beketov MA, Kefford BJ, Schäfer RB, Liess M (2013) Pesticides reduce regional biodiversity of stream invertebrates. *Proc Natl Acad Sci U S A* 110:11039–11043. doi:10.1073/pnas.1305618110
- Belzunces LP (2006) Rapport d'étude du programme Etude comparée des impacts de trois classes d'insecticides néonicotinoïdes chez l'abeille. Programme communautaire sur l'apiculture Année 2006
- Belzunces LP, Tchamitchian S, Brunet JL (2012) Neural effects of insecticides in the honey bee. *Apidologie* 43:348–370
- Benzidane Y, Touinsi S, Motte E, Jadas-Hécart A, Communal PY, Leduc L, Thany SH (2010) Effect of thiamethoxam on cockroach locomotor activity is associated with its metabolite clothianidin. *Pest Manag Sci* 66:1351–1359
- Berghahn R, Mohr S, Hübner V, Schmiediche R, Schmiedling I, Svetich-Will E, Schmidt R (2012) Effects of repeated insecticide pulses on macroinvertebrate drift in indoor stream mesocosms. *Aquat Toxicol* 122:56–66
- Biocca M, Conte E, Pulcini P, Marinelli E, Pochi D (2011) Sowing simulation tests of a pneumatic drill equipped with systems aimed at reducing the emission of abrasion dust from maize dressed seed. *J Environ Sci Health B* 46:438–448
- Bloem S, Mizell RF, Bloem KA, Hight SD, Carpenter JE (2005) Laboratory evaluation of insecticides for control of the invasive *Cactoblastis cactorum* (Lepidoptera: Pyralidae). *Fla Entomol* 88: 395–400
- Boggs CL (2003) Environmental variation, life histories, and allocation. In: Boggs CL, Watt WB, Ehrlich PR (eds) *Butterflies: ecology and evolution taking flight*. The University of Chicago Press, Chicago, pp 185–206
- Bonmatin JM, Moineau I, Charvet R, Colin ME, Fleche C, Bengsch ER et al (2005) Behaviour of imidacloprid in fields: toxicity for honey bees. In: Lichtfouse E, Schwarzbauer J, Robert D (eds) *In environmental chemistry: green chemistry and pollutants in ecosystems*. Springer, New York, pp 483–494
- Bonmatin J-M, Giorio C, Girolami V, Goulson D, Kreutzweiser D, Krupke C, Liess M, Long E, Marzaro M, Mitchell E, Noome D, Simon-Delso N, Tapparo A (2014) Environmental fate and exposure; neonicotinoids and fipronil. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3332-7 (this issue)
- Bordereau-Dubois B, List O, Calas-List D, Marques O, Communal PY, Thany SH, Lapié B (2012) Transmembrane potential polarization, calcium influx and receptor conformational state modulate the sensitivity of the imidacloprid-insensitive neuronal insect nAChR to the neonicotinoid insecticides. *J Pharmacol Exp Ther*. doi:10.1124/jpet.111.188060
- Bortolotti L, Montanari R, Marcelino J, Medrzycki P, Maini S, Porrini C (2003) Effect of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. *Bull Insect* 56:63–67
- Bosch J, Kemp WP (2006) Bee population returns and cherry yields in an orchard pollinated with *Osmia lignaria* (Hymenoptera: Megachilidae). *J Econ Entomol* 99:408–413
- Bostanian NJ, Hardman JM, Ventard E, Racette G (2005) The intrinsic toxicity of several neonicotinoids to *Lygus lineolaris* and *Hyaliodes vitripennis*, a phytophagous and a predaceous mired. *Pest Manag Sci* 61:991–996
- Böttger R, Feibicke M, Schaller J, Dudel G (2013) Effects of low-dosed imidacloprid pulses on the functional role of the caged amphipod *Gammarus roeseli* in stream mesocosms. *Ecotoxicol Environ Saf* 93:93–100
- Braun G, Bicker G (1992) Habituation of an appetitive reflex in the honeybee. *J Neurophysiol* 67:588–598
- Breeze TD, Bailey AP, Balcombe KG et al (2011) Pollination services in the UK: how important are honeybees? *Agric Ecosyst Environ* 142: 137–143
- Brittain C, Bommarco R, Vighi M, Settele J, Potts SG (2010a) Organic farming in isolated landscapes does not benefit flower-visiting insects and pollination. *Biol Conserv* 143:1860–1867. doi:10.1016/j.biocon.2010.04.029
- Brittain CA, Vighi M, Bommarco R, Settele J, Potts SG (2010b) Impacts of a pesticide on pollinator species richness at different spatial scales. *Basic App Ecol* 11:106–115
- Brooks DR, Bate JE, Clark SJ, Monteith DT, Andrews C, Corbett SJ, Beaumont DA, Chapman JW (2012) Large carabid beetle declines in a United Kingdom monitoring network increases evidence for a widespread loss in insect biodiversity. *J Appl Ecol* 49:1009–1019
- Broznic D, Marinic J, Tota M, Juresic GC, Petkovic O, Milin C (2012) Hysteretic behaviour of imidacloprid sorption-desorption in soils of Croatian coastal regions. *Soil Sediment Contam* 21:850–871
- Brunet JL, Badiou A, Belzunces LP (2005) In vivo metabolic fate of [¹⁴C]-acetamiprid in six biological compartments of the honeybee *Apis mellifera* L. *Pest Manag Sci* 61:742–748
- Brunner JF, Beers EH, Dunley JE, Doerr M, Granger K (2005) Role of neonicotinyl insecticides in Washington apple integrated pest management. Part I. Control of lepidopteran pests. *J Insect Sci* 5
- Bryden J, Gill RJ, Mitton RAA, Raine NE, Jansen VAA (2013) Chronic sublethal stress causes bee colony failure. *Ecol Lett* 16:1463–1493
- Byrne FJ, Toscano NC (2007) Lethal toxicity of systemic residues of imidacloprid against *Homalodisca vitripennis* (Homoptera: Cicadellidae) eggs and its parasitoid *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae). *Biol Control* 43:130–135
- Calderone NW (2012) Insect pollinated crops, insect pollinators and us agriculture: trend analysis of aggregate data for the period 1992–2009. *PLoS One* 7:e37235. doi:10.1371/journal.pone.0037235
- Canadian Council of Ministers of the Environment (2007) Canadian water quality guidelines: IMIDACLOPRID. Scientific supporting document. ISBN 978-1-896997-71-1 PDF
- Capowiez Y, Berard A (2006) Assessment of the effects of imidacloprid on the behaviour of two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*) using 2D terraria. *Ecotoxicol Environ Saf* 64:198–206
- Capowiez Y, Rault M, Mazzia C, Belzunces L (2003) Earthworm behaviour as a biomarker—a case study using imidacloprid. *Pedobiologia* 47:542–547
- Capowiez Y, Rault M, Costagliola G, Mazzia C (2005) Lethal and sublethal effects of imidacloprid on two earthworm species

- (*Aporrectodea nocturna* and *Allolobophora icterica*). Biol Fertil Soils 41:135–143
- Capowiez Y, Bastardie F, Costagliola G (2006) Sublethal effects of imidacloprid on the burrowing behaviour of two earthworm species: modifications of the 3D burrow systems in artificial cores and consequences on gas diffusion in soil. Soil Biol Biochem 38:285–293
- Capowiez Y, Dittbrenner N, Rault M, Triebkorn R, Hedde M, Mazzia C (2010) Earthworm cast production as a new behavioural biomarker for toxicity testing. Environ Pollut 158:388–393
- Casida JE (2011) Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance. J Agric Food Chem 59:2923–2931
- Chagnon M, Gingras J, de Oliveira D (1993) Complementary aspects of strawberry pollination by honey and indigenous bees (Hymenoptera). J Econ Entomol 86:416–420
- Charmillot PJ, Pasquier D, Salamin C, Ter-Hovannesian A (2007) Ovicidal and larvicidal effectiveness of insecticides applied by dipping apples on the small fruit tortrix *Grapholita lobarzewskii*. Pest Manag Sci 63:677–681
- Charpentier G, Louat F, Bonmatin JM, Marchand PA, Vannier F, Locker D, Decoville M (2014) Lethal and sublethal effects of imidacloprid, after chronic exposure, on the insect model *Drosophila melanogaster*. Environ Sci Technol 48(7):4096–4102
- Chen RZ, Klein MG (2012) Efficacy of insecticides against the rice stem-borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae), and use of sex pheromones to time accurately the yearly application. Int J Pest Manag 58:353–359
- Chen XD, Culbert E, Herbert V, Stark JD (2010) Mixture effects of the adjuvant R-11 and the insecticide imidacloprid on population growth rate and other parameters of *Ceriodaphnia dubia*. Ecotoxicol Environ Saf 73:132–137
- Cheng XA, Chang C, Dai SM (2010) Responses of striped stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae), from Taiwan to a range of insecticides. Pest Manag Sci 66:762–766
- Chowdhury S, Mukhopadhyay S, Bhattacharyya A (2012) Degradation dynamics of the insecticide: Clothianidin (Dantop 50 % WDG) in a tea field ecosystem. Bull Environ Contam Toxicol 89:340–343
- Cichon LB, Soleno J, Anguiano OL, Garrido SA, Montagna CM (2013) Evaluation of cytochrome P450 activity in field populations of *Cydia pomonella* (Lepidoptera: Tortricidae) resistant to azinphosmethyl, acetamiprid, and thiacloprid. J Econ Entomol 106:939–944
- Coleman DC, Ingham ER (1988) Carbon, nitrogen, phosphorus and sulphur cycling in terrestrial ecosystems. Biogeochemistry 5:3–6
- Colin ME, Bonmatin JM, Moineau I, Gaimon C, Brun S, Vermandere JP (2004) A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Arch Environ Contam Toxicol 47:387–395
- Couvillon MJ, Barton SN, Cohen JA, Fabricius OK, Kaercher MH, Cooper LS, Silk MJ, Helantera H, Ratnieks FLW (2010) Alarm pheromones do not mediate rapid shifts in honey bee guard acceptance threshold. J Chem Ecol 36:1306–1308
- Cox L, Koskinen WC, Yen PY (1997) Sorption-desorption of imidacloprid and its metabolites in soils. J Agric Food Chem 45:1468–1472
- Cox L, Hermosin MC, Cornejo J (2004) Influence of organic amendments on sorption and dissipation of imidacloprid in soils. Int J Environ Anal Chem 84:95–102
- Cresswell JE (2011) A meta-analysis of experiments testing the effects of neonicotinoid insecticide (imidacloprid) on honey bees. Ecotoxicology 20:149–154
- Cresswell JE, Thompson H (2012) Comment on “A common pesticide decreases foraging success and survival in honey bees”. Science 337:1453
- Cresswell JE, Desneux N, van Engelsdorp D (2012a) Dietary traces of neonicotinoid pesticides as a cause of population declines in honey bees: an evaluation by Hill’s epidemiological criteria. Pest Manag Sci 68:819–827
- Cresswell JE, Page CJ, Uygun MB, Holmbergh M, Li Y, Wheeler JG, Laycock I, Pook CJ, De Ibarra NH, Smirnov N, Tyler CR (2012b) Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). Zoology 115:365–371
- Cutler GC, Scott-Dupree C (2007) Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. J Econ Entomol 100:765–772
- Davis BNK, Lakhani KH, Yates TJ (1991a) The hazards of insecticides to butterflies of field margins. Agric Ecosyst Environ 36:151–161
- Davis BNK, Lakhani KH, Yates TJ, Frost AJ (1991b) Bioassays of insecticide spray drift: the effects of wind speed on the mortality of *Pieris brassicae* larvae (Lepidoptera) caused by diflubenzuron. Agric Ecosyst Environ 36:141–149
- Davis BNK, Lakhani KH, Yates TJ, Frost AJ, Plant RA (1993) Insecticide drift from ground-based, hydraulic spraying of peas and brussels sprouts: bioassays for determining buffer zones. Agric Ecosyst Environ 43:93–108
- Decourtye A, Devillers J (2010) Ecotoxicology of neonicotinoids insecticides in the bees. In: Thany S (ed) Insect nicotinic acetylcholine receptors. Landes Bioscience, St. Austin
- Decourtye A, Lacassie E, Pham-Delegue MH (2003) Learning performances of honeybees (*Apis mellifera* L.) are differentially affected by imidacloprid according to the season. Pest Manag Sci 59:269–278
- Decourtye A, Armengaud C, Renou M, Devillers J, Cluzeau S, Gauthier M, Pham-Delègue MH (2004a) Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). Pestic Biochem Physiol 78:83–92
- Decourtye A, Devillers J, Cluzeau S, Charretton M, Pham-Delègue MH (2004b) Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicol Environ Saf 57:410–419
- Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, Cluzeau S, Pham-Delegue MH (2005) Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. Arch Environ Contam Toxicol 48:242–250
- Decourtye A, Mader E, Desneux N (2010) Landscape enhancement of floral resources for honey bees in agro-ecosystems. Apidologie 41:264–277
- DEFRA (2007) Assessment of the risk posed to honeybees by systemic pesticides. PS2322, CSL York, UK
- DEFRA (2009) Intermittent exposure in terrestrial invertebrates—a case study with honeybees. PS2341, CSL York, UK
- Dégise P, Grünwald B, Gauthier M (2002) The insecticide imidacloprid is a partial agonist of the nicotinic receptor of honeybee Kenyon cells. Neurosci Lett 321:13–16
- Delbeke E, Vercruysse P, Tirry L, de Clercq P, Degheele D (1997) Toxicity of diflubenzuron, pyriproxyfen, imidacloprid and diafenthiuron to the predatory bug *Orius laevis* (Het.: Anthoridae). Entomophaga 42:349–358
- Derecka K, Blythe MJ, Malla S, Genereux DP, Guffanti A, Pavan P, Moles A, Snart C, Ryder T, Ortori CA, Barrett DA, Schuster E, Stöger R (2013) Transient exposure to low levels of insecticide affects metabolic networks of honeybee larvae. PLoS One 8:e68191
- Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. Ann Rev Entomol 52:81–106
- Detzel A, Wink M (1993) Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. Chemoecology 4:8–18
- Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G, Pennacchio F (2013) Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. Proc Natl Acad Sci U S A 110:18466–18471. doi:10.1073/pnas.1314923110
- Diez-Rodriguez GI, de Baptista GC, Trevizani LR, Haddad ML, Nava DE (2006) Residues of thiamethoxam, aldicarb and its metabolites

- in coffee leaves and effect on the control of *Leucoptera coffeella* (Guerin-Meneville) (Lepidoptera: Lyonetiidae). *Neotropical Entomol* 35:257–263
- Dilling C, Lambdin P, Grant J, Rhea R (2009) Community response of insects associated with eastern hemlock to imidacloprid and horticultural oil treatments. *Environ Entomol* 38:53–66
- Dittbrenner N, Triebkorn R, Moser I, Capowiez Y (2010) Physiological and behavioural effects of imidacloprid on two ecologically relevant earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa*). *Ecotoxicology* 19:1567–1573
- Dittbrenner N, Schmitt H, Capowiez Y, Triebkorn R (2011a) Sensitivity of *Eisenia fetida* in comparison to *Aporrectodea caliginosa* and *Lumbricus terrestris* after imidacloprid exposure. Body mass change and histopathology. *J Soils Sediments* 11:1000–1010
- Dittbrenner N, Moser I, Triebkorn R, Capowiez Y (2011b) Assessment of short and long-term effects of imidacloprid on the burrowing behaviour of two earthworm species (*Aporrectodea caliginosa* and *Lumbricus terrestris*) by using 2D and 3D post-exposure techniques. *Chemosphere* 84:1349–1355
- Dittbrenner N, Capowiez Y, Kohler H, Triebkorn R (2012) Stress protein response (Hsp70) and avoidance behaviour in *Eisenia fetida*, *Aporrectodea caliginosa*, and *Lumbricus terrestris* when exposed to imidacloprid. *J Soils Sediments* 12:198–206
- Dively G, Hooks C (2010) Use patterns of neonicotinoid insecticides on cucurbit crops and their potential exposure to honey bees. Progress report, strategic agricultural initiative grants program, EPA region 3
- Doffou NM, Ochou OG, Kouassi P (2011) Susceptibility of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae) to insecticides used on cotton crops in Côte d'Ivoire, West Africa. Implications in insecticide resistance pest management strategies. *Resistant Pest Manag Newsl* 20:10–15
- Dogramaci M, Tingey WM (2008) Comparison of insecticide resistance in a North American field population and a laboratory colony of potato tuberworm (Lepidoptera: Gelechiidae). *J Pest Sci* 81:17–22
- Dondero F, Negri A, Boatti L, Marsano F, Mignone F, Viarengo A (2010) Transcriptomic and proteomic effects of a neonicotinoid insecticide mixture in the marine mussel (*Mytilus galloprovincialis*, Lam.). *Sci Total Environ* 15:3775–3786
- Donnarumma L, Pulcini P, Pochi D, Rosati S, Lusco L, Conte E (2011) Preliminary study on persistence in soil and residues in maize of imidacloprid. *J Environ Sci Health B* 46:469–472
- Dunley JE, Brunner JF, Doerr MD, Beers EH (2006) Resistance and cross-resistance in populations of the leafrollers, *Choristoneura rosaceana* and *Pandemis pyrusana*, in Washington apples. *J Insect Sci* 6
- Dupuis JP, Gauthier M, Raymond-Delpech V (2011) Expression patterns of nicotinic subunits alpha 2, alpha 7, alpha 8, and beta 1 affect the kinetics and pharmacology of ACh-induced currents in adult bee olfactory neuropiles. *J Neurophysiol* 106:1604–1613
- Durham EW, Scharf ME, Siegfried BD (2001) Toxicity and neurophysiological effects of fipronil and its oxidative sulfone metabolite on European corn borer larvae (Lepidoptera: Crambidae). *Pestic Biochem Physiol* 71:97–106
- Durham EW, Siegfried BD, Scharf ME (2002) In vivo and in vitro metabolism of fipronil by larvae of the European corn borer *Ostrinia nubilalis*. *Pest Manag Sci* 58:799–804
- Easton AH, Goulson D (2013) The neonicotinoid insecticide imidacloprid repels pollinating flies and beetles at field-realistic concentrations. *PLoS One* 8:e54819
- Edwards CA, Bohlen PJ (1996) Biology and ecology of earthworms, 3rd edn. Chapman and Hall, London
- EFSA (2006) Draft Assessment Report (DAR). Draft assessment report: initial risk assessment provided by the rapporteur member State Germany for the existing active substance imidacloprid
- Eisenback BM, Salom SM, Kok LT, Lagalante AF (2010) Lethal and sublethal effects of imidacloprid on Hemlock woolly Adelgid (Hemiptera: Adelgidae) and two introduced predator species. *J Econ Entomol* 103:1222–1234
- El Hassani AK, Dacher M, Gauthier M, Armengaud C (2005) Effects of sublethal doses of fipronil on the behavior of the honeybee (*Apis mellifera*). *Pharmacol Biochem Behav* 82:30–39
- El Hassani AK, Dacher M, Gary V, Lambin M, Gauthier M, Armengaud C (2008) Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). *Arch Environ Contam Toxicol* 54:653–661
- El Hassani AK, Dupuis JP, Gauthier M, Armengaud C (2009) Glutamatergic and GABAergic effects of fipronil on olfactory learning and memory in the honeybee. *Invert Neurosci* 9:91–100
- Elbert A, Becker B, Hartwig J, Erdelen C (1991) Imidacloprid—a new systemic insecticide. *Plant Prot Nachr Bayer* 44:113–136
- Epstein LH, Robinson JL, Roemmich JN, Marusewski A (2011) Slow rates of habituation predict greater zBMI gains over 12 months in lean children. *Eat Behav* 12:214–218
- European Commission (2010) Commission Directive 2010/21/EU of 12 March 2010 amending Annex I to Council Directive 91/414/EEC as regards the specific provisions relating to clothianidin, thiamethoxam, fipronil and imidacloprid
- Fagin D (2012) The learning curve. *Nature* 490:462–465. doi:10.1038/490462a
- Fang Q, Huang CH, Ye GY, Yao HW, Cheng JA et al (2008) Differential fipronil susceptibility and metabolism in two rice stem borers from China. *J Econ Entomol* 101:1415–1420
- Farooqui T (2013) A potential link among biogenic amines based pesticides, learning and memory, and colony collapse disorder: a unique hypothesis. *Neurochem Int* 62:122–136
- Faucon JP, Aurieres C, Drajnudel P, Mathieu L, Ribiere M, Martel AC, Zeggane S, Chauzat MP, Aubert MFA (2005) Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. *Pest Manag Sci* 61:111–125
- Feber RE, Firbank LG, Johnson PJ, Macdonald DW (1997) The effects of organic farming on pest and non-pest butterfly abundance. *Agric Ecosyst Environ* 64:133–139
- Feltham H, Park K, Goulson D (2014) Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. *Ecotoxicology* 37:301–308
- Fischer J, Müller T, Spatz A-K, Greggers U, Grünewald B et al (2014) Neonicotinoids interfere with specific components of navigation in honeybees. *PLoS One* 9:e91364
- Ford KA, Casida JE (2006) Unique and common metabolites of thiamethoxam, clothianidin, and dinotefuran in mice. *Chem Res Toxicol* 19:1549–1556
- Fossen M (2006) Environmental fate of imidacloprid. Environmental monitoring department of pesticide regulation, California, USA
- Franklin MT, Winston ML, Morandin LA (2004) Effects of clothianidin on *Bombus impatiens* (Hymenoptera: Apidae) colony health and foraging behaviour. *J Econ Entomol* 97:369–373
- Frantzios G, Paptiki K, Sidiropoulou B, Lazaridis G, Theophilidis G, Mavragani-Tsapidou P (2008) Evaluation of insecticidal and genotoxic effects of imidacloprid and acetochlor in *Drosophila melanogaster*. *J Appl Entomol* 132:583–590
- Freemark K, Boutin C (1995) Impacts of agricultural herbicide use on terrestrial wildlife in temperate landscapes: a review with special reference to North America. *Agric Ecosyst Environ* 52:67–91
- Funayama K, Ohsumi S (2007) Control effect of neonicotinoid insecticides on apple leaf miner, *Phyllonorycter ringoniella* (Lepidoptera: Gracillariidae). *Annu Rep Soc Plant Protect N Jpn* 58:156–158
- Galvanho JP, Carrera MP, Moreira DDO, Erthal M, Silva CP, Samuels RI (2013) Imidacloprid inhibits behavioral defences of the leaf-cutting ant *Acromyrmex subterraneus subterraneus* (Hymenoptera:

- Formicidae). *J Insect Behav* 26:1–13. doi:10.1007/S10905-012-9328-6
- Gauthier M (2010) State of the art on insect nicotinic acetylcholine receptor function in learning and memory. In: Thany S (ed) *Insect nicotinic acetylcholine receptors*. Springer, Berlin
- Gawleta N, Zimmermann Y, Eltz T (2005) Repellent foraging scent recognition across bee families. *Apidologie* 36:325–330
- Geiger F, Bengtsson J, Berendse F, Weisser WW, Emmerson M, Morales MB, Ceryngier P, Liira J, Tschamtké T, Winqvist C, Eggers S, Bommarco R, Pärt T, Bretagnolle V, Plantegenest M, Clement LW, Dennis C, Palmer C, Oñate JJ, Guerrero I, Hawro V, Aavik T, Thies C, Flohre A, Hänke S, Fischer C, Goedhart PW, Inchausti P (2010) Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic Appl Ecol* 11:97–105
- Gill RJ, Ramos-Rodriguez O, Raine NE (2012) Combined pesticide exposure severely affects individual- and colony-level traits in bees. *Nature* 491:105–108
- Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Bernardo AD, Tapparo A (2009) Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees. *J Econ Entomol* 102:1808–1815
- Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A (2012) Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. *J Appl Entomol* 136:17–26
- Giurfa M (1993) The repellent scent-mark of the honeybee *Apis mellifera ligustica* and its role as communication cue during foraging. *Insect Soc* 40:59–67
- Gomez-Eyles JL, Svendsen C, Lister L, Martin H, Hodson ME, Spurgeon DJ (2009) Measuring and modelling mixture toxicity of imidacloprid and thiacloprid on *Caenorhabditis elegans* and *Eisenia fetida*. *Ecotoxicol Environ Saf* 72:71–79
- Goulson D (2003) Effects of introduced bees on native ecosystems. *Annu Rev Ecol Evol Syst* 34:1–26
- Goulson D (2010) *Bumblebees: behaviour, ecology and conservation*. Oxford University Press, Oxford
- Goulson D, Chapman JW, Hughes WOH (2001) Discrimination of unrewarding flowers by bees; direct detection of rewards and use of repellent scent marks. *J Insect Behav* 14:669–678
- Gradish AE, Scott-Dupree CD, Shipp L, Harris CR, Ferguson G (2010) Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae). *Pest Manag Sci* 66:142–146
- Graystock P, Yates K, Darvill B, Goulson D, Hughes WOH (2013a) Emerging dangers: deadly effects of an emergent parasite in a new pollinator host. *J Invertebr Pathol* 114:114–119
- Graystock P, Yates K, Evison S, Darvill B, Goulson D, Hughes WOH (2013b) The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies. *J Appl Ecol* 50:1207–1215
- Greatti M, Sabatini AG, Barbattini R, Rossi S, Stravisi A (2003) Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results. *Bull Insect* 56:69–72
- Greenleaf S, Kremen C (2006) Wild bees enhance honey bees' pollination of hybrid sunflower. *Proc Natl Acad Sci U S A* 103:13890–13895
- Gregor A, Ellis JD (2011) Cell death localization in situ in laboratory reared honey bee (*Apis mellifera* L.) larvae treated with pesticides. *Pestic Biochem Physiol* 99:200–207
- Gross M (2008) Pesticides linked to bee deaths. *Curr Biol* Vol 18:684
- Guez D, Suchail S, Gauthier M, Maleszka R, Belzunces LP (2001) Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). *Neurobiol Learn Mem* 76:183–191
- Guez D, Belzunces LP, Maleszka R (2003) Effects of imidacloprid metabolites on habituation in honeybees suggest the existence of two subtypes of nicotinic receptors differentially expressed during adult development. *Pharmacol Biochem Behav* 75:217–222
- Gupta S, Gajbhiye VT (2007) Persistence of acetamiprid in soil. *Bull Environ Contam Toxicol* 78:349–352
- Han P, Niu CY, Lei CL, Cui JJ, Desneux N (2010) Use of an innovative T-tube maze assay and the proboscis extension response assay to assess sublethal effects of GM products and pesticides on learning capacity of the honey bee *Apis mellifera* L. *Ecotoxicology* 19:1612–1619
- Hatjina F, Papaefthymiou C, Charistos L, Dogaroglu T, Bouga M, Emmanouil C, Arnold G (2013) Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo. *Apidologie* 44:467–480
- Hayasaka D, Korenaga T, Suzuki K, Saito F, Sánchez-Bayo F, Goka K (2012) Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. *Ecotoxicol Environ Saf* 80:355–62. doi:10.1016/j.ecoenv.2012.04.004
- Haynes KF (1988) Sublethal effects of neurotoxic insecticides on insect behavior. *Annu Rev Entomol* 33:149–168
- He YP, Gao CF, Cao MZ, Chen WM, Huang LQ et al (2007) Survey of susceptibilities to monosultap, triazophos, fipronil, and abamectin in *Chilo suppressalis* (Lepidoptera: Crambidae). *J Econ Entomol* 100:1854–1861
- He YP, Gao CF, Chen WM, Huang LQ, Zhou WJ et al (2008) Comparison of dose responses and resistance ratios in four populations of the rice stem borer, *Chilo suppressalis* (Lepidoptera: Pyralidae), to 20 insecticides. *Pest Manag Sci* 64:308–315
- He YP, Zhang JF, Gao CF, Su JY, Chen JM et al (2013) Regression analysis of dynamics of insecticide resistance in field populations of *Chilo suppressalis* (Lepidoptera: Crambidae) during 2002–2011 in China. *J Econ Entomol* 106:1832–1837
- Henry M, Beguin M, Requier F, Rollin O, Odoux JF, Aupinel P, Aptel J, Tchamitchian S, Decourtye A (2012a) A common pesticide decreases foraging success and survival in honey bees. *Science* 336:348–350
- Henry M, Beguin M, Requier F, Rollin O, Odoux J, Aupinel P, Aptel J, Tchamitchian S, Decourtye A (2012b) Response to comment on “A common pesticide decreases foraging success and survival in honey bees”. *Science* 337:1453
- Hill TA, Foster RE (2000) Effect of insecticides on the diamondback moth (Lepidoptera: Plutellidae) and its parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). *J Econ Entomol* 93:763–768
- Hoi KK, Daborn PJ, Battlay P, Robin C, Batterham P, O'Hair RA, Donald WA (2014) Dissecting the insect metabolic machinery using twin ion mass spectrometry: a single P450 enzyme metabolizes the insecticide imidacloprid in vivo. *Anal Chem* 86(7):3525–3532
- Huang SJ, Xu JF, Han ZJ (2006) Baseline toxicity data of insecticides against the common cutworm *Spodoptera litura* (Fabricius) and a comparison of resistance monitoring methods. *Int J Pest Manag* 52:209–213
- Illarionov AI (1991) Toxic effect of some insecticides on the honeybee. *Agrokhimiva* 8:121–125
- Iwasa T, Motoyama N, Ambrose JT, Roe MR (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Prot* 23:371–378
- James DG, Price TS (2002) Fecundity in twospotted spider mite (Acari: Tetranychidae) is increased by direct and systemic exposure to imidacloprid. *J Econ Entomol* 95:729–732
- Jeschke P, Nauen R, Schindler M, Elbert A (2010) Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* 59:2897–2908
- Jingui H, Thuyet DQ, Ueda T, Watanabe H (2013) Effect of imidacloprid and fipronil pesticide application on *Sympetrum infuscatum* (Libellulidae: Odonata) larvae and adults. *Paddy Water Environ* 11:277–284

- Jones MM, Robertson JL, Weinzierl RA (2010) Susceptibility of Oriental fruit moth (Lepidoptera: Tortricidae) larvae to selected reduced-risk insecticides. *J Econ Entomol* 103:1815–1820
- Jones MM, Robertson JL, Weinzierl RA (2012) Toxicity of thiamethoxam and mixtures of chlorantraniliprole plus acetamiprid, esfenvalerate, or thiamethoxam to neonates of oriental fruit moth (Lepidoptera: Tortricidae). *J Econ Entomol* 105:1426–1431
- Kalajdzic P, Oehler S, Reczko M, Pavlidi N, Vontas J, Hatzigeorgiou AG, Savakis C (2012) Use of mutagenesis, genetic mapping and next generation transcriptomics to investigate insecticide resistance mechanisms. *PLoS One* 7:e40296. doi:10.1371/journal.pone.0040296
- Kalajdzic P, Markaki M, Oehler S, Savakis C (2013) Imidacloprid does not induce Cyp genes involved in insecticide resistance of a mutant *Drosophila melanogaster* line. *Food Chem Toxicol* 60:355–359. doi:10.1016/j.fct.2013.07.080
- Kather R, Drijfhout FP, Martin SJ (2011) Task group differences in cuticular lipids in the honey bee *Apis mellifera*. *J Chem Ecol* 37: 205–212
- Khani A, Ahmadi F, Ghadamyari M (2012) Side effects of imidacloprid and abamectin on the Mealybug destroyer, *Cryptolaemus montrouzieri*. *Trakia J Sci* 10:30–35
- Kilpatrick AL, Hagerty AM, Turnipseed SG, Sullivan MJ, Bridges WC (2005) Activity of selected neonicotinoids and dicofol on non-target arthropods in cotton: implications in insect management. *J Econ Entomol* 98:814–820
- Kinkler H, Löser S, Rehnelt K (1987) 10 Jahre Erforschung des Moselapollfalter (*Parnassius apollo vinningensis* STICHEL 1899, Lepidoptera, Papilionidae) im modernen Weinbaugebiet der Mosel—ein Beitrag zu seiner Rettung. *Commun Assoc rheinisch-westfälischer Lepidopterologen* 5:74–96
- Klein AM, Vaissie're BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. *Proc R Soc B* 274:303–313
- Kluser S, Neumann P, Chauzat MP, Pettis JS (2011) UNEP emerging issues: global honey bee colony disorder and other threats to insect pollinators. <http://www.unep.org>. Accessed 25 October 2012
- Knight AL (2010) Cross-resistance between azinphos-methyl and acetamiprid in populations of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), from Washington State. *Pest Manag Sci* 66:865–874
- Knight ME, Martin AP, Bishop S, Osborne JL, Hale RJ, Sanderson RA, Goulson D (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species. *Mol Ecol* 14: 1811–1820
- Knoepf JD, Vose JM, Michael JL, Reynolds BC (2012) Imidacloprid movement in soils and impacts on soil microarthropods in Southern Appalachian eastern hemlock stands. *J Environ Qual* 41:469–478
- Knollengberg WG, Merritt RW, Lawson DL (1985) Consumption of leaf litter by *Lumbricus terrestris* (Oligochaeta) on a Michigan woodland floodplain. *Am Midl Nat J* 113:1–6
- Kramarz P, Stark JD (2003) Population level effects of cadmium and the insecticide, imidacloprid to the Parasitoid, *Aphidius ervi* after exposure through its host, the pea aphid, *Acyrtosiphon pisum*. *Biol Control* 27:310–314
- Kreutzweiser DP, Sibley PK (2013) Aquatic communities: pesticide impacts. In: Jorgensen SE (ed) *Encyclopedia of environmental management*. Taylor and Francis, New York, pp 312–321
- Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA, Thompson DG (2008a) Are leaves that fall from imidacloprid-treated maple trees to control Asian longhorned beetles toxic to non-target decomposer organisms? *J Environ Qual* 37:639–646
- Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA, Holmes SB, Thompson DG (2008b) Effects on litter-dwelling earthworms and microbial decomposition of soil-applied imidacloprid for control of wood-boring insects. *Pest Manag Sci* 64:112–118
- Kreutzweiser DP, Thompson DG, Scarr TA (2009) Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates. *Ecotoxicol Environ Saf* 72:1053–1057
- Krischik VA, Landmark AL, Heimpel GE (2007) Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae). *Environ Entomol* 36:1238–1245
- Kromp B (1999) Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. *Agric Ecosyst Environ* 74:187–228
- Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One* 7:e29268
- Kullik SA, Sears MK et al (2011) Sublethal effects of Cry 1F Bt corn and clothianidin on black cutworm (Lepidoptera: Noctuidae) larval development. *J Econ Entomol* 104:484–93
- Kunkel BA, Held DW, Potter AD (1999) Impact of halofenozide, imidacloprid, and bendiocarb on beneficial invertebrates and predatory activity in turfgrass. *J Econ Entomol* 92:922–930
- Kunkel BA, Held DW, Potter DA (2001) Lethal and sub-lethal effects of bendiocarb, halofenozide, and imidacloprid on *Harpalus pennsylvanicus* (Coleoptera: Carabidae) following different modes of exposure in turfgrass. *J Econ Entomol* 94:60–67
- Kurwadkar ST, Dewinne D, Wheat R, McGahan DG, Mitchell FL (2013) Time dependent sorption behaviour of dinotefuran, imidacloprid and thiamethoxam. *J Environ Sci Health B* 48:237–242
- Lambin M, Armengaud C, Raymond S, Gauthier M (2001) Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. *Arch Insect Biochem Physiol* 48:129–134
- Landis DA, Wratten SD, Gurr GM (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annu Rev Entomol* 45:175–201
- Larson JL, Redmond CT, Potter DA (2012) Comparative impact of an anthranilic diamide and other insecticidal chemistries on beneficial invertebrates and ecosystem services in turfgrass. *Pest Manag Sci* 68:740–748
- Laycock I, Lenthall KM, Barratt AT, Cresswell JE (2012a) Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). *Ecotoxicology* 21:1937–1945
- Laycock I, Lenthall KM, Barratt AT, Cresswell JE (2012b) Erratum to: Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). *Ecotoxicology* 21: 1946–1946
- LeBlanc HMK, Culp JM, Baird DJ, Alexander AC, Cessna AJ (2012) Single versus combined lethal effects of three agricultural insecticides on larvae of the freshwater insect *Chironomus dilutes*. *Arch Environ Contam Toxicol* 63:378–390
- Lee KW (1985) *Earthworms: their ecology and relationship with soils and land use*. Academic, Sydney
- Lee JC, Menalled FD, Landis DA (2001) Refuge habitats modify impact of insecticide disturbance on carabid beetle communities. *J Appl Ecol* 38:472–483
- Li AG, Yang YH, Wu SW, Li C, Wu YD (2006) Investigation of resistance mechanisms to fipronil in diamondback moth (Lepidoptera: Plutellidae). *J Econ Entomol* 99:914–919
- Li XT, Huang QC, Yuan JZ, Tang ZH (2007) Fipronil resistance mechanisms in the rice stem borer, *Chilo suppressalis* Walker. *Pestic Biochem Physiol* 89:169–174
- Liang GM, Chen W, Liu TTX (2003) Effects of three neem-based insecticides on diamondback moth (Lepidoptera: Plutellidae). *Crop Prot* 22:333–340
- Liess M, Beketov M (2011) Traits and stress—keys to identify community effects at low toxicant level. *Ecotoxicology* 20:1328–1340
- Liess M, Beketov MA (2012) Rebuttal related to “Traits and stress—keys to identify community effects of low levels of toxicants in test systems”. *Ecotoxicology* 21:300–303

- Liess M, von der Ohe PC (2005) Analyzing effects of pesticides on invertebrate communities in streams. *Environ Toxicol Chem* 24: 954–965
- Liess M, Schäfer RB, Schriever CA (2008) The footprint of pesticide stress in communities—species traits reveal community effects of toxicants. *Sci Total Environ* 406:484–90
- Liess M, Foit K, Becker A, Hassold E, Dolciotti I, Kattwinkel M, Duquesne S (2013) Culmination of low-dose pesticide effects. *Environ Sci Tech* 47:8862–8868
- Lintott DR (1992) NTN 33893 (240 FS formulation): acute toxicity to the mysid, *Mysidopsis bahia*, under flow-through conditions. Toxicon Environmental Sciences, Jupiter, Florida (performing laboratory). Miles Incorporated, Kansas City, Missouri (submitting company). 43 pp. Miles Report No. 103845
- Little EE (1990) Behavioural toxicology; stimulating challenges for a growing discipline. *Environ Toxicol Chem* 9:1–2
- Longley M, Sotherton NW (1997) Factors determining the effects of pesticides upon butterflies inhabiting arable farmland. *Agric Ecosyst Environ* 61:1–12
- Lucas E, Giroux S, Demougeot S, Duchesne RM, Coderre D (2004) Compatibility of a natural enemy, *Coleomegilla maculata lengi* (Col., Coccinellidae) and four insecticides used against the Colorado potato beetle (Col., Chrysomelidae). *J Appl Entomol* 128:233–239
- Lukancic S, Zibrat U, Mezek T, Jerebic A, Simic T, Brancelj A (2010) Effects of exposing two non-target crustacean species, *Asellus aquaticus* L., and *Gammarus fossarum* Koch., to atrazine and imidacloprid. *Bull Environ Contam Toxicol* 84(1):85–90. doi:10.1007/s00128-009-9854-x
- Luo Y, Zang Y, Zhong Y, Kong Z (1999) Toxicological study of two novel pesticides on earthworm *Eisenia fetida*. *Chemosphere* 39: 2347–2356
- Lydy M, Belden J, Wheelock C, Hammock B, Denton D (2004) Challenges in regulating pesticide mixtures. *Ecol Soc* 9:1
- Magalhaes LC, Walgenbach JF (2011) Life stage toxicity and residual activity of insecticides to codling moth and Oriental fruit moth (Lepidoptera: Tortricidae). *J Econ Entomol* 104:1950–1959
- Maisonnasse A, Lenoir JC, Beslay D, Crauser D, Le Conte Y (2010) E-b-Ocimene, a volatile brood pheromone involved in social regulation in the honey bee colony (*Apis mellifera*). *PLoS One* 5
- Mani S, Medrzycki P, Porrini C (2010) The puzzle of honey bee losses: a brief review. *Bull Insect* 63:153–160
- Mann RS, Uppal SK, Sharma S, Mann KK (2009) Soil efficacy of fipronil to early stage pests of sugarcane, and its effect on development on *Chilo infuscatellus* Snellen (Crambidae: Lepidoptera). *Int J Pest Manag* 55:307–315
- Marzaro M, Vivan L, Targa A, Mazzon L, Mori N, Greatti M, Petrucco Toffolo E, Di Bernardo A, Giorio C, Marton D, Tapparo A, Girolami V (2011) Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat. *Bull Insect* 64:118–125
- Mason R, Tennekes HA, Sanchez-Bayo F, Jepsen PU (2013) Immune suppression by neonicotinoid insecticides at the root of global wildlife declines. *J Environ Immunol Toxicol* 1:3–12
- Maus C, Nauen R (2010) Response to the publication: Tennekes H.A. The significance of the Druckey-Küpfmüller equation for risk assessment—the toxicity of neonicotinoid insecticides is reinforced by exposure time. *Toxicology* 280:176–177. doi:10.1016/j.tox.2010.11.014
- Maxim L, van der Sluijs JP (2007) Uncertainty: cause or effect of stakeholders' debates? Analysis of a case study: the risk for honeybees of the insecticide Gaucho®. *Sci Total Environ* 376:1–17
- Maxim L, van der Sluijs JP (2013) Seed-dressing systemic insecticides and honeybees, chapter 16. In: European Environment Agency (ed.) Late lessons from early warnings: science, precaution, innovation. European Environment Agency (EEA) report 1/2013, Copenhagen. p. 401–438
- Mayer DF, Lunden JD (1997) Effects of imidacloprid insecticide on three bee pollinators. *Hortic Sci* 29:93–97
- McKern JA, Johnson DT, Lewis BA (2007) Biology and control of the raspberry crown borer (Lepidoptera: Sesiidae). *J Econ Entomol* 100: 398–404
- Medrzycki P, Montanari R, Bortolotti L, Sabatini AG, Maini S, Porrini C (2003) Effects of imidacloprid administered in sublethal doses on honey bee behaviour. Laboratory tests. *Bull Insect* 56:59–62
- Medrzycki P, Sgolastra F, Bogo G, Tosi S, Venturi S (2011) Influence of some experimental conditions on the results of laboratory toxicological tests on honeybees. XI International Symposium “Hazards of pesticides to bees” Wageningen, The Netherlands, November 2–4
- Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G, Smaghe G (2010) Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology* 19:207–215
- Morandin LA, Winston ML (2003) Effects of novel pesticides on bumble bee (Hymenoptera: Apidae) colony health and foraging ability. *Environ Entomol* 32:555–563
- Moser SE, Obrycki JJ (2009) Non-target effects of neonicotinoid seed treatments; mortality of coccinellid larvae related to zoophytophagy. *Biol Control* 51:487–492
- Mostert MA, Schoeman AS, van der Merwe M (2000) The toxicity of five insecticides to earthworms of the Pheretima group, using an artificial soil test. *Pest Manag Sci* 56:1093–1097
- Mostert MA, Schoeman AS, van der Merwe M (2002) The relative toxicities of insecticides to earthworms of the Pheretima group (Oligochaeta). *Pest Manag Sci* 58:446–450
- Mota-Sanchez D, Wise JC, Poppen RV, Gut LJ, Hollingworth RM (2008) Resistance of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), larvae in Michigan to insecticides with different modes of action and the impact on field residual activity. *Pest Manag Sci* 64:881–890
- Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, van Engelsdorp D, Pettis JS (2010) High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLoS One* 5:e9754
- Nauen R, Ebbinghaus-Kintscher U, Schmuck R (2001) Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). *Pest Manag Sci* 57:577–586
- Nauen R, Ebbinghaus-Kintscher U, Salgado VL, Kaussmann M (2003) Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pestic Biochem Physiol* 76:55–69
- Neumann P, Carreck NL (2010) Honey bee colony loss. *J Apic Res* 49:1–6, special issue
- Nguyen BK et al (2009) Does imidacloprid seed-treated maize have an impact on honey bee mortality? *J Econ Entomol* 102:616–623
- Nielsen SA, Brodsgaard CJ, Hansen H (2000) Effects on detoxification enzymes in different life stages of honey bees (*Apis mellifera* L., Hymenoptera: Apidae) treated with a synthetic pyrethroid (flumethrin). *Altern Lab Anim* 28:437–443
- Ninsin KD (2004a) Acetamiprid resistance and cross-resistance in the diamondback moth, *Plutella xylostella*. *Pest Manag Sci* 60:839–841
- Ninsin KD (2004b) Selection for resistance to acetamiprid and various other insecticides in the diamondback moth, *Plutella xylostella* (L.) (Lep., Plutellidae). *J Appl Entomol* 128:445–451
- Ninsin KD, Miyata T (2003) Monitoring acetamiprid resistance in the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae). *Appl Entomol Zool* 38:517–521
- Ninsin KD, Tanaka T (2005) Synergism and stability of acetamiprid resistance in a laboratory colony of *Plutella xylostella*. *Pest Manag Sci* 61:723–727
- Ninsin KD, Mo JC, Miyata T (2000) Decreased susceptibilities of four field populations of the diamondback moth, *Plutella xylostella* (L.)

- (Lepidoptera: Yponomeutidae), to acetamiprid. *Appl Entomol Zool* 35:591–595
- Nyman AM, Hintermeister A, Schirmer K, Ashauer R (2013) The insecticide imidacloprid causes mortality of the freshwater Amphipod *Gammarus pulex* by interfering with feeding behavior. *PLoS One* 8: e62472
- Oldroyd PB (2007) What's killing American honey bees? *PLoS Biol* 5: e168
- Oliveira RA, Roat TC, Carvalho SM, Malaspina O (2013) Side-effects of thiamethoxam on the brain and midgut of the Africanized honeybee *Apis mellifera* (Hymenoptera: Apidae). *Environ Toxicol* 28 doi: 10.1002/tox.21842
- Osborne JL, Martin AP, Shortall CR, Todd AD, Goulson D, Knight ME, Hale RJ, Sanderson RA (2008) Quantifying and comparing bumblebee nest densities in gardens and countryside habitats. *J Appl Ecol* 45:784–792
- Osterberg JS, Darnell KM, Blickley TM, Romano JA, Rittschof D (2012) Acute toxicity and sub-lethal effects of common pesticides in post-larval and juvenile blue crabs, *Callinectes sapidus*. *J Exp Mar Biol Ecol* 424–425:5–14
- Papachristos DP, Milonas PG (2008) Adverse effects of soil applied insecticides on the predatory coccinellid *Hippodamia undecimnotata* (Coleoptera: Coccinellidae). *Biol Control* 47:77–81
- Paradis D, Bérail G, Bonmatin JM, Belzunces LP (2013) Sensitive analytical methods for 22 relevant insecticides of 3 chemical families in honey by GC-MS/MS and LC-MS/MS. *Anal Bioanal Chem* 406:621–633
- Peck DC (2009) Comparative impacts of white grub (Coleoptera: Scarabaeidae) control products on the abundance of non-target soil-active arthropods in turfgrass. *Pedobiologia* 52:287–299
- Peck DC, Olmstead D (2010) Neonicotinoid insecticides disrupt predation on the eggs of turf-infesting scarab beetles. *Bull Entomol Res* 100:689–700
- Pedibhotla VK, Hall FR, Holmsen J (1999) Deposit characteristics and toxicity of fipronil formulations for tobacco budworm (Lepidoptera: Noctuidae) control on cotton. *Crop Prot* 18:493–499
- Pestana JLT, Alexander AC, Culp JM, Baird DJ, Cessna AJ, Soares A (2009) Structural and functional responses of benthic invertebrates to imidacloprid in outdoor stream mesocosms. *Environ Pollut* 157: 2328–2334
- Pettis JS, van Engelsdorp D, Johnson J, Dively G (2012) Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften* 99:153–158
- Pilling E, Campbell P, Coulson M, Ruddle N, Tornier I (2013) A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. *PLoS One* 8:e77193
- Pochi D, Biocca M, Fanigliulo R, Pulcini P, Conte E (2012) Potential exposure of bees, *Apis mellifera* L., to particulate matter and pesticides derived from seed dressing during maize sowing. *Bull Environ Contam Toxicol* 89:354–61
- Prabhaker N, Morse JG, Castle SJ, Naranjo SE, Henneberry TJ, Toscano NC (2007) Toxicity of seven foliar insecticides to four insect parasitoids attacking citrus and cotton pests. *J Econ Entomol* 100:1053–1061
- Prabhaker N, Castle SJ, Naranjo SE, Toscano NC, Morse JG (2011) Compatibility of two systemic neonicotinoids, imidacloprid and thiamethoxam, with various natural enemies of agricultural pests. *J Econ Entomol* 104:773–781
- Rahmani S, Bandani AR, Sabahi Q (2013) Effects of thiamethoxam in sublethal concentrations, on life expectancy (ex) and some other biological characteristics of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). *Int Res J App Basic Sci* 4:556–560
- Ramasubramanian T (2013) Persistence and dissipation kinetics of clothianidin in the soil of tropical sugarcane ecosystem. *Water Air Soil Pollut* 224:1468
- Ramirez-Romero R, Chauvaux J, Pham-Delegue MH (2005) Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. *Apidologie* 36: 601–611
- Rasmussen JJ, McKnight US, Loinaz MC, Thomsen NI, Olsson ME, Bjerg PL, Binning PJ, Kronvang B (2013) A catchment scale evaluation of multiple stressor effects in headwater streams. *Sci Total Environ* 442:420–31
- Reinhard J, Srinivasan MV, Zhang SW (2004) Scent-triggered navigation in honeybees. *Nature* 427:411–411
- Reyes M, Franck P, Charmillot PJ, Ioriatti C, Olivares J et al (2007) Diversity of insecticide resistance mechanisms and spectrum in European populations of the Codling moth, *Cydia pomonella*. *Pest Manag Sci* 63:890–902
- Rhainds M, Sadof C (2009) Control of bagworms (Lepidoptera: Psychidae) using contact and soil-applied systemic insecticides. *J Econ Entomol* 102:1164–1169
- Riaz MA, Chandor-Proust A, Dauphin-Villemant C, Poupardin R, Jones CM, Strode C, Régent-Kloeckner M, David JP, Reynaud S (2013) Molecular mechanisms associated with increased tolerance to the neonicotinoid insecticide imidacloprid in the dengue vector *Aedes aegypti*. *Aquat Toxicol* 126:326–337. doi:10.1016/j.aquatox.2012.09.010
- Richarz N, Neumann D, Wipking W (1989) Untersuchungen zur Ökologie des Apollofalters (Pamassius apollo vinningensis Stichel 1899, Lepidoptera, Papilionidae) im Weinbaugebiet der unteren Mosel. *Mitt der Assoc Rheinisch-Westfälischer Lepidopterologen* 5:108–259
- Rodrick GB (2008) Effect of temperature, salinity, and pesticides on oyster hemocyte activity. *Florida Water Resour J* 86:4–14. doi:10.2175/SJWP(2008)1
- Rogers MA, Krischik VA, Martin LA (2007) Effect of soil application of imidacloprid on survival of adult green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), used for biological control in greenhouse. *Biol Control* 42:172–177
- Rondeau G, Sanchez-Bayo F, Tennekes HA, Decourtye A, Ramirez-Romero R, Desneux N (2014) Delayed and time-cumulative toxicity of imidacloprid in bees, ants and termites. *Nat Sc Rep* 4:5566. doi: 10.1038/srep05566
- Rundlöf M, Bengtsson J, Smith HG (2008) Local and landscape effects of organic farming on butterfly species richness and abundance. *J Appl Ecol* 45:813–820
- Rust MK, Reiersen DA, Klotz JH (2004) Delayed toxicity as a critical factor in the efficacy of aqueous baits for controlling Argentine ants (Hymenoptera: Formicidae). *J Econ Entomol* 97:1017–1024
- Sánchez-Bayo F (2006) Comparative acute toxicity of organic pollutants and reference values for crustaceans. I. Branchiopoda, Copepoda and Ostracoda. *Environ Pollut* 139:385–420
- Sánchez-Bayo F (2009) From simple toxicological models to prediction of toxic effects in time. *Ecotoxicology* 18:343–354
- Sanchez-Bayo F, Goka K (2014) Pesticide residues and bees—a risk assessment. *PLoS One* 9:e94482
- Sanchez-Bayo F, Kouchi G (2012) Evaluation of suitable endpoints for assessing the impacts of toxicants at the community level. *Ecotoxicology* 21:667–680. doi:10.1007/s10646-011-0823-x
- Saour G (2008) Effect of thiacloprid against the potato tuber moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae). *J Pest Sci* 81:3–8
- Sardo AM, Soares AMVM (2010) Assessment of the effects of the pesticide imidacloprid on the behaviour of the aquatic Oligochaete *Lumbriculus variegatus*. *Arch Environ Contam Toxicol* 58:648–656
- Sarkar MA, Roy S, Kole RK, Chowdhury A (2001) Persistence and metabolism of imidacloprid in different soils of West Bengal. *Pest Manag Sci* 57:598–602

- Sayyed AH, Crickmore N (2007) Selection of a field population of diamondback moth (Lepidoptera: Plutellidae) with acetamiprid maintains, but does not increase, cross-resistance to pyrethroids. *J Econ Entomol* 100:932–938
- Sayyed AH, Wright DJ (2004) Fipronil resistance in the diamondback moth (Lepidoptera: Plutellidae): inheritance and number of genes involved. *J Econ Entomol* 97:2043–2050
- Schäfer RB, vd Ohe P, Rasmussen J, Kefford B, Beketov M, Schulz R, Liess M (2012) Thresholds for the effects of pesticides on invertebrate communities and leaf breakdown in stream ecosystems. *Environ Sci Tech* 46:5134–5142
- Schmidt A (1997) Zur aktuellen situation des mosel-apollofalters *parnassius apollo vinningensis* Stichel, 1899 (Lep., Papilionidae). *Melanargia* 9:38–47
- Schmuck R (2004) Effects of a chronic dietary exposure of the honeybee *Apis mellifera* (Hymenoptera: Apidae) to imidacloprid. *Arch Environ Contam Toxicol* 47:471–478
- Schmuck R, Schoning R, Stork A, Schramel O (2001) Risk posed to honeybees (*Apis mellifera* L. Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Manag Sci* 57:225–238
- Schmuck R, Stadler T, Schmidt HW (2003) Field relevance of a synergistic effect observed in the laboratory between an EBI fungicide and a chloronicotinyl insecticide in the honeybee (*Apis mellifera* L., Hymenoptera). *Pest Manag Sci* 59:279–286
- Schneider CW, Tautz J, Grünewald B, Fuchs S (2012) RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PLoS One* 7:e30023
- Scholer J, Krischik V (2014) Chronic exposure of imidacloprid and clothianidin reduce queen survival, foraging, and nectar storing in colonies of *Bombus impatiens*. *PLoS One* 9:e91573
- Scott-Dupree CD, Conroy L, Harris CR (2009) Impact of currently used or potentially useful insecticides for canola agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymenoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). *J Econ Entomol* 102:177–182
- Seagraves MP, Lundgren JG (2012) Effects of neonicotinoid seed treatments on soybean aphid and its natural enemies. *J Pest Sci* 85:125–132
- Sergio M (2013) GMO and pesticide experiments in Hawaii: the poisoning of paradise. *Huffington Post*. http://www.huffingtonpost.com/maggie-sergio/gmo-pesticide-experiments_b_3513496.html
- Setamou M, Rodriguez D, Saldana R, Schwarzlose G, Palrang D et al (2010) Efficacy and uptake of soil-applied imidacloprid in the control of Asian citrus psyllid and a citrus leafminer, two foliar-feeding citrus pests. *J Econ Entomol* 103:1711–1719
- Sgolastra F, Renzi T, Draghetti S, Medrzycki P, Lodesani M, Maini S, Pottini C (2012) Effects of neonicotinoid dust from maize seed-dressing on honey bees. *Bull Insect* 65:273–280
- Sheehan C, Kirwan L, Connolly J, Bolger T (2008) The effects of earthworm functional diversity on microbial biomass and the microbial community level physiological profile of soils. *Eur J Soil Biol* 44:65–70
- Shi ZH, Guo SJ, Lin WC, Liu SS (2004) Evaluation of selective toxicity of five pesticides against *Plutella xylostella* (Lep: Plutellidae) and their side-effects against *Cotesia plutellae* (Hym: Braconidae) and *Oomyzus sokolowskii* (Hym: Eulophidae). *Pest Manag Sci* 60:1213–1219
- Siegfried BD, Spencer T, Marcon PCRG (1999) Susceptibility of European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Pyralidae) neonate larvae to fipronil. *J Agric Urban Entomol* 16: 273–278
- Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin J-M, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, Van Dyck H, Van Praagh J, Van der Sluijs JP, Whitehorn PR, Wiemers M (2014) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environ Sci Pollut Res* (this issue)
- Sinha SN, Lakhani KH, Davis BNK (1990) Studies on the toxicity of insecticidal drift to the first instar larvae of the large white butterfly *Pieris brassicae* (Lepidoptera: Pieridae). *Ann Appl Biol* 116:27–41
- Smith SF, Krischick VA (1999) Effects of systemic imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Environ Entomol* 28:1189–1195
- Smith JF, Catchot AL, Musser FR, Gore J (2013) Effects of aldicarb and neonicotinoid seed treatments on twospotted spider mite on cotton. *J Econ Entomol* 106:807–815
- Smodis Skerl MI, Velikonja Bolta S, Basa Cesnik H, Gregorc A (2009) Residues of pesticides in honeybee (*Apis mellifera carnica*) bee bread and in pollen loads from treated apple orchards. *Bull Environ Contam Toxicol* 83:374–7
- Song MY, Brown JJ (1998) Osmotic effects as a factor modifying insecticide toxicity on *Aedes* and *Artemia*. *Ecotoxicol Environ Saf* 41:195–202
- Song MY, Stark JD, Brown JJ (1997) Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. *Environ Toxicol Chem* 16:2494–2500
- Stapel JO, Cortesero AM, Lewis WJ (2000) Disruptive sublethal effects of insecticides on biological control: altered foraging ability and life span of a parasitoid after feeding on extrafloral nectar of cotton treated with systemic insecticides. *Biol Control* 17:243–249
- Stara J, Kocourek F (2007) Insecticidal resistance and cross-resistance in populations of *Cydia pomonella* (Lepidoptera: Tortricidae) in central Europe. *J Econ Entomol* 100:1587–95
- Stark JD, Banks JE (2003) Population-level effects of pesticides and other toxicants on arthropods. *Annu Rev Entomol* 48:505–519
- Stark JD, Jepson PC, Mayer DF (1995) Limitations to use of topical toxicity data for predictions of pesticide side effects in the field. *J Econ Entomol* 88:1081–1088
- Stamer K, Goh KS (2012) Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010–2011. *Bull Environ Contam Toxicol*, 1–6
- Stokstad E (2007) The case of the empty hives. *Science* 316:970–972
- Stoughton SJ, Liber K, Culp J, Cessna A (2008) Acute and chronic toxicity of imidacloprid to the aquatic invertebrates *Chironomus tentans* and *Hyalella azteca* under constant- and pulse-exposure conditions. *Arch Environ Contam Toxicol* 54:662–673
- Stygar D, Michalczyk K, Dolezych B, Nakonieczny M, Migula P et al (2013) Digestive enzymes activity in subsequent generations of *Cameraria ohridella* larvae harvested from horse chestnut trees after treatment with imidacloprid. *Pestic Biochem Physiol* 105:5–12
- Suchail S, Guez D, Belzunces LP (2000) Characteristics of imidacloprid toxicity in two *Apis mellifera* subspecies. *Environ Toxicol Chem* 19: 1901–1905
- Suchail S, Guez D, Belzunces LP (2001) Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ Toxicol Chem* 20:2482–2486
- Suchail S, De Sousa G, Rahmani R, Belzunces LP (2004a) In vivo distribution and metabolism of [¹⁴C]-imidacloprid in different compartments of *Apis mellifera* L. *Pest Manag Sci* 60:1056–1062
- Suchail S, Debrauwer L, Belzunces LP (2004b) Metabolism of imidacloprid in *Apis mellifera*. *Pest Manag Sci* 60:291–296
- Symington CA (2003) Lethal and sublethal effects of pesticides on the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) and its parasitoid *Orgilus lepidus* Muesebeck (Hymenoptera: Braconidae). *Crop Prot* 22:513–519
- Szczepaniak A, Creary SF, Laskowski KL, Nyrop JP, Raupp MJ (2011) Neonicotinoid insecticide imidacloprid causes outbreaks of spider mites on elm trees in urban landscapes. *PLoS One* 6:e20018

- Szczepaniec A, Raupp MJ, Parker RD, Kerns D, Eubanks MD (2013) Neonicotinoid insecticides alter induced defenses and increase susceptibility to spider mites in distantly related crop plants. *PLoS One* 8:e62620
- Tapparo A, Marton D, Giorio C, Zanella A, Solda' L, Marzaro M, Vivan L, Girolami V (2012) Assessment of the environmental exposure of honeybees to the particulate matter containing neonicotinoid insecticides coming from corn coated seeds. *Environ Sci Tech* 46:2592–2599
- Tarmann G (2009) Die Vinschger Trockenrasen - Ein Zustandsbericht auf basis der bioindikatoren Tagfalter und widderchen (Lepidoptera: Rhopalocera, Zygaenidae). *Sci Yearb Tyrolean State Mus* 2:307–350
- Tasei JN, Lerin J, Ripault G (2000) Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. *Pest Manag Sci* 56:784–788
- Tasei JN, Ripault G, Rivault E (2001) Hazards of imidacloprid seed coating to *Bombus terrestris* (Hymenoptera: Apidae) when applied to sunflower. *J Econ Entomol* 94:623–627
- Taverner PD, Sutton C, Cunningham NM, Dyson C, Lucas N et al (2011) Efficacy of several insecticides alone and with horticultural mineral oils on light brown apple moth (Lepidoptera: Tortricidae) eggs. *J Econ Entomol* 104:220–224
- Taverner PD, Sutton C, Cunningham NM, Myers SW (2012) The potential of mineral oils alone and with reduced rates of insecticides for the control of light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), on nursery plants. *Crop Prot* 42:83–87
- Teeters BS, Johnson RM, Ellis MD, Siegfried BD (2012) Using video-tracking to assess sublethal effects of pesticides on honey bees (*Apis mellifera* L.). *Environ Toxicol Chem* 31:1349–1354
- Tennekes HA (2010) The significance of the Druckrey-Küpfmüller equation for risk assessment—the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time. *Toxicology* 276:1–4
- Tennekes HA (2011) The significance of the Druckrey-Küpfmüller equation for risk assessment—the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time: responding to a letter to the editor by Drs. C. Maus and R. Nauen of Bayer CropScience AG. *Toxicology* 280:173–175
- Tennekes HA, Sánchez-Bayo F (2012) Time-dependent toxicity of neonicotinoids and other toxicants: implications for a new approach to risk assessment. *J Environ Anal Toxicol* S4:001. doi:10.4172/2161-0525.S4-001
- Tennekes HA, Sánchez-Bayo F (2013) The molecular basis of simple relationships between exposure concentration and toxic effects with time. *Toxicology* 309:39–51
- Thany SH, Gauthier M (2005) Nicotine injected into the antennal lobes induces a rapid modulation of sucrose threshold and improves short-term memory in the honeybee *Apis mellifera*. *Brain Res* 1039:216–219
- Thany SH, Lenaers G, Crozatier M, Armengaud C, Gauthier M (2003) Identification and localization of the nicotinic acetylcholine receptor alpha3 mRNA in the brain of the honeybee, *Apis mellifera*. *Insect Mol Biol* 12:255–262
- Thompson HM, Maus C (2007) The relevance of sublethal effects in honey bee testing for pesticide risk assessment. *Pest Manag Sci* 63:1058–1061
- Tomé HVV, Martins GF, Lima MAP, Campos LAO, Guedes RNC (2012) Imidacloprid-induced impairment of mushroom bodies and behavior of the native stingless bee *Melipona quadrifasciata anthidioides*. *PLoS One* 7:e38406
- Tomizawa M, Maltby D, Talley TT, Durkin KA, Medzihradsky KF, Burlingame AL, Taylor P, Casida JE (2008) Atypical nicotinic agonist bound conformations conferring subtype selectivity. *Proc Natl Acad Sci U S A* 105:1728–1732
- Tu C, Wang Y, Duan W, Hertl P, Tradway L, Brandenburg R, Lee D, Snell M, Hu S (2011) Effects of fungicides and insecticides on feeding behavior and community dynamics of earthworms: implications for casting control in turfgrass systems. *Appl Soil Ecol* 47:31–36
- Valdovinos-Núñez J, Quezada-Euán JG, Ancona-Xiu P, Moo-Valle H, Carmona A, Sanchez ER (2009) Comparative toxicity of pesticides to stingless bees (Hymenoptera: Apidae: Meliponini). *J Econ Entomol* 102:1737–1742
- Van den Brink PJ, Roelsma J, van Nes EH, Scheffer M, Brock TCM (2002) PERPEST model: a case-based reasoning approach to predict ecological risks of pesticides. *Environ Toxicol Chem* 21:2500–2506
- Van der Sluijs JP, Simon-Delso S, Goulson D, Maxim L, Bonmatin JM, Belzunces LP (2013) Neonicotinoids, bee disorders and the sustainability of pollinator services. *Curr Opin Environ Sustain* 5:293–305
- Van der Zee R, Pisa L, Andonov S, Brodschneider R, Charriere JD et al (2012) Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008–9 and 2009–10. *J Apic Res* 51:91–114. doi:10.3896/ibra.1.51.1.12
- Van Dijk TC, van Staalduinen MA, van der Sluijs JP (2013) Macro-invertebrate decline in surface water polluted with imidacloprid. *PLoS One* 8:e62374. doi:10.1371/journal.pone.0062374
- Van Engelsdorp D, Meixner MD (2010) A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J Invertebr Pathol* 103:80–95
- Vandame R, Meled M, Colin ME, Belzunces LP (1995) Alteration of the homing-flight in the honey bee *Apis mellifera* L. exposed to sublethal dose of deltamethrin. *Environ Toxicol Chem* 14:855–860
- Vidau C, Diogon M, Aufauvre J, Fontbonne R, Vigues B, Brunet JL, Texier C, Biron DG, Blot N, El Alaoui H, Belzunces LP, Delbac F (2011) Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. *PLoS One* 6:e21550. doi:10.1371/journal.pone.0021550
- Vijver MG, van den Brink PJ (2014) Macro-invertebrate decline in surface water polluted with imidacloprid: a rebuttal and some new analyses. *PLoS One* 9:e89837
- Villanueva-Jimenez JA, Hoy MA (1998) Toxicity of pesticides to the citrus leafminer and its parasitoid *Ageniaspis citricola* evaluated to assess their suitability for an IPM program in citrus nurseries. *Biocontrol* 43:357–388
- Volkov EM, Nurullin LF, Nikolsky E, Vyskocil F (2007) Miniature excitatory synaptic ion currents in the earthworm *Lumbricus terrestris* body wall muscles. *Physiol Res* 56:655–658
- Voudouris CC, Sauphanor B, Franck P, Reyes M, Mamuris Z et al (2011) Insecticide resistance status of the codling moth *Cydia pomonella* (Lepidoptera: Tortricidae) from Greece. *Pestic Biochem Physiol* 100:229–238
- Wang M, Grimm V (2010) Population models in pesticide risk assessment: lessons from assessing population-level effects, recovery, and alternative exposure scenarios from modeling a small mammal. *Environ Toxicol Chem* 29:1292–1300
- Wang AH, Wu JC, Yu YS, Liu JL, Yue JF et al (2005) Selective insecticide-induced stimulation on fecundity and biochemical changes in *Tryporyza incertulas* (Lepidoptera: Pyralidae). *J Econ Entomol* 98:1144–1149
- Wang Y, Cang T, Zhao X, Yu R, Chen L, Wu C, Wang Q (2012a) Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*. *Ecotoxicol Environ Saf* 79:122–128
- Wang Y, Wu S, Chen L, Wu C, Yu R, Wang Q, Zhao X (2012b) Toxicity assessment of 45 pesticides to the epigeic earthworm *Eisenia fetida*. *Chemosphere* 88:484–491
- Ward GS (1990) NTN 33893 technical: acute toxicity to the mysid, *Mysidopsis bahia*, under flow-through test conditions. Toxicon Environmental Sciences, Jupiter, Florida (performing laboratory).

- Mobay Corporation, Kansas City, Missouri (submitting laboratory). Mobay Report No. 100355. 46 pp
- Ward GS 1991. NTN 33893 technical: chronic toxicity to the mysid *Mysidopsis bahia* under flow-through conditions. Toxicon Environmental Sciences, Jupiter, FL. 87 pp. Miles Report No. 101347
- Weibull AC, Bengtsson J, Nohlgren E (2000) Diversity of butterflies in the agricultural landscape: the role of farming system and landscape heterogeneity. *Ecography* 23:743–750
- Whitehorn PR, O'Connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336:351–352
- Wise JC, Jenkins PE, Vander Poppen R, Isaacs R (2010) Activity of broad-spectrum and reduced-risk insecticides on various life stages of cranberry fruitworm (Lepidoptera: Pyralidae) in highbush blueberry. *J Econ Entomol* 103:1720–1728
- Wu JY, Anelli CM, Sheppard WS (2011) Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. *PLoS One* 6:e14720
- Wu JY, Smart MD, Anelli CM, Sheppard WS (2012) Honey bees (*Apis mellifera*) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to *Nosema* (Microsporidia) infection. *J Invertebr Pathol* 109:326–329
- Yamada T, Yamada K, Wada N (2012) Influence of dinotefuran and clothianidin on a bee colony. *Japan J Clin Ecol* 21:10–23
- Yang EC, Chuang YC, Chen YL, Chang LH (2008) Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J Econ Entomol* 101:1743–1748
- Yang EC, Chang HC, Wu WY, Chen YW (2012) Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval stage. *PLoS One* 7:e49472
- Youn YN, Seo MJ, Shin JG, Jang C, Yu YM (2003) Toxicity of greenhouse pesticides to multicolored Asian lady beetles, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biol Control* 28:164–170
- Yu YS, Xue S, Wu JC, Wang F, Liu JL et al (2007a) Distribution of imidacloprid residues in different parts of rice plants and its effect on larvae and adult females of *Chilo suppressalis* (Lepidoptera: Pyralidae). *J Econ Entomol* 100:375–380
- Yu YS, Xue S, Wu JC, Wang F, Yang GQ (2007b) Changes in levels of juvenile hormone and molting hormone in larvae and adult females of *Chilo suppressalis* (Lepidoptera: Pyralidae) after imidacloprid applications to rice. *J Econ Entomol* 100:1188–1193
- Yue B, Wilde GE, Arthur F (2003) Evaluation of thiamethoxam and imidacloprid as seed treatments to control European corn borer and Indianmeal moth (Lepidoptera: Pyralidae) larvae. *J Econ Entomol* 96:503–509
- Zang Y, Zhong Y, Luo Y, Kong ZM (2000) Genotoxicity of two novel pesticides for the earthworm, *Eisenia fetida*. *Environ Pollut* 108: 271–278
- Zeng CX, Wang JJ (2010) Influence of exposure to imidacloprid on survivorship, reproduction and vitellin content of the carmine spider mite, *Tetranychus cinnabarinus*. *J Insect Sci* 10:20
- Zhou LJ, Huang JG, Xu HH (2011) Monitoring resistance of field populations of diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) to five insecticides in South China: a ten-year case study. *Crop Prot* 30:272–278

A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife

David Gibbons · Christy Morrissey · Pierre Mineau

Received: 7 April 2014 / Accepted: 6 June 2014 / Published online: 18 June 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract Concerns over the role of pesticides affecting vertebrate wildlife populations have recently focussed on systemic products which exert broad-spectrum toxicity. Given that the neonicotinoids have become the fastest-growing class of insecticides globally, we review here 150 studies of their direct (toxic) and indirect (e.g. food chain) effects on vertebrate wildlife—mammals, birds, fish, amphibians and reptiles. We focus on two neonicotinoids, imidacloprid and clothianidin, and a third insecticide, fipronil, which also acts in the same systemic manner. Imidacloprid and fipronil were found to be toxic to many birds and most fish, respectively. All three insecticides exert sub-lethal effects, ranging from genotoxic and cytotoxic effects, and impaired immune function, to reduced growth and reproductive success, often at concentrations well below those associated with mortality. Use of imidacloprid and clothianidin as seed treatments on some crops poses risks to small birds, and ingestion of even a few treated seeds could cause mortality or reproductive impairment to sensitive bird species. In contrast, environmental concentrations of imidacloprid and clothianidin appear to be

at levels below those which will cause mortality to freshwater vertebrates, although sub-lethal effects may occur. Some recorded environmental concentrations of fipronil, however, may be sufficiently high to harm fish. Indirect effects are rarely considered in risk assessment processes and there is a paucity of data, despite the potential to exert population-level effects. Our research revealed two field case studies of indirect effects. In one, reductions in invertebrate prey from both imidacloprid and fipronil uses led to impaired growth in a fish species, and in another, reductions in populations in two lizard species were linked to effects of fipronil on termite prey. Evidence presented here suggests that the systemic insecticides, neonicotinoids and fipronil, are capable of exerting direct and indirect effects on terrestrial and aquatic vertebrate wildlife, thus warranting further review of their environmental safety.

Keywords Pesticide · Neonicotinoid · Imidacloprid · Clothianidin · Fipronil · Vertebrate · Wildlife · Mammals · Birds · Fish · Amphibians · Reptiles · Risk assessment

Responsible editor: Philippe Garrigues

D. Gibbons (✉)
RSPB Centre for Conservation Science, RSPB, The Lodge, Sandy,
Bedfordshire SG19 2DL, UK
e-mail: david.gibbons@rspb.org.uk

C. Morrissey
Department of Biology, University of Saskatchewan, 112 Science
Place, Saskatoon, Saskatchewan S7N 5E2, Canada

C. Morrissey
School of Environment and Sustainability, University of
Saskatchewan, 117 Science Place, Saskatoon, Saskatchewan S7N
5E2, Canada

P. Mineau
Pierre Mineau Consulting, 124 Creekside Drive, Salt Spring
Island V8K 2E4, Canada

Overview of impacts of pesticides on vertebrate wildlife

Although vertebrates are the intended target of only 2 % of pesticides on the market, the unintentional impacts of pesticides on vertebrate populations have been marked and are well documented (e.g. Sánchez-Bayo 2011). Pesticides can exert their impact on vertebrates either directly, through their toxicity, or indirectly, for example, by reducing their food supply.

Direct effects may be the result of several different exposure pathways: through ingestion of the formulated product (e.g. birds eating seeds coated with insecticide; Avery et al. 1997; Prosser and Hart 2005), through uptake via the skin following a spray event (Mineau 2011) or by eating

contaminated prey. Probably the most notable example among the latter exposure pathway was the dramatic impact that organochlorine pesticides, especially DDT and its metabolite DDE, had on populations of birds of prey (Ratcliffe 1967; Newton 1995). Depending on the extent of intoxication, direct effects of pesticides can either kill vertebrates outright or exert sub-lethal effects, for example, on growth and reproduction (Sánchez-Bayo 2011). Progress since the organo-chlorine era has helped ensure that compounds that are currently being developed and registered are generally less persistent and do not as readily bio-accumulate in food webs.

More recently, however, interest has turned to investigating the potential for *indirect effects* which are typically mediated through loss in quantity or quality of prey associated with pesticide use, or through habitat modification (Sotherton and Holland 2002; Boatman et al. 2004; Morris et al. 2005). This is especially the case in jurisdictions where the use of highly toxic pesticides has been controlled and the frequency of direct impacts reduced (Mineau et al. 1999).

Over the last 2 decades, a new class of insecticides, the neonicotinoids, has become the most important and fastest growing of the five major chemical classes of insecticides on the global market (Jeschke and Nauen 2008; Jeschke et al. 2011; Tomizawa and Casida 2011; Casida and Durkin 2013). When used as plant protection products, neonicotinoids act by becoming distributed systemically throughout the growing plant following seed or soil applications. Another recent insecticide, fipronil, a phenyl-pyrazole (fiprole) rather than a neonicotinoid, also acts in the same manner and has a similar toxicity and persistence profile (Grant et al. 1998). Consequently, the neonicotinoids and fipronil are sometimes jointly termed ‘systemic insecticides’, although there are also older products which could be termed ‘systemic’, for example, the organo-phosphorous insecticide acephate and the organo-arsenical, monosodium methanearsonate. Neonicotinoids are, in particular, commonly applied as seed treatments. The use of seed treatments as a convenient and effective application method has widespread appeal in the farming industry. Consequently, systemic seed treatments are now used on the majority of agricultural crops worldwide (Garthwaite et al. 2003; Jeschke et al. 2011).

Here, we build on the reviews of others (e.g. Goulson 2013; Köhler and Triebkorn 2013; Mineau and Palmer 2013) to examine the evidence and potential for direct and indirect effects of two common systemic neonicotinoid insecticides, imidacloprid and clothianidin, along with fipronil on vertebrate wildlife.

Mode of action of the systemic insecticides

Neonicotinoids work by interfering with neural transmission in the central nervous system. They bind to the nicotinic

acetylcholine receptors (*nAChR*) in the postsynaptic neuron, acting as ‘false neurotransmitters’ (agonists). This interference with acetylcholine neurotransmitter signalling causes continuous activation of the receptor, leading to symptoms of neurotoxicity. Neonicotinoids have greater affinity for, and thus bind more strongly to, insect than mammalian or other vertebrate receptors, so their toxicity to mammals is lower than it is to insects and the reversibility of intoxication higher (Tomizawa and Casida 2005; Jeschke et al. 2011). Fipronil works similarly, but instead binds to the gamma-aminobutyric acid (GABA) receptors, resulting in similar continuous central nervous system activity (Tingle et al. 2000, 2003). As with neonicotinoids, fipronil has a lower affinity to vertebrate than to invertebrate receptors (Grant et al. 1998). Despite the lower toxicity of these products to vertebrates than to invertebrates, there is still ample evidence that vertebrates show toxic effects, albeit at markedly higher concentrations than for many target and non-target invertebrate species (e.g. Tingle et al. 2000, 2003; Cox 2001; SERA 2005; DeCant and Barrett 2010; Mineau and Palmer 2013).

Materials and methods

To assess the likely impacts of neonicotinoids and fipronil on vertebrates, a literature search was undertaken using Web of Science and Google Scholar. Search terms were [product] and [taxon], where [product] was either neonicotinoid, imidacloprid, thiacloprid, clothianidin, thiamethoxam, acetamiprid, nitenpyram, dinotefuran or fipronil; and [taxon] was either vertebrate*, mammal*, bird*, reptile*, amphibian* and fish*. In addition, specific searches were made on a few common toxicity test species (e.g. rat) and by following up references cited in the publications found by the search. The review also draws heavily on the recently published report by Mineau and Palmer (2013) on the direct and indirect toxicity of neonicotinoids to birds. Several industry studies, which have not been formally published but which were part of product approval processes, were reviewed by Mineau and Palmer and have been included here. While industry studies have been reviewed by regulators and may receive as much critical review as in the open peer-reviewed literature, emphasis here is on published reports and the primary literature.

The following information was extracted from each study: the product used, its dose and whether or not it was presented as a single dose (acute) or over a period of time (chronic; e.g. over 30 days); the effects on individual organisms, specifically whether there was an impact on survival, reproduction, growth and development, or other sub-lethal effects, such as neurobehavioural, genotoxic, cytotoxic, and immunotoxic; the impact on populations of the animal (e.g. local populations); the type of study, separated into laboratory or field; and finally whether it was a study of direct toxic effects, or indirect

effects (e.g. leading to changes in food availability). In some cases, individual studies covered more than one species, and each is treated here as a separate species impact study.

The great majority of the studies were laboratory-based (139/152=91 %) and most (146, 96 %) were direct toxicity studies. While common in ecotoxicology, the lack of field testing and over-reliance on laboratory direct toxicity testing limit our ability to interpret the findings under field-realistic conditions. Field experiments have provided some of the most compelling evidence of the impact of neonicotinoids on populations in their natural environment (e.g. Whitehorn et al. 2012), and there is an increasing recognition that maintaining ecological complexity in field studies is desirable (Suryanarayanan 2013).

The most common study taxa were mammals (58), birds (47) and fish (32), with substantially fewer studies of amphibians (12) and reptiles (3). Within these individual taxa, the most commonly studied mammals were rat, *Rattus norvegicus*, (39) and mouse, *Mus musculus*, (9); the most commonly studied birds were northern bobwhite quail, *Colinus virginianus*, (8) and mallard, *Anas platyrhynchos*, (6), the two test species mandated by regulatory approval schemes in North America; and the most commonly tested fish were rainbow trout, *Oncorhynchus mykiss*, (6) and Nile tilapia, *Oreochromis niloticus*, (6).

Most of these studies investigated the effects of the two neonicotinoids, imidacloprid (72) and clothianidin (19), as well as fipronil (47); between them, these three insecticides accounted for 91 % of all studies. Given the paucity of information collated for the other neonicotinoids, this review concentrates on these three products alone.

The direct effects of neonicotinoids and fipronil on vertebrate wildlife

Toxicity to vertebrates

Standard toxicity testing for pesticides on terrestrial vertebrates is through an acute (<96 h) study. Test organisms are given the product by gavage (i.e. through a feeding tube) or through the diet in varying concentrations, and the estimated dose of pesticide associated with death of half of the test subjects is recorded and expressed as a proportion of bodyweight (i.e. the 50 % lethal dose, LD₅₀, expressed as milligrams of pesticide per kilogram of bodyweight). Toxicity for aquatic organisms is typically measured as the LC₅₀ or the concentration in water (e.g. mg/L) which is toxic to the test organisms. Numerous LD₅₀ and LC₅₀ tests have been undertaken for vertebrates, and those that were located as part of this review are shown for imidacloprid, clothianidin and fipronil in Table 1. As can be seen, the relative toxicity of these products varies, both among products and among species.

The US Environmental Protection Agency has developed an ecotoxicity classification based on LD₅₀ and LC₅₀ assessments (US EPA 2012). They classify the acute toxicity of a given product on a particular species as either practically non-toxic, slightly toxic, moderately toxic, highly toxic, or very highly toxic based on lethality dose ranges. Sub-lethal or reproductive effects are not included in this classification. By US EPA's definitions, and within the highly restricted range of species assessed, imidacloprid shows moderate to high toxicity to birds, particularly for smaller-bodied species such as house sparrows, *Passer domesticus*, and canaries, *Serinus canaria*, and approaches very high toxicity to grey partridge, *Perdix perdix*. It is moderately toxic to rats and mice, but practically non-toxic to fish (with the exception of rainbow trout, especially their fry) and amphibians. Clothianidin's toxicity ranges from moderate to practically non-toxic for both birds and mammals, whereas for the fish studied, it varies from slightly toxic to practically non-toxic. By contrast, for all fish species studied, fipronil is either highly or very highly toxic (e.g. bluegill sunfish, *Lepomis macrochirus*). Fipronil is in addition highly toxic to the three game birds studied (red-legged partridge, *Alectoris rufa*, ring-necked pheasant, *Phasianus colchicus*, and northern bobwhite quail), and moderately toxic to mice and rats.

One of the serious failings of current risk assessments is the underestimation of interspecies variation in insecticide susceptibility that is apparent from Table 1. Too few species are typically tested to derive the true variation in response from the vast array of exposed species in the wild. Mineau and Palmer (2013) discuss this at length for neonicotinoids and propose improved thresholds derived from species sensitivity distributions and estimated 'hazard doses' (HD₅—the LD₅₀ value for a species at the 5 % tail of the sensitivity distribution).

Impacts on growth, development and reproduction of vertebrates

While not necessarily causing mortality among adults, intoxication by imidacloprid, clothianidin and fipronil can reduce the growth, development and reproduction of individual vertebrates (Table 2). Reproductive effects are manifest in a variety of ways among mammals, but especially as reduced sperm production, adverse effects on the fertilization process, reduced rates of pregnancy, higher rates of embryo death, stillbirth and premature birth, and reduced weights of offspring. Among birds, testicular anomalies and reduced fertilization success, reduced eggshell thickness and embryo size, reduced hatching success and chick survival, and chick developmental abnormalities have all been reported. Weight loss, or impaired weight gain, sometimes associated with reduction or cessation of feeding, occurred within all taxa studied.

Table 1 Single (acute) dose LD₅₀ (for mammals birds and reptiles, mg/kg) and LC₅₀ (for fish and amphibia, mg/L) for imidacloprid, clothianidin and fipronil

Taxon	Species	Imidacloprid	Clothianidin	Fipronil
Mammal	Rat, <i>Rattus norvegicus</i>	425–475 (MT) ^a	5,000 (PNT) ⁱ	97 (MT) ^l
	Mouse, <i>Mus musculus</i>	131–300 (MT) ^a	>389 (MT) ⁱ	95 (MT) ^m
Bird	Mallard, <i>Anas platyrhynchos</i>	283 (MT) ^b	>752 (ST) ^j	2,150 (PNT) ^l
	Ring-necked pheasant, <i>Phasianus colchicus</i>			31 (HT) ^l
	Grey partridge, <i>Perdix perdix</i>	13.9 (HT) ^c		
	Red-legged partridge, <i>Alectoris rufa</i>			34 (HT) ^l
	Northern bobwhite quail, <i>Colinus virginianus</i>	152 (MT) ^a	>2,000 (PNT) ^k	11.3 (HT) ^l
	Japanese quail, <i>Coturnix japonica</i>	31 (HT) ^a	423 (MT) ^k	
	Feral pigeon, <i>Columba livia</i>	25–50 (HT) ^a		>2,000 (PNT) ^l
	House sparrow, <i>Passer domesticus</i>	41 (HT) ^a		
	Field sparrow, <i>Spizella pusilla</i>			1,120 (ST) ^l
	Canary, <i>Serinus canaria</i>	25–50 (HT) ^a		
	Zebra finch, <i>Taeniopygia guttata</i>			310 (MT) ⁿ
Fish	Bluegill sunfish, <i>Lepomis macrochirus</i>	105 (PNT) ^a	>117 (PNT) ⁱ	0.083 (VHT) ^l
	Japanese carp, <i>Cyprinus carpio</i>			0.34 (HT) ^l
	Nile tilapia, <i>Oreochromis niloticus</i>			0.042–0.147 (VHT–HT) ^l
	Rainbow trout, <i>Oncorhynchus mykiss</i>	>83–211 (ST–PNT) ^a	>105 (PNT) ⁱ	0.246 (HT) ^l
	Rainbow trout (fry)	1.2 (MT) ^d		
	Sheepshead minnow, <i>Cyprinodon variegatus</i>	161 (PNT) ^a	>93.6 (ST) ⁱ	0.13 (HT) ^l
	Zebrafish, <i>Danio rerio</i>	241 (PNT) ^e		
	Black-spotted pond frog, <i>Rana nigromaculata</i>	129–219 (PNT) ^{a,f}		
Amphibia	Indian rice frog, <i>Rana limnocharis</i>	82–366 (ST–PNT) ^{a,f,g}		
	Western chorus frog, <i>Pseudacris triseriata</i>	194 (PNT) ^h		
	American toad, <i>Bufo americanus</i>	234 (PNT) ^h		
Reptile	Fringe-toed lizard, <i>Acanthodactylus dumerili</i>			30 (HT) ^o

Toxicity classification follows US EPA (2012): PNT practically non-toxic, ST slightly toxic, MT moderately toxic, HT highly toxic, VHT very highly toxic. For birds, mammals and reptiles: PNT >2,000, ST 501–2,000, MT 51–500, HT 10–50, VHT <10. For aquatic organisms, fish and amphibia: PNT >100, ST >10–100, MT >1–10, HT 0.1–1, VHT <0.1. Note that kg in the LD₅₀ units refers to body weight of the dosed animal. Source references denoted by superscripts are as follows: ^aSERA 2005, ^bFossen 2006, ^cGrolleau 1991 in Anon 2012, ^dCox 2001, ^eTisler et al. 2009, ^fFeng et al. 2004, ^gNian 2009, ^hHoward et al. 2003, ⁱDeCant and Barrett 2010, ^jEuropean Commission 2005, ^kMineau and Palmer 2013, ^lTingle et al. 2003, ^mConnelly 2011, ⁿKitulagodage et al. 2008 (NB : a formulation of fipronil containing the dispersant solvent diacetone alcohol was sevenfold more toxic than technical grade fipronil itself), ^oPeveling and Demba 2003 (NB: 42 %, rather than 50 %, mortality)

Most of the studies found were required for pesticide registration purposes. In birds, a reproductive test is frequently conducted on standard test species such as the northern bobwhite quail or the mallard. This is a truncated test, which consists of feeding a constant concentration of the pesticide to the study animals and then collecting the eggs and incubating them artificially. There is therefore no inclusion of endpoints to assess the ability of the dosed birds to incubate, hatch or raise their young. The test is a hybrid between single life stage chronic toxicity and a test of true reproductive effects, and has been the subject of analysis and criticism (Mineau et al. 1994, 1996; Mineau 2005). Because of the longer duration of the test, and the occasional pair that fails to bond, spurious variance is introduced, thus decreasing the power to detect reproductive deficits in limited sample sizes. On the other hand, because the birds are offered contaminated diet only, with no other food choice, the test may overestimate

realistic exposure in the wild. However, it remains the only test available with which to model non-acute risk in avian wildlife.

Other sub-lethal impacts on vertebrates

A range of other effects of these insecticides have been documented for vertebrates (Table 2), outside of those reported on survival, growth and development, and reproduction. Among mammals—principally rats and mice—these include genotoxic and cytotoxic effects, neuro-behavioural disorders of offspring (including those dosed in utero), lesions of the thyroid, retinal atrophy, reduced movement, and increased measures of anxiety and fear. House sparrows can become uncoordinated and unable to fly, and studies of Japanese quail and red-legged partridges have reported DNA breakages and a reduced immune response, respectively. Similarly, studies of

Table 2 Other studies of the direct effects of imidacloprid, clothianidin and fipronil on vertebrates

Taxon and species	Effect on:	Imidacloprid	Clothianidin	Fipronil	Source and detailed effect
Mammal					
Rat, <i>Rattus norvegicus</i>	Reproduction	2, 19, 90 mg/kg/day ^{a,b,c}	24, 31.2–36.8 mg/kg/day ^{d,e}	280 mg/kg ^f 26–28 mg/kg/day ^g	^a Bal et al. 2012; reduced sperm production ^b Cox 2001; reduced weight offspring ^c Gawade et al. 2013; abortions, soft tissue abnormalities and skeletal alterations ^d Bal et al. 2013; no effect on sperm concentration, mobility or morphology, but reduced weight of epididymis and seminal vesicles ^e DeCant and Barrett 2010; stillbirths and delayed sexual maturation ^f Ohi et al. 2004; reduced levels of pregnancy ^g Tingle et al. 2003; range of effects including reduced fertility and decreased litter size
Rat, <i>Rattus norvegicus</i>	Growth and development	10, 17, 25, 100 mg/kg/day ^{a,b,c,d}	31.2 mg/kg/day ^e 32 mg/kg ^f	20 mg/kg/day ^g	^a Cox 2001; reduced weight gain ^b Cox 2001; thyroid lesions ^c Bhardwaj et al. 2010; reduced weight and locomotor ability ^d Cox 2001; atrophy of retina ^e DeCant and Barrett 2010; reduced weight gain of offspring ^f Bal et al. 2012; reduced body weight and impact on reproductive organs ^g Tingle et al. 2003; reduced food consumption and reduced weight gain
Rat, <i>Rattus norvegicus</i>	Genotoxic	300 mg/kg ^a	24 mg/kg/day ^b (NE)		^a Demia et al. 2007; significant effect on in vitro micronucleus induction in rat erythrocytes ^b Bal et al. 2013; no effect on sperm DNA fragmentation
Rat, <i>Rattus norvegicus</i>	Cytotoxic	<400 mg/kg ^a 0.21, 1, 20, 45 mg/kg/day ^{b,c,d,e}			^a Nellore et al. 2010; blocks to the cholinergic enzyme system ^b Mohany et al. 2011; oxidative stress and hepatotoxicity, i.e. heavily congested central vein and blood sinusoids in liver ^c Duzguner and Erdogan 2012; oxidative stress and inflammation caused by altering antioxidant systems ^d Kapoor et al. 2010; oxidative stress ^e Toor et al. 2013; hepatotoxicity—dilations of central vein and sinusoids between hepatocytes in liver
Rat, <i>Rattus norvegicus</i>	Neurobehavioural	337 mg/kg ^a	>2mM ^b 18–66 mg/kg/day ^c	<30, 140–280 ^d mg/kg ^{d,e}	^a Abou-Donia 2008; offspring dosed in utero, led to neurobehavioural deficits

Table 2 (continued)

Taxon and species	Effect on:	Imidacloprid	Clothianidin	Fipronil	Source and detailed effect
Rat, <i>Rattus norvegicus</i>	Immunotoxic	0.21, 90 mg/kg/day ^{a,b}			^b de Oliveira et al. 2010; increased release of dopamine ^c Tanaka 2012; adverse neurobehavioural impacts on pups ^d Martins 2009; reduced movement ^e Tercariol and Godinho 2011; increased emotion and fear
Mouse, <i>Mus musculus</i>	Reproduction	5 mM ^a	18–66 mg/kg/day (NE) ^b		^a Mohany et al. 2011; significant effect on leukocyte count, immunoglobulins and phagocytic activity ^b Gawade et al. 2013; compromised immunity ^a Gu et al. 2013; no impact on sperm mobility, but fertilisation process and zygotes adversely affected ^b Tanaka 2012; no effect on litter size or weight Tanaka 2012; no effect on litter size or weight
Mouse, <i>Mus musculus</i>	Growth and development		18–66 mg/kg/day (NE)		Gu et al. 2013; no effect on DNA integrity
Mouse, <i>Mus musculus</i>	Genotoxic	5 mM (NE)			Badgajar et al. 2013; suppressed cell-mediated immune response and prominent histopathological alterations in spleen and liver
Mouse, <i>Mus musculus</i>	Immunotoxic	10 mg/kg/day			^a Cox 2001; increased frequency of miscarriage ^b DeCant and Barrett 2010; increase in premature births
Rabbit, <i>Sylvilagus sp.</i>	Reproduction	72 mg/kg/day ^a	>25 mg/kg/day ^b		
Sheep, <i>Ovis aries</i>	Growth and development			0.5 mg/kg/day (NE)	Leghait et al. 2010; no thyroid disruption
Cow, <i>Bos primigenius</i>	Cytotoxic	1 mg/kg/day (NE)			Kaur et al. 2006; some modest impacts on plasma biochemistry, but mostly no impact on range of other blood measures
Bird					
Mallard, <i>Anas platyrhynchos</i>	Reproduction	16 mg/kg/day	>35 mg/kg/day (NE)		Adapted from figures in Mineau and Palmer (2013)*; various effects on reproduction
Chicken, <i>Gallus gallus domesticus</i>	Growth and development			37.5 mg/kg	Kitulagodage et al. 2011b; reduced feeding and body mass, and developmental abnormalities of chicks
Chicken, <i>Gallus gallus domesticus</i>	Neurobehavioural			37.5 mg/kg	Kitulagodage et al. 2011b; behavioural abnormalities of chicks
Red-legged partridge, <i>Alectoris rufa</i>	Survival	31.9–53.4 mg/kg/day			Lopez-Antia et al. 2013; reduced chick survival at low dose, and reduced adult survival at high dose
Red-legged partridge, <i>Alectoris rufa</i>	Reproduction	31.9 mg/kg/day			Lopez-Antia et al. 2013; reduced fertilisation rate and chick survival

Table 2 (continued)

Taxon and species	Effect on:	Imidacloprid	Clothianidin	Fipronil	Source and detailed effect
Red-legged partridge, <i>Alectoris rufa</i>	Immunotoxic	53.4 mg/kg/day	>52 mg/kg/day		Lopez-Antia et al. 2013; reduced immune response
Northern bobwhite quail, <i>Colinus virginianus</i>	Reproduction				Adapted from figures in Mineau and Palmer (2013)*; various effects on reproduction
Northern bobwhite quail, <i>Colinus virginianus</i>	Growth and development	24 mg/kg/day ^a		11 mg/kg ^b	^a Adapted from figures in Mineau and Palmer (2013)*; various effects on weight ^b Kitulagodage et al. 2011a; birds stopped feeding so lost weight
Japanese quail, <i>Coturnix japonica</i>	Reproduction	1 mg/kg/day			Tokumoto et al. 2013; testicular anomalies; reductions in embryo length when those males mated with un-dosed females
Japanese quail, <i>Coturnix japonica</i>	Genotoxic	1 mg/kg/day			Tokumoto et al. 2013; increased breakage of DNA in males
House sparrow, <i>Passer domesticus</i>	Neurobehavioural	6 mg/kg			Cox 2001; in-coordination, inability to fly
Zebra finch, <i>Taeniopygia guttata</i>	Reproduction			>1 mg/kg	Kitulagodage et al. 2011b; reduced hatching success
Fish					
Japanese carp, <i>Cyprinus carpio</i>	Growth & development			REC (NE)	Clasen et al. 2012; no impact on growth or survival, though biochemical changes
Zebrafish, <i>Danio rerio</i>	Reproduction	320 mg/L (NE)			Tisler et al. 2009; no effect on embryos observed
Zebrafish, <i>Danio rerio</i>	Growth and development			0.33 mg/L	Stehr et al. 2006; notochord degeneration
Zebrafish, <i>Danio rerio</i>	Neurobehavioural			0.33 mg/L	Stehr et al. 2006; locomotor defects in embryos and larvae
Fathead minnow, <i>Pimephales promelas</i>	Growth and development		20 mg/L		DeCant and Barrett 2010; reduced weight and length
Fathead minnow, <i>Pimephales promelas</i>	Genotoxic			0.03 mg/L	Beggel et al. 2012; changes in gene transcription
Fathead minnow, <i>Pimephales promelas</i>	Neurobehavioural			0.14 mg/L	Beggel et al. 2010; impaired swimming; formulation more toxic than technical grade
Nile tilapia, <i>Oreochromis niloticus</i>	Growth and development	0.134, <1.34 mg/L ^{a,b}			^a Lauan and Ocampo 2013; extensive disintegration of testicular tissue. ^b Ocampo and Sagun 2007; changes to gonads
Medaka, <i>Oryzias latipes</i>	Immunotoxic	0.03–0.24 mg/L (1.5*REC)			Sanchez-Bayo and Goka 2005; juveniles stressed, led to ectoparasite infestation, when concentrations high early in the experiment
Silver catfish, <i>Rhamdia quelen</i>	Genotoxic			0.0002 mg/L (NE)	Ghisi et al. 2011; no genotoxic effects
Silver catfish, <i>Rhamdia quelen</i>	Cytotoxic			0.0002 mg/L	Ghisi et al. 2011; erythrocyte damage

Table 2 (continued)

Taxon and species	Effect on:	Imidacloprid	Clothianidin	Fipronil	Source and detailed effect
Amphibia Black-spotted pond frog, <i>Rana nigromaculata</i>	Genotoxic	0.05 mg/L			Feng et al. 2004; DNA damage at very low concentrations

Acute toxicity studies are given in Table 1 and not repeated here. Dosage could either be acute or chronic, the latter shown as /day (per day). All studies demonstrated deleterious effects at the given dosage, except those marked NE (no effect). Studies marked REC were field-based, with insecticides applied at the manufacturer's recommended rate; all others are of direct toxicity under laboratory conditions. 'dermal' = dermal application. Only studies for which dosage information was readily available are listed. * Lowest feed concentrations causing an effect were transformed to a daily dose assuming an average consumption of 21- and 67-g laboratory feed per day for bobwhite quail and mallard, respectively, and average body weights of 210 and 100 g, respectively

fish have reported changes in gene transcription, erythrocyte damage, disintegration of gonadal tissue, impaired swimming, notochord degeneration and locomotor defects in embryos and larvae. In one case, medaka fish, *Oryzias latipes*, in experimental rice fields became physiologically stressed (characterized by increased anaerobic metabolism leading to hyperglycemia) following exposure to imidacloprid at 1.5 times the commercially recommended rate of application, and subsequently became susceptible to infestation by the protozoan ectoparasite, *Cychochaeta (Trichodina) domerguei* (Sánchez-Bayo and Goka 2005). While the majority of studies documented deleterious impacts from neonicotinoid or fipronil exposure, effective doses have not typically been matched to realistic field exposure conditions.

Many of these, perhaps, more subtle sub-lethal effects (Table 2) occur at much lower concentrations than lethal effects (Table 1). Thus, while single oral doses of 425–475 and 5,000 mg/kg of imidacloprid and clothianidin, respectively, will kill rats, lower daily doses of 0.21–100 and 18–66 mg/kg/day have consistently caused a range of sub-lethal effects. For example, a daily dose of 10–19 or 31 mg/kg/day of imidacloprid and clothianidin, respectively, will cause reduced growth of young rats and, in the case of clothianidin, a greater frequency of stillbirths. Even doses as low as 0.21 and 2.0 mg/kg/day of imidacloprid have been shown to have immunotoxic effects and reduce sperm production, respectively. Similarly, while a single oral dose of 41 mg/kg of imidacloprid will cause mortality in house sparrows, a substantially lower dose (6 mg/kg) can induce uncoordinated behaviour and an inability to fly. While imidacloprid is highly toxic to Japanese quail, with an LD₅₀ of 31 mg/kg, chronic daily doses of only 1 mg/kg/day can lead to testicular anomalies, DNA damage in males, and reductions in embryo size when those males are mated with control females. The black-spotted pond frog has an LC₅₀ of 129–219 mg/L of imidacloprid, but DNA damage occurs at a much lower concentration, 0.05 mg/L. Given the high toxicity of fipronil to fish, it is perhaps not surprising that the lowest recorded concentration of that insecticide to affect a vertebrate was of 0.0002 mg/L (0.2 µg/L); the effect being erythrocyte damage in silver catfish, *Rhamdia quelen*. While it is difficult to extrapolate such sub-organism effects to fitness-related measures in individuals and population-level responses, they offer insight into potential mechanisms underpinning direct toxicity.

Different families of pesticides rarely elicit sub-lethal effects at doses below 1/10 of the lethal dose (Callahan and Mineau 2008). But, in the case of imidacloprid, signs of severe debilitation (e.g. ataxia) were observed a full order of magnitude below lethal doses. Review of available laboratory data here suggests that some effects can be detected at even lower doses (1/1,000). This apparent feature of these insecticides is of toxicological concern with respect to vertebrates, increasing

the probability that wild species can be affected under field-realistic exposure conditions.

Are vertebrates at risk in their natural environment?

Risks to aquatic vertebrates

Various measured or estimated environmental concentrations of imidacloprid, clothianidin and fipronil in the aquatic environment are available. For imidacloprid, these include 0–0.22 µg/L (Lamers et al. 2011); mean and maximum values of 0.016 and 0.27 µg/L, respectively (Main et al. 2014); 0.13–0.14 µg/L (Stoughton et al. 2008); 0–3.3 µg/L (Starnes and Goh 2012); 1–14 µg/L (Jemec et al. 2007); <15 µg/L (Kreuger et al. 2010); 17–36 µg/L (Fossen 2006); and up to 49 µg/L (Hayasaka et al. 2012). Higher concentrations of imidacloprid have been more rarely recorded in the aquatic environment. In one study in the Netherlands, while 98 % of 1,465 measurements ranged from 0 to 8.1 µg/L, the remaining 2 % were up to 320 µg/L (Van Dijk et al. 2013). Similarly, in a study in experimental rice fields, the concentration of imidacloprid immediately after application was 240 µg/L, but fell to 5 µg/L within a week (Sánchez-Bayo and Goka 2005). For clothianidin, DeCant and Barrett (2010) estimated concentrations of 0.5–3.0 µg/L for standing water surrounding two crops, while Main et al. (2014) measured mean and maximum concentrations of 0.14 and 3.1 µg/L, respectively, in water bodies beside canola fields. Measurements for fipronil in the aquatic environment have been reported at 0.17 µg/L (Stark and Vargas 2005); a median of 0.23 and range of 0.004–6.4 µg/L (Mize et al. 2008); 1 µg/L (Hayasaka et al. 2012); and 0.15–5 µg/L (Wirth et al. 2004).

Imidacloprid LC₅₀ measurements for fish and amphibia (Table 1) range from 1,200 to 366,000 µg/L, and for clothianidin, from 94,000 to 117,000 µg/L (fish only). Thus, except in the most extreme cases, environmental concentrations are from approximately 2 to 7 orders of magnitude lower than LC₅₀ measurements for fish and amphibians, so it is unlikely that the mortality rates of these taxa will be directly affected by these two insecticides under normal exposure. However, the possibility of sub-lethal effects, e.g. physiological stress and damage to DNA, cannot be ruled out (Table 2). For fipronil, there is a greater apparent risk to fish survival, as some of the highest environmental concentrations are within an order of magnitude of their LC₅₀ values (Table 1), especially for bluegill sunfish and Nile tilapia. Sub-organism effects may also be apparent, for example, erythrocyte damage and alterations to gene transcription (Table 2).

Risks to terrestrial vertebrates

Determining the exposure risks to terrestrial vertebrates is more complex than to aquatic species given that there are

several routes of exposure, e.g. from ingestion of treated seed; from residues in or on the crop and soil; from drinking water, nearby vegetation or invertebrates; from dermal exposure due to direct overspray or contact with treated surfaces; from inhalation; and even from preening. Concentrations to which terrestrial taxa can be exposed vary markedly within and between these different pathways, based on habitat requirements and movement between contaminated and uncontaminated patches.

Treated seeds contain some of the highest concentrations of neonicotinoids, with a typical individual canola (oilseed rape), beet or corn seed calculated to contain 0.17, 0.9 or 1 mg of active ingredient, respectively (Goulson 2013). Application rates vary widely by crop but, for example, canola seeds treated with clothianidin have recommended application rates of 4.0 g a.i./kg of canola seed, while corn is almost double, at 7.5 g a.i./kg seed. Given these high concentrations, and that many granivorous species eat crop seeds, the most likely route of exposure to terrestrial animals is probably through the consumption of treated seeds.

Residues in crops and surrounding soil may be lower but still pose a risk to wildlife consumers that feed on the treated plants or ingest soil. For example, Bonmatin et al. (2005) found residues of 2.1–6.6 µg/kg of imidacloprid in seed-treated maize plants. Substantially higher concentrations of 1.0–12.4 mg/kg of imidacloprid have been detected in seed-treated sugar beet leaves (Rouchaud et al. 1994). Ground-dwelling species may also be exposed via the soil. Anon (cited in Goulson 2013) found concentrations of 18–60 µg/kg of imidacloprid in soil following several years of repeated applications as a seed treatment on winter wheat. Donnarumma et al. (2011) measured concentrations of 652 µg/kg of imidacloprid in soil 30 days after sowing of dressed maize seeds, falling to 11 µg/kg at harvest. Following soil drenching (i.e. applying a diluted insecticide directly to the base of a plant), Cowles et al. (2006) found concentrations of 120–220 µg/kg of imidacloprid in hemlock, *Tsuga Canadensis*, tissue. Cutler and Scott-Dupree (2007) found residues of 0.5–2.6 µg/kg of clothianidin in seed-treated canola plants, while Krupke et al. (2012) found residues of 1–9 µg/kg of clothianidin on natural vegetation surrounding seed-treated maize fields. Krupke et al. (2012) also detected concentrations of 6.3 µg/kg of clothianidin in soil in fields sown with seed-treated maize.

The US EPA modelled the estimated daily intake of clothianidin, assuming that mammals and birds only eat a diet of treated seeds (DeCant and Barrett 2010). This risk modelling approach showed that clothianidin, at least when used to treat oilseed rape and cotton seeds, could reduce the survival of small birds and mammals (DeCant and Barrett 2010).

Similar approaches have been developed for other routes of exposure beyond ingestion of seed treatments (e.g. SERA 2005; US EPA 2012). For example, risk modelling for

imidacloprid suggests hazards to birds and mammals consuming vegetation, grass and even insects. In particular, it predicts that foliar spraying may lead to substantial mortality of sensitive bird species (SERA 2005). In its 2008 re-assessment of imidacloprid, the US EPA (2008) reported an incident where grubs surfacing after a lawn treatment appear to have poisoned young robins, *Turdus migratorius*.

A more detailed assessment of the risk of intoxication of birds following the consumption of neonicotinoid-treated seed is given by Mineau and Palmer (2013). Their analysis suggests that the risks of acute intoxication with imidacloprid applied on maize, oilseeds or cereals are comparably high, such that birds need only to ingest a few treated seeds. The risk of acute intoxication with clothianidin in maize is highest, whereas for oilseeds or cereals, birds would need to ingest more, largely because application rates are lower. In principle, there are ways in which this risk could be mitigated, for example, by burying seeds below the soil surface, but this is rarely 100 % effective due to spillage (de Leeuw et al. 1995; Pascual et al. 1999). Whether or not birds avoid eating treated seeds (Avery et al. 1998), or the extent to which they may remove a substantial proportion of the toxicant by discarding outer seed husks (Avery et al. 1997) have been debated. However, incidents of bird poisoning with imidacloprid-treated seed have been documented (Berny et al. 1999), suggesting that the calculated risk may be real.

The potential risk to birds from eating neonicotinoid-treated seeds can be illustrated by the following example in which we calculate the relative risk for two granivorous species, a grey partridge, *Perdix perdix* (mass ~390 g) and a house sparrow (mass ~34 g) (<http://blx1.bto.org/birdfacts/results/bob3670.htm>), feeding on a field recently sown with imidacloprid-treated beet seed, each containing 0.9 mg of imidacloprid (Anon 2012). Imidacloprid is highly toxic to both species, with a LD₅₀ of 13.9 mg/kg of body weight for grey partridge and 41 mg/kg for house sparrow (Table 1). Consequently, ingestion of just 6 and 1.5 seeds would have a 50 % chance of killing an individual foraging partridge and sparrow, respectively. Less than a quarter of a seed could have a sub-lethal effect on a house sparrow, as 6 mg/kg is sufficient to reduce flying ability (Table 2; Cox 2001). While de Leeuw et al. (1995) suggest that only 0.17 % of beet seeds remain on the soil surface after sowing, at a maximum drilling rate of 130,000 seeds per hectare (Anon 2012), 6 and 1.5 seeds would be found on the surface in areas of approximately 270 and 70 m², respectively, well within the daily foraging ranges of each species. Areas of accidentally spilled seed could contain much higher densities. While individual partridges and sparrows may not ingest treated seeds (i.e. as the brightly coloured seed coatings may deter birds if they represent a novel food source), these calculations suggest that there is a potential risk of imidacloprid-treated seeds to affect sensitive bird species, consistent with conclusions drawn by DeCant and Barrett

(2010), Mineau and Palmer (2013) and Goulson (2013). Anecdotal observations of blackbirds and sparrows foraging in fields recently seeded with neonicotinoid-treated crops suggest that the calculated risks are further plausible (C. Morrissey personal observation).

The indirect effects of pesticides on vertebrate wildlife

While rarely considered in ecological risk assessments, concerns about the impacts of pesticide use on vertebrates have more recently turned to the widespread potential for indirect effects (Sotherton and Holland 2002; Boatman et al. 2004). Observations of farmland and grassland bird declines and range contractions correlate well with agricultural intensification, including increased pesticide use (Chamberlain et al. 2000; Morris et al. 2005; Ghilain and Bélisle 2008; Robillard et al. 2013; Mineau and Whiteside 2013). Tennekes (2010) and Mason et al. (2012) have recently suggested, albeit with little supporting evidence, that neonicotinoid insecticides may be contributing to declines of insectivorous birds in Europe, and of fish, amphibians, bats and birds around the world, respectively. Tennekes (2010) hypothesized that neonicotinoids were acting indirectly on bird populations, by reducing the abundance of their insect prey. Mason et al. (2012) suggested that neonicotinoids have suppressed the immune system of vertebrates (and invertebrates) making them more prone to infectious disease and other stressors.

Indirect effects of pesticides on vertebrates are most commonly exerted in one of three ways: (1) through reductions of plant seed food for granivores following herbicide applications (e.g. Gibbons et al. 2006); (2) through the loss of insect host plants following herbicide applications and the secondary impacts for dependent insects and insectivores, (e.g. Potts 1986); or (3) through reductions in arthropod prey for insectivores following applications of insecticides—or fungicides with insecticidal properties (e.g. Martin et al. 2000; Morris et al. 2005; Poulin et al. 2010).

Indirect effects are inherently difficult to measure and frequently suffer from limitations of correlative inferences. Boatman et al. (2004) highlighted three criteria for conclusively inferring a causal link between pesticides and their indirect actions on vertebrate wildlife. Conclusive studies should document negative effects on (1) food quality and quantity, (2) reproduction, condition or survivorship of the vertebrate consumer and (3) concomitant vertebrate population declines. The only documented case where indirect effects were definitively shown using the full range of these criteria in a fully replicated field experiment was for the grey partridge in Britain (Rands 1985) following several decades of intensive study. Population modelling showed that declines in grey partridge populations could be wholly explained by

herbicide-induced reductions in prey availability in tandem with reduced growth and survival of grey partridge chicks (reviewed by Potts 1986). Other studies, however, have revealed consistent effects on one or more of these three criteria, suggesting that the indirect effects of pesticides may be more prevalent than documented in the literature.

Studies reporting effects on consumers through food reductions

Pesticide applications, in temperate regions, directly overlap with the seasonal production of invertebrates and the breeding seasons of a range of numerous vertebrate species. Food supply (i.e. abundance and availability) is widely accepted as affecting habitat selection, reproductive success and survival in vertebrates, with extensive supporting evidence for birds in particular (Simons and Martin 1990; Johansson and Blomqvist 1996; Brickle et al. 2000; Moller 2001; Hole et al. 2002; Nagy and Holmes 2004, 2005; Boatman et al. 2004; Morris et al. 2005; Britschgi et al. 2006; Hart et al. 2006; Zanette et al. 2006; Golawski and Meissner 2008; Selås et al. 2008; Dunn et al. 2010; Poulin et al. 2010). Across Europe and North America, dramatic and widespread declines have been observed in populations of birds associated with farmland and wetland habitats (Beauchamp et al. 1996; Donald et al. 2001; Benton et al. 2002; Boatman et al. 2004), with arthropod abundance showing similar trends (Benton et al. 2002). In Canada and the USA, however, species loss has been more strongly correlated with pesticide use than agricultural area or intensification measures alone (Gibbs et al. 2009; Mineau and Whiteside 2013).

Reductions in invertebrate food abundance caused by insecticide use has been linked to reductions in reproductive success of at least four farmland passerines in the UK: corn bunting, *Miliaria calandra*, yellowhammer, *Emberiza citrinella*, whinchat, *Saxicola rubetra*, and reed bunting, *Emberiza schoeniclus* (Brickle et al. 2000; Brickle and Peach 2004; Morris et al. 2005; Hart et al. 2006; Dunn et al. 2010; but see Bradbury et al. 2000, 2003). Although declines in bird populations in the UK have been coincident with invertebrate losses, changes in invertebrate abundance alone do not fully explain population trends for these species. In fact, the nesting success of these species increased during time periods when populations were declining (Siriwardena et al. 2000). Population declines of seed eaters have instead been linked to reduced over-winter survival, likely as a consequence of reduced seed availability (Siriwardena et al. 2000; Butler et al. 2010).

Indirect effects of neonicotinoids and fipronil

We found only six studies that have investigated the indirect effects of neonicotinoids and fipronil on vertebrate wildlife

(Table 3). All were field rather than laboratory-based studies. Of these studies, one found a beneficial, indirect effect. Female Cape ground squirrels, *Xerus inauris*, benefited from ectoparasite removal with fipronil and had fourfold higher breeding success (Hillegass et al. 2010). A number of studies have shown that reducing parasite burdens can enhance vertebrate breeding success (e.g. Hudson et al. 1992). However, interpretation of the effect of fipronil was not straightforward, as endoparasites were simultaneously removed with ivermectin, and researchers could not distinguish the effects of the two products.

In two further field studies, both in experimental rice fields, imidacloprid and/or fipronil was applied at the recommended commercial rates. While one study found no effect of fipronil on growth or survival of Japanese carp, *Cyprinus carpio* (Clasen et al. 2012), the other found that both imidacloprid and fipronil applications reduced the growth of both adult and fry medaka fish, *Oryzias latipes* (Hayasaka et al. 2012). Hayasaka et al. (2012) suggest that this is most likely an indirect effect, through a reduction in the abundance of medaka prey. The concentrations were probably too low (approximately 0.001 to 0.05 mg/L) to exert a direct toxic effect on medaka but assumed sufficiently high to reduce the abundance of their invertebrate prey.

Population-level studies investigating indirect impacts of neonicotinoids and fipronil on vertebrate species are rare. Only three such studies were found during this review, and all were of local—rather than national or regional—populations (Table 3). All were field studies that applied either imidacloprid or fipronil at recommended commercial rates using sprays or soil drenching, rather than seed treatments.

Falcone and DeWald (2010) investigated the impact of a single soil drenching application with imidacloprid on eastern hemlock, *Tsuga Canadensis*, as part of a campaign to reduce numbers of an exotic insect pest. While the soil drenching had (surprisingly) no impact on the woolly adelgid (*Adelges tsugae*) pest, populations of non-target hemiptera and lepidoptera were reduced. Despite lepidopteran larvae being important in the diet of three neotropical migrant insectivorous bird species, bird numbers were not affected in the following year. Norelius and Lockwood (1999) undertook a similar study, this time spraying with fipronil to control a grasshopper outbreak. While grasshopper numbers were markedly reduced, populations of insectivorous prairie birds that commonly consume the grasshoppers were slightly, but not significantly, reduced a month after spraying. The lack of clear population-level effects in both these studies may have been related to birds seeking food outside treated areas in compensation, although this seems unlikely, at least for the Norelius and Lockwood (1999) study, as the home ranges of the birds studied (few hectares) were small compared to the total treated area (few hundred hectares). Alternatively, population-level effects could have been masked in such

Table 3 Indirect effects of imidacloprid and fipronil on vertebrates

Taxon and Species	Effect on:	Imidacloprid	Fipronil	Source and detailed effect
Mammal				
Lesser hedgehog tenrec, <i>Echinops telfairi</i>	Population		REC	Peveling et al. 2003; marked reduction in harvester termite prey may eventually lead to tenrec decline
Cape ground squirrel, <i>Xerus inauris</i>	Reproduction		0.7 mg/kg; REC (POS)	Hillegass et al. 2010; removal of ectoparasites (with fipronil) and endoparasites boosted breeding success; unable to determine impact of fipronil alone
Bird				
3 neotropical migrant insectivores	Population	REC (NE)		Falcone and DeWald 2010; spraying reduced lepidopteran prey, but not populations of black-throated green warbler (<i>Dendroica virens</i>), black-throated blue warbler (<i>D. caerulescens</i>) and blue-headed vireo (<i>Vireo solitarius</i>)
38 species, of which 33 were insectivores	Population		REC (NE)	Norelius and Lockwood 1999; marked reduction in grasshoppers, but not in bird densities; 34 bird species studied, most abundant were horned lark, <i>Eremophila alpestris</i> , western meadowlark, <i>Sturnella neglecta</i> , and lark sparrow, <i>Chondestes grammacus</i>
Fish				
Medaka, <i>Oryzias latipes</i>	Growth & development	0.001 mg/L; REC	0.001–0.05 mg/L; REC	Hayasaka et al. 2012; reduced growth of both adults and fry
Japanese carp, <i>Cyprinus carpus</i>	Growth and survival		REC (NE)	Clasen et al. 2012; no effect on growth and survival of Japanese carp
Reptile				
Madagascar iguana, <i>Chalarodon madagascariensis</i>	Population		REC ⁷	Peveling et al. 2003; marked reduction in harvester termite prey led to decline in iguana population
A skink, <i>Mabuy elegans</i>	Population		REC ⁷	Peveling et al. 2003; marked reduction in harvester termite prey led to decline in skink population

All other studies demonstrated deleterious effects

REC insecticide applied at manufacturer's recommended rate, NE no effect at the given dosage, POS positive effect at the given dosage

relatively small-scale field trials if birds had immigrated into the treated plots from surrounding un-treated areas. Neither study, however, measured breeding success or impacts on chick survival which may be more plausible than effects on adult survival.

In contrast, Peveling et al. (2003) documented how fipronil spraying to control a plague of migratory locusts in Madagascar halved populations of the harvester termite, *Coarctotermes clepsydra*. Consequently, populations of two lizard species, the Madagascar iguana, *Chalarodon madagascariensis*, and a skink, *Mabuy elegans*, declined, because termites form an important part of the diet of both species, while the lesser hedgehog tenrec, *Echinops telfairi*, may have also been affected. To date, this is the only study that has demonstrated a

population-level impact of a systemic insecticide on a vertebrate population, where its effect was exerted indirectly through the food chain. While Tingle et al. (2003) report that a study of fipronil spraying to control locusts in Madagascar may have caused population declines of two bird species, Madagascar bee-eater, *Merops superciliosus*, and Madagascar kestrel, *Falco newtoni*, (but no effect on two others, Madagascar bush lark, *Mirafra hova*, and Madagascar cisticola, *Cisticola cherina*), sample sizes were too small to be conclusive, and it was not possible to distinguish between direct and indirect effects.

While it is possible to use laboratory toxicity studies to inform models on the indirect effects of a pesticide on vertebrate populations, such models are very data-demanding and

case studies are rare (see e.g. Watkinson et al. 2000). Systemic insecticides are known to affect invertebrate populations (e.g. Whitehorn et al. 2012; Van Dijk et al. 2013), but the lack of evidence for, and difficulty in determining, comparable indirect effects on vertebrates is an issue in ecotoxicology. There remains an essential need to determine if a causal link between loss of insect prey through pesticide use and the decline of vertebrate populations exists. This is especially true in North America and Europe where neonicotinoids are being used in large quantities and over vast areas.

Conclusions

Neonicotinoid and fipronil insecticides can exert their impact on vertebrates either directly, through their overt toxicity, or indirectly, for example, by reducing their food supply. Marked variation exists among taxa and different systemic insecticides in acute toxicity (as measured by LD₅₀ and LC₅₀), while a range of sub-lethal effects can occur at concentrations orders of magnitude below those causing lethality. Overall, at concentrations relevant to field exposure scenarios from seed treatments (birds) or water concentrations (fish), imidacloprid and clothianidin can be considered a risk to granivorous bird species, while fipronil may pose a similar risk to sensitive fish species. Except in the most extreme cases, however, concentrations of imidacloprid and clothianidin that fish and amphibians are exposed to appear to be substantially below thresholds to cause mortality, although sub-lethal effects have not been widely studied.

Despite the lack of research and the difficulty in assigning causation, indirect effects may be as—or even more—important than direct toxic effects on vertebrates, as modern systemic insecticides are more effective at killing the invertebrate prey of vertebrates than the vertebrates themselves. Given the data here, current risk assessment procedures for neonicotinoids and other systemic pesticides need to consider the associated risks from both direct and indirect effects to vertebrate wildlife.

Acknowledgments This manuscript benefited from discussions in the International Task Force on Systemic Pesticides (TFSP) during its plenary meetings in Paris (2010), Bath (2011), Cambridge (2012), Padua (2012), Louvain-la-Neuve (2013) and Legnaro (2013). We thank the members of the TFSP for their advice and encouragement, particularly Dominique Noome, Maarten Bijleveld van Lexmond, Jeroen van der Sluijs, Noa Simon Delso and Jean-Marc Bonmatin. We also thank Brigitte Poulin and Barnett Rattner for generously providing a critical review of an earlier version of the manuscript. The work of the TFSP was funded by Triodos Foundation's 'Support Fund for Independent Research on Bee Decline and Systemic Pesticides'. This Support Fund was created from donations by Adessium Foundation (The Netherlands), Act Beyond Trust (Japan), Universiteit Utrecht (Netherlands), Stichting Triodos Foundation (The Netherlands), Gesellschaft fuer Schmetterlingsschutz (Germany), M.A.O.C. Gravin van Bylandt Stichting (The Netherlands), Zukunft

Stiftung Landwirtschaft (Germany), Study Association Storm (Student Association Environmental Sciences Utrecht University) and citizens. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript, and none of the authors received funding from any of these sources. The authors declare no conflicts of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Abou-Donia MB, Goldstein LB, Bullman S, Tu T, Khan WA, Dechkovskaia AM, Abdel-Rahman AA (2008) Imidacloprid induces neurobehavioral deficits and increases expression of glial fibrillary acidic protein in the motor cortex and hippocampus in offspring rats following in utero exposure. *J Toxicol Environ Health A* 71:119–130
- Anon (2012) Addendum 7 to the draft assessment report; confirmatory data; imidacloprid. EU Commission
- Avery ML, Fischer DL, Primus TM (1997) Assessing the hazard to granivorous birds feeding on chemically treated seeds. *Pestic Sci* 49:362–366
- Avery ML, Primus TM, Mihaich EM, Decker DG, Humphrey JS (1998) Consumption of fipronil-treated rice seed does not affect captive blackbirds. *Pest Manag Sci* 52:91–96
- Badgular PC, Jain SK, Singh A, Punia JS, Gupta RP, Chandratre GA (2013) Immunotoxic effects of imidacloprid following 28 days of oral exposure in BALB/c mice. *Environ Toxicol Pharmacol* 35:408–418
- Bal R, Türk G, Yılmaz Ö, Etem E, Kuloğlu T, Baydaş G, Naziroğlu M (2012) Effects of clothianidin exposure on sperm quality, testicular apoptosis and fatty acid composition in developing male rats. *Cell Biol Toxicol* 28:187–200
- Bal R, Türk G, Tuzcu M, Yılmaz Ö, Kuloğlu T, Baydaş G, Naziroğlu M, Yener Z, Etem E, Tuzcu Z (2013) Effects of the neonicotinoid insecticide, clothianidin, on the reproductive organ system in adult male rats. *Drug Chem Toxicol* 36:421–429
- Beauchamp WD, Koford RR, Nudds TD, Clark RG, Johnson DH (1996) Long-term declines in nest success of prairie ducks. *J Wildl Manag* 60:247–257
- Beggel S, Werner I, Connon RE, Geist JP (2010) Sublethal toxicity of commercial insecticide formulations and their active ingredients to larval fathead minnow (*Pimephales promelas*). *Sci Total Environ* 408(16):3169–3175
- Beggel S, Werner I, Connon RE, Geist JP (2012) Impacts of the phenylpyrazole insecticide fipronil on larval fish: time-series gene transcription responses in fathead minnow (*Pimephales promelas*) following short-term exposure. *Sci Total Environ* 426:160–165
- Benton TG, Bryant DN, Cole L, Crick HQP (2002) Linking agricultural practice to insect and bird populations: a historical study over three decades. *J Appl Ecol* 39:673–687
- Berny PJ, Buronfosse F, Videmann B, Buronfosse T (1999) Evaluation of the toxicity of imidacloprid in wild birds. A new high performance thin layer chromatography (HPTLC) method for the analysis of liver and crop samples in suspected poisoning cases. *J Liq Chrom Rel Technol* 22:1547–1559
- Bhardwaj S, Srivastava MK, Kapoor U, Srivastava LP (2010) A 90 days oral toxicity of imidacloprid in female rats: morphological,

- biochemical and histopathological evaluations. *Food Chem Toxicol* 48:1185–1190
- Boatman ND, Brickle NW, Hart JD, Milsom TP, Morris AJ, Murray AWA, Murray KA, Pobertson PA (2004) Evidence for the indirect effects of pesticides on farmland birds. *Ibis* 146:131–143
- Bonmatin JM, Marchand PA, Charvet R, Moineau I, Bengsch ER, Colin ME (2005) Quantification of imidacloprid uptake in maize crops. *J Agric Food Chem* 53:5336–5341
- Bradbury RB, Kyrkos A, Morris AJ, Clark SC, Perkins AJ, Wilson JD (2000) Habitat associations and breeding success of yellowhammers on lowland farmland. *J Appl Ecol* 37:789–805
- Bradbury RB, Wilson JD, Moorcroft D, Morris A, Perkins AJ (2003) Habitat and weather are weak correlates of nestling condition and growth rates of four UK farmland passerines. *Ibis* 145:295–306
- Brickle NW, Peach WJ (2004) The breeding ecology of Reed Buntings *Emberiza schoeniclus* in farmland and wetland habitats in lowland England. *Ibis* 146:69–77
- Brickle NW, Harper DGC, Aebischer NJ, Cockayne SH (2000) Effects of agricultural intensification on the breeding success of corn buntings *Miliaria calandra*. *J Appl Ecol* 37:742–755
- Britschgi A, Spaar R, Arlettaz R (2006) Impact of grassland farming intensification on the breeding ecology of an indicator insectivorous passerine, the Whinchat *Saxicola rubetra*: Lessons for overall Alpine meadowland management. *Biol Conserv* 130:193–205
- Butler SJ, Mattison EHA, Glithero NJ, Robinson LJ, Atkinson PW, Gillings S, Vickery JA, Norris K (2010) Resource availability and the persistence of seed-eating bird populations in agricultural landscapes: a mechanistic modelling approach. *J Appl Ecol* 47:67–75
- Callahan J, Mineau P (2008) Evaluation of clinical sign data from avian acute oral toxicity studies. Appendix 11; Scientific opinion of the panel on plant protection products and their residues on risk assessment for birds and mammals. EFSA J 734, 10pp
- Casida JE, Durkin KA (2013) Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annu Rev Entomol* 58:99–117
- Chamberlain DE, Fuller RJ, Bunce RGH, Duckworth JC, Shrubbs M (2000) Changes in the abundance of farmland birds in relation to the timing of agricultural intensification in England and Wales. *J Appl Ecol* 37:771–788
- Clasen B, Loro VL, Cattaneo R, Moraes B, Lopes T, de Avila LA, Zanella R, Reimche GB, Baldisserotto B (2012) Effects of the commercial formulation containing fipronil on the non-target organism *Cyprinus carpio*: Implications for rice-fish cultivation. *Ecotoxicol Environ Saf* 77:45–51
- Connelly P (2011) Environmental fate of fipronil. Californian Environmental Protection Agency, Sacramento
- Cowles RS, Montgomery ME, Cheah CAS-J (2006) Activity and residues of imidacloprid applied to soil and tree trunks to control Hemlock Woolly Adelgid (Hemiptera: Adelgidae) in Forests. *J Econ Entomol* 99:1258–1267
- Cox C (2001) Insecticide factsheet: imidacloprid. *J Pestic Reform* 21:15–21
- Cutler GC, Scott-Dupree CD (2007) Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. *J Econ Entomol* 100:765–772
- de Leeuw J, Gorree M, de Snoo GR, Jams WLM, van der Poll RJ, Luttik R (1995) Risks of granules of treated seeds to birds on arable fields. GML report no. 118. Centre of Environmental Science, Leiden University, Leiden, ISSN 1381–1703
- de Oliveira IM, Nunes BV, Barbosa DR, Pallares AM, Faro LR (2010) Effects of the neonicotinoids thiamethoxam and clothianidin on in vivo dopamine release in rat striatum. *Toxicol Lett* 192:294–297
- DeCant J, Barrett M (2010) Environmental fate and ecological risk assessment for the registration of clothianidin for use as a seed treatment on mustard seed (oilseed and condiment) and cotton. United States Environmental Protection Agency, Washington
- Demsia G, Vlastos D, Goumenou M, Matthopoulos DP (2007) Assessment of the genotoxicity of imidacloprid and metalaxyl in cultured human lymphocytes and rat bone-marrow. *Mutat Res* 634:32–39
- Donald PF, Green RE, Heath MF (2001) Agricultural intensification and the collapse of Europe's farmland bird populations. *Proc R Soc B* 268:25–29
- Donnarumma L, Pulcini P, Pochi D, Rosati S, Lusco L, Conte E (2011) Preliminary study of persistence in soil and residues in maize of imidacloprid. *J Environ Sci Health B* 46:469–472
- Dunn JC, Hamer KC, Benton TG (2010) Fear for the family has negative consequences: indirect effects of nest predators on chick growth in a farmland bird. *J Appl Ecol* 47:994–1002
- Duzguner V, Erdogan S (2012) Chronic exposure to imidacloprid induces inflammation and oxidative stress in the liver and central nervous system of rats. *Pestic Biochem Physiol* 104:58–64
- European Commission (2005) Clothianidin. SANCO/10533/05—Final, 26 pp
- Falcone JF, DeWald LE (2010) Comparisons of arthropod and avian assemblages in insecticide-treated and untreated eastern hemlock (*Tsuga canadensis* [L.] Carr) stands in Great Smoky Mountains National Park, USA. *For Ecol Manag* 260:856–863
- Feng S, Kong Z, Wang X, Zhao L, Peng P (2004) Acute toxicity and genotoxicity of two novel pesticides on amphibian, *Rana N Hallowell*. *Chemosphere* 56:457–463
- Fossen M (2006) Environmental fate of imidacloprid. Department of Pesticide Regulation, Sacramento
- Garthwaite DG, Thomas MR, Dawson A, Stoddart H (2003) Pesticide Usage Survey Report 187: arable crops in Great Britain 2002. Department for Environment, Food and Rural Affairs, London
- Gawade L, Dadarkar SS, Husain R, Gatne M (2013) A detailed study of developmental immunotoxicity of imidacloprid in Wistar rats. *Food Chem Toxicol* 51:61–70
- Ghilain A, Bélsile M (2008) Breeding success of tree swallows along a gradient of agricultural intensification. *Ecol Appl* 18:1140–1154
- Ghisi D d C, Ramsdorf WA, Vinicius M, Ferraro M, Almeida MIM, Ribeiro CA d O, Cestari MM (2011) Evaluation of genotoxicity in *Rhamdia quelen* (Pisces, Siluriformes) after sub-chronic contamination with Fipronil. *Environ Monit Assess* 180:589–599
- Gibbons DW, Bohan DA, Rothery P, Stuart RC, Houghton AJ, Scott RJ, Wilson JD, Perry JN, Clark SJ, Dawson RJG, Firkbank L (2006) Weed seed resources for birds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. *Proc R Soc B* 273:1921–1928
- Gibbs KE, Mackey RL, Currie DJ (2009) Human land use, agriculture, pesticides and losses of imperiled species. *Divers Distrib* 15:242–253
- Golawski A, Meissner W (2008) The influence of territory characteristics and food supply on the breeding performance of the Red-backed Shrike (*Lanius collurio*) in an extensively farmed region of eastern Poland. *Ecol Res* 23:347–353
- Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. *J Appl Ecol* 50:977–987
- Grant DB, Chalmers AE, Wolff MA, Hoffman HB, Bushey DF, Kuhr RJ, Motoyama N (1998) Fipronil: action at the GABA receptor. In: Kuhr RJ, Motoyama N (eds) Pesticides and the Future: minimizing chronic exposure of humans and the environment. IOS Press, Amsterdam, pp 147–156
- Gu YH, Li Y, Huang XF, Zheng JF, Yang J, Diao H, Yuan Y, Xu Y, Liu M, Shi HJ, Xu WP (2013) Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development in vitro. *PLoS ONE* e70111
- Hart JD, Milsom TP, Fisher G, Wilkins V, Moreby SJ, Murray AWA, Robertson PA (2006) The relationship between yellowhammer breeding performance, arthropod abundance and insecticide applications on arable farmland. *J Appl Ecol* 43:81–91
- Hayasaka D, Korenaga T, Suzuki K, Saito F, Sanchez-Bayo F, Goka K (2012) Cumulative ecological impacts of two successive annual

- treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. *Ecotoxicol Environ Saf* 80:355–362
- Hillegass MA, Waterman JM, Roth JD (2010) Parasite removal increases reproductive success in a social African ground squirrel. *Behav Ecol* 21:696–700
- Hole DG, Whittingham MJ, Bradbury RB, Anderson GQA, Lee PLM, Wilson JD, Krebs JR (2002) Widespread local house-sparrow extinctions. *Nature* 418:931–932
- Howard JH, Julian SE, Ferrigan J (2003) Golf course maintenance: impact of pesticides on amphibians. *Golf Course Mon*, September 94–101
- Hudson PJ, Newborn D, Dobson AP (1992) Regulation and stability of a free-living host-parasite system: *Trichostrongylus tenuis* in red grouse. 1. Monitoring and parasite reduction experiments. *J Anim Ecol* 61:477–486
- Jemec A, Tišler T, Drobne D, Sepčič K, Fournier D, Trebše P (2007) Comparative toxicity of imidacloprid, of its commercial liquid formulation and of diazinon to a non-target arthropod, the microcrustacean *Daphnia magna*. *Chemosphere* 68:1408–1418
- Jeschke P, Nauen R (2008) Neonicotinoids—from zero to hero in insecticide chemistry. *Pest Manag Sci* 64:1084–1098
- Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* 59:2897–2908
- Johansson OC, Blomqvist D (1996) Habitat selection and diet of lapwing, *Vanellus vanellus*, chicks on coastal farmland in SW Sweden. *J Appl Ecol* 33:1030–1040
- Kapoor U, Srivastava MK, Bhardwaj S, Srivastava LP (2010) Effect of imidacloprid on antioxidant enzymes and lipid peroxidation in female rats to derive its No Observed Effect Level (NOEL). *J Toxicol Sci* 35:577–581
- Kaur B, Sandhu HS, Kaur R (2006) Toxic effects of subacute oral exposure of imidacloprid on biochemical parameters in crossbred cow calves. *Toxicol Int* 13:43–47
- Kitulagodage M, Astheimer LB, Buttemer WA (2008) Diacetone alcohol, a dispersant solvent, contributes to acute toxicity of a fipronil-based insecticide in a passerine bird. *Ecotoxicol Environ Saf* 71:597–600
- Kitulagodage M, Buttemer WA, Astheimer LB (2011a) Adverse effects of fipronil on avian reproduction and development: maternal transfer of fipronil to eggs in zebra finch *Taeniopygia guttata* and in ovo exposure in chickens *Gallus domesticus*. *Ecotoxicol* 20:653–660
- Kitulagodage M, Isanhardt J, Buttemer WA, Hooper MJ, Astheimer LB (2011b) Fipronil toxicity in northern bobwhite quail *Colinus virginianus*: reduced feeding behaviour and sulfone metabolite formation. *Chemosphere* 83:524–530
- Köhler H-R, Triebkorn R (2013) Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond. *Science* 341:759–765
- Kreuger J, Graaf S, Patring J, Adielsson S (2010) Pesticides in surface water in areas with open ground and greenhouse horticultural crops in Sweden, 2008. Swedish University of Agricultural Science. http://www-mv.slu.se/webfiles/vv/CKB/Ekohydrologi_117_ENG.pdf. Accessed 4 Feb 2013
- Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS ONE* 7:e29268. doi:10.1371/journal.pone.0029268
- Lamers M, Anyusheva M, La N, Nguten V, Streck T (2011) Pesticide pollution in surface and groundwater by paddy rice cultivation: a case study from Northern Vietnam. *Clean Soil Air Water* 39:356–361
- Lauan MCB, Ocampo PP (2013) Low-dose effects of carbaryl, chlorpyrifos and imidacloprid on the gonad and plasma testosterone level of male juvenile and adult Nile tilapia (*Oreochromis niloticus* Linnaeus). *Asia Life Sci* 1:239–250
- Leghait J, Gayraud V, Toutain P-L, Picard-Hagen N, Viguié C (2010) Is the mechanism of fipronil-induced thyroid disruption specific to the rat: re-evaluation of fipronil thyroid toxicity in sheep? *Toxicol Lett* 194:351–357
- Lopez-Antia A, Ortiz-Santaliestra ME, Mougeot F, Mateo R (2013) Experimental exposure of red-legged partridges (*Alectoris rufa*) to seeds coated with imidacloprid, thiram and difenoconazole. *Ecotoxicol* 22:125–138. doi:10.1007/s10646-012-1009-x
- Main AR, Headley JV, Peru KM, Michel NL, Cessna AJ, Morrissey CA (2014) Widespread use and frequent detection of neonicotinoid insecticides in wetlands of Canada's Prairie Pothole Region. *PLoS ONE* 9:e92821
- Martin PA, Johnson DL, Forsyth DJ, Hill BD (2000) Effects of two grasshopper control insecticides on food resources and reproductive success of two species of grassland songbirds. *Environ Toxicol Chem* 19:2987–2996
- Martins AP (2009) Neurobehavioural effects of acute fipronil administration in rats. PhD thesis, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo
- Mason R, Tennekes H, Sánchez-Bayo F, Jepsen PU (2012) Immune suppression by neonicotinoid insecticides at the root of global wildlife declines. *J Environ Immunol Toxicol* 1:3–12
- Mineau P (2005) A review and analysis of study endpoints relevant to the assessment of 'long term' pesticide toxicity in avian and mammalian wildlife. *Ecotoxicol* 14:775–799
- Mineau P (2011) Barking up the wrong perch: why we should stop ignoring non-dietary routes of pesticide exposure in birds. *Integr Environ Assess Manag* 7:297–305
- Mineau P, Palmer C (2013) The impact of the nation's most widely used insecticides on birds. American Bird Conservancy, USA
- Mineau P, Whiteside M (2013) Pesticide acute toxicity is a better correlate of U.S. grassland bird declines than agricultural intensification. *PLoS ONE* 8:e57457
- Mineau P, Boersma DC, Collins B (1994) An analysis of avian reproduction studies submitted for pesticide registration. *Ecotoxicol Environ Saf* 29:304–329
- Mineau P, Balcomb R, Bennett R, Dobson S, Fry M, Jaber M, Leopold A, Munk R, Ringer B, Rispin A, Sileo L, Solecki R, Thompson H (1996) Testing for effects on reproduction. In: Report of the SETAC/OECD Workshop on Avian Toxicity Testing. Inter-Organizational Programme for the Sound Management of Chemicals, OECD Environmental Health and Safety Publications, Series on Testing and Assessment No. 5, pp 44–62
- Mineau P, Fletcher MR, Glaser LC, Thomas NJ, Brassard C, Wilson LC, Elliott JE, Lyon LA, Henny CJ, Bollinger T, Porter SL (1999) Poisoning of raptors with organophosphorous and carbamate pesticides with emphasis on Canada, the United States and the United Kingdom. *J Raptor Res* 33:1–37
- Mize SV, Porter SD, Demcheck DK (2008) Influence of fipronil compounds and rice cultivation land-use intensity on macro-invertebrate communities in streams of southwestern Louisiana, USA. *Environ Pollut* 152:491–503
- Mohany M, Badr G, Refaat I, El-Feki M (2011) Immunological and histological effects of exposure to imidacloprid insecticide in male albino rats. *Afr J Pharm Pharmacol* 5:2106–2114
- Moller AP (2001) The effect of dairy farming on barn swallow *Hirundo rustica* abundance, distribution and reproduction. *J Appl Ecol* 38:378–389
- Morris AJ, Wilson JD, Whittingham MJ, Bradbury RB (2005) Indirect effects of pesticides on breeding yellowhammer (*Emberiza citrinella*). *Agric Ecosyst Environ* 106:1–16
- Nagy LR, Holmes RT (2004) Factors influencing fecundity in migratory songbirds: is nest predation the most important? *J Avian Biol* 35:487–491
- Nagy LR, Holmes RT (2005) Food limits annual fecundity of a migratory songbird: an experimental study. *Ecol* 86:675–681
- Nellore K, Raj K, Usha Rani CT, Jacob DP (2010) Studies on the effect of imidacloprid toxicity on the acetylcholine esterase activity levels in different regions of the brain of the albino rat. *Int J Agric Environ Biotechnol* 3:377–380

- Newton I (1995) The contribution of some recent research on birds to ecological understanding. *J Anim Ecol* 64:675–695
- Nian Y (2009) Study on toxicity of triazophos, trichlorophon and imidacloprid on *Rana limnocharis* tadpole. *J Anhui Agric Sci* 2009:18
- Norelius EE, Lockwood JA (1999) The effects of reduced agent-area insecticide treatments for rangeland grasshopper (Orthoptera: Acrididae) control on bird densities. *Arch Environ Contam Toxicol* 37:519–528
- Ocampo PP, Sagun VG (2007) Gonadal changes in male tilapia (*Oreochromis niloticus* Linn.) after exposure to imidacloprid insecticide. *Philippine Entomol* 21:199–200
- Ohi M, Dalsenter PR, Andrade AJM, Nascimento AJ (2004) Reproductive adverse effects of fipronil in Wistar rats. *Toxicol Lett* 146:121–127
- Pascual JA, Hart ADM, Saunders PJ, McKay HV, Kilpatrick J, Prosser P (1999) Agricultural methods to reduce the risk to birds from cereal seed treatments on fenlands in eastern England. I. Sowing depth. *Agric Ecosyst Environ* 72:59–73
- Peveling R, Demba SA (2003) Toxicity and pathogenicity of *Metarhizium anisopliae* var. *acridum* (Deuteromycotina, Hyphomycetes) and fipronil to the fringe-toed lizard *Acanthodactylus dumerili* (Squamata: Lacertidae). *Environ Toxicol Chem* 22:1437–1447
- Peveling R, McWilliam AN, Nagel P, Rasolomanana H, Raholijaona, Rakotomianina L, Ravoninjatovo A, Dewhurst CF, Gibson G, Rafanomezana S, Tingle CCD (2003) Impact of locust control on harvester termites and endemic vertebrate predators in Madagascar. *J Appl Ecol* 40:729–741
- Potts GR (1986) The partridge—pesticides, predation and conservation. Collins, London
- Poulin B, Lefebvre G, Paz L (2010) Red flag for green spray: adverse trophic effects of *Bti* on breeding birds. *J Appl Ecol* 47:884–889
- Prosser P, Hart ADM (2005) Assessing potential exposure of birds to pesticide-treated seeds. *Ecotoxicol* 14:679–691
- Rands MRW (1985) Pesticide use on cereals and the survival of grey partridge chicks: a field experiment. *J Appl Ecol* 22:49–54
- Ratcliffe DA (1967) Decrease in eggshell weight in certain birds of prey. *Nature* 215:208–210
- Robillard A, Garant D, Bélisle M (2013) The swallow and the sparrow: how agricultural intensification affects abundance, nest site selection and competitive interactions. *Landsc Ecol* 28:201–215
- Rouchaud J, Gustin F, Wauters A (1994) Soil biodegradation and leaf transfer of insecticide imidacloprid applied in seed dressing in sugar beet crops. *Bull Environ Contam Toxicol* 53:344–350
- Sánchez-Bayo F (2011) Impacts of agricultural pesticides on terrestrial ecosystems. In: Sánchez-Bayo F (ed) *Ecological impacts of toxic chemicals*. Bentham Science Publishers Ltd, USA, pp 63–87
- Sánchez-Bayo F, Goka K (2005) Unexpected effects of zinc pyrethione and imidacloprid on Japanese medaka fish (*Oryzias latipes*). *Aquat Toxicol* 74:285–293
- Selås V, Steen R, Kobro S, Lislevand T, Stenberg I (2008) Direct and indirect weather impacts on spring populations of lesser spotted woodpecker (*Dendrocopos minor*) in Norway. *Scand J For Res* 23:148–153
- SERA (2005) Imidacloprid—human health and ecological risk assessment—final report. Report from Syracuse Environmental Research Associates to USDA, Forest Service
- Simons LS, Martin TE (1990) Food limitation of avian reproduction: an experiment with the Cactus Wren. *Ecol* 71:869–876
- Siriwardena GM, Ballie SR, Crick HQP, Wilson JD (2000) The importance of variation in the breeding performance of seed-eating birds in determining their population trends on farmland. *J Appl Ecol* 37: 128–148
- Sotherton N, Holland J (2002) Indirect effects of pesticides on farmland wildlife. In: Hoffman DJ, Rattner BA, Allen Burton G, Cairns J (eds) *Handbook of ecotoxicology*, 2nd edn. CRC Press Ltd, USA, pp 1173–1196
- Stark JD, Vargas RI (2005) Toxicity and hazard assessment of fipronil to *Daphnia Pulex*. *Ecotoxicol Environ Saf* 62:11–16
- Stamer K, Goh KS (2012) Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010–2011. *Bull Environ Contam Toxicol* 88: 316–321
- Stehr CM, Linbo TL, Incardona JP, Scholz NL (2006) The developmental neurotoxicity of fipronil: notochord degeneration and locomotor defects in zebrafish embryos and larvae. *Toxicol Sci* 92:270–278
- Stoughton SJ, Liber K, Culp J, Cessna A (2008) Acute and chronic toxicity of imidacloprid to the aquatic invertebrates *Chironomus tentans* and *Hyalella azteca* under constant- and pulse-exposure conditions. *Archives Environ Contam Toxicol* 54:662–673
- Suryanarayanan S (2013) Balancing control and complexity in field studies of neonicotinoids and honey bee health. *Insects* 4:153–167
- Tanaka T (2012) Reproductive and neurobehavioral effects of clothianidin administered to mice in the diet. *Birth Defects Res B* 95:151–159. doi:10.1002/bdrb.20349
- Tennekes H (2010) The systemic insecticides: a disaster in the making. ETS Nederland BV, Zutphen, The Netherlands
- Terçaol PRG, Godinho AF (2011) Behavioural effects of acute exposure to the insecticide fipronil. *Pestic Biochem Physiol* 99:221–225
- Tingle CCD, Rother JA, Dewhurst CF, Lauer S, King WJ (2000) Health and environmental effects of fipronil. Briefing paper for Pesticides Action Network, UK
- Tingle CCD, Rother JA, Dewhurst CF, Lauer S, King WJ (2003) Fipronil: environmental fate, ecotoxicology and human health concerns. *Rev Environ Contam Toxicol* 176:1–66
- Tišler T, Jemec A, Mozetič B, Trebše P (2009) Hazard identification of imidacloprid to aquatic environment. *Chemosphere* 76:907–914
- Tokumoto J, Danjo M, Kobayashi Y, Kinoshita K, Omotegara T, Tatsumi A, Hashiguchi M, Sekijima T, Kamisoyama H, Yokoyama T, Kitagawa H, Hoshi N (2013) Effects of exposure to clothianidin on the reproductive system of male quails. *J Vet Med Sci* 75:755–760
- Tomizawa M, Casida JE (2005) Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annu Rev Pharmacol Toxicol* 45: 247–268
- Tomizawa M, Casida JF (2011) Neonicotinoid insecticides: highlights of a Symposium on Strategic Molecular Designs. *J Agric Food Chem* 59:2883–2886
- Toor HK, Sangha GK, Khera KS (2013) Imidacloprid induced histological and biochemical alterations in liver of female albino rats. *Pestic Biochem Physiol* 105:1–4
- US EPA (2008) Imidacloprid summary document registration review: initial docket December 2008. Docket Number: EPA-HQ-OPP-2008-0844
- US EPA (2012) United States Environmental Protection Agency Ecological Risk Assessment. http://www.epa.gov/oppefed1/ecorisk_ders/toera_analysis_eco.htm. Accessed 27 Oct 2012
- Van Dijk T, Van Staalduinen MA, Van der Sluijs JP (2013) Macro-invertebrate decline in surface water polluted with imidacloprid. *PLoS ONE* 8:e62374. doi:10.1371/journal.pone.0062374
- Watkinson AR, Freckleton RP, Robinson RA, Sutherland WJ (2000) Predictions of biodiversity response to genetically modified herbicide-tolerant crops. *Science* 289:1554–1557
- Whitehorn PR, O'Connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336:351–352
- Wirth EF, Pennington PL, Lawton JC, DeLorenzo ME, Bearden D, Shaddix B, Sivertsen S, Fulton MH (2004) The effects of the contemporary-use insecticide Fipronil in an estuarine mesocosm. *Environ Pollut* 131:365–371
- Zanette L, Clinchy M, Smith JNM (2006) Combined food and predator effects on songbird nest survival and annual reproductive success: results from a bi-factorial experiment. *Oecol* 147:632–640

Risks of large-scale use of systemic insecticides to ecosystem functioning and services

Madeleine Chagnon • David Kreutzweiser • Edward A.D. Mitchell •
Christy A. Morrissey • Dominique A. Noome • Jeroen P. Van der Sluijs

Received: 29 April 2014 / Accepted: 1 July 2014 / Published online: 19 July 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract Large-scale use of the persistent and potent neonicotinoid and fipronil insecticides has raised concerns about risks to ecosystem functions provided by a wide range of species and environments affected by these insecticides. The concept of ecosystem services is widely used in decision making in the context of valuing the service potentials, benefits, and use values that well-functioning ecosystems provide to humans and the biosphere and, as an endpoint (value to be protected), in ecological risk assessment of chemicals. Neonicotinoid insecticides are frequently detected in soil and water and are also found in air, as dust particles during sowing of crops and aerosols during spraying. These environmental media provide essential resources to support biodiversity, but are known to be threatened by long-term or repeated contamination by neonicotinoids and fipronil. We review the state of knowledge regarding the potential impacts of these insecticides on

ecosystem functioning and services provided by terrestrial and aquatic ecosystems including soil and freshwater functions, fisheries, biological pest control, and pollination services. Empirical studies examining the specific impacts of neonicotinoids and fipronil to ecosystem services have focused largely on the negative impacts to beneficial insect species (honeybees) and the impact on pollination service of food crops. However, here we document broader evidence of the effects on ecosystem functions regulating soil and water quality, pest control, pollination, ecosystem resilience, and community diversity. In particular, microbes, invertebrates, and fish play critical roles as decomposers, pollinators, consumers, and predators, which collectively maintain healthy communities and ecosystem integrity. Several examples in this review demonstrate evidence of the negative impacts of systemic insecticides on decomposition, nutrient cycling, soil respiration, and

Responsible editor: Philippe Garrigues

M. Chagnon (✉)
Département des sciences biologiques, Université du Québec à
Montréal, Case Postale 8888, Succursale Centre-Ville, Montréal,
Québec H3C 3P8, Canada
e-mail: chagnon.madeleine@uqam.ca

D. Kreutzweiser
Canadian Forest Service, Natural Resources Canada, 1219 Queen St.
East, Sault Ste. Marie, Ontario P6A 2E5, Canada

E. A. Mitchell
Laboratory of Soil Biology, University of Neuchâtel,
Rue Emile Argand 11, 2000 Neuchâtel, Switzerland

E. A. Mitchell
Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58,
2000 Neuchâtel, Switzerland

C. A. Morrissey
Department of Biology and School of Environment and
Sustainability, University of Saskatchewan, 112 Science Place,
Saskatoon, Saskatchewan S7N 5E2, Canada

D. A. Noome
Task Force on Systemic Pesticides, 46, Pertuis-du-Sault,
2000 Neuchâtel, Switzerland

D. A. Noome
Kasungu National Park, c/o Lifupa Conservation Lodge,
Private Bag 151, Lilongwe, Malawi

J. P. Van der Sluijs
Environmental Sciences, Utrecht University, Heidelberglaan 2,
3584 CS Utrecht, The Netherlands

J. P. Van der Sluijs
Centre for the Study of the Sciences and the Humanities, University
of Bergen, Postboks 7805, N-5020 Bergen, Norway

invertebrate populations valued by humans. Invertebrates, particularly earthworms that are important for soil processes, wild and domestic insect pollinators which are important for plant and crop production, and several freshwater taxa which are involved in aquatic nutrient cycling, were all found to be highly susceptible to lethal and sublethal effects of neonicotinoids and/or fipronil at environmentally relevant concentrations. By contrast, most microbes and fish do not appear to be as sensitive under normal exposure scenarios, though the effects on fish may be important in certain realms such as combined fish-rice farming systems and through food chain effects. We highlight the economic and cultural concerns around agriculture and aquaculture production and the role these insecticides may have in threatening food security. Overall, we recommend improved sustainable agricultural practices that restrict systemic insecticide use to maintain and support several ecosystem services that humans fundamentally depend on.

Keywords Ecosystem services · Soil ecosystem · Neonicotinoids · Pollinators · Freshwater · Rice paddies

Introduction

Other papers in this special issue have shown that neonicotinoid insecticides and fipronil are presently used on a very large scale (e.g., Simon-Delso et al. 2014, this issue) and are highly persistent, and repeated application can lead to buildup of environmental concentrations in soils. They have high runoff and leaching potential to surface and groundwaters and have been detected frequently in the global environment (Bonmatin et al. 2014, this issue). Evidence is mounting that they have direct and indirect impacts at field realistic environmental concentrations on a wide range of nontarget species, mainly invertebrates (Pisa et al. 2014, this issue) but also on vertebrates (Gibbons et al. 2014, this issue). Although studies directly assessing impacts to ecosystem functions and services are limited, here we review the present state of knowledge on the potential risks posed by neonicotinoids and fipronil.

The concept of ecosystem services is widely used in decision making in the context of valuing the service potentials, benefits, and use values that well-functioning ecosystems provide to humans and the biosphere (Spangenberg et al. 2014a, b). Ecosystem services were initially defined as “benefits people obtain from ecosystems” as popularized by the United Nations Environment Program (UNEP 2003) and the Millennium Ecosystem Assessment (MEA 2003, 2005). They are seen as critical to the functioning of the Earth’s life support system, which consists of habitats, ecological systems, and processes that provide services that contribute to human welfare (Costanza et al. 1997). Under the MEA framework (among others), ecosystem services have been categorized into provisioning services (e.g., food, wood, fiber, clean water), regulating services

(e.g., climate control, detoxification, water purification, pollination, seed dispersal, pest and disease regulation, herbivory, and weed control), supporting services (e.g., soil formation, nutrient cycling, pollination, soil quality, food web support, waste treatment, and remediation), and cultural services (e.g., recreation, esthetic, or spiritual value).

The wide application of neonicotinoid systemic pesticides, their persistence in soil and water, and potential for uptake by crops and wild plants expose a wide range of species, which are important in providing valuable ecosystem services. This paper addresses the risks to ecosystem functioning and services from the growing use of systemic neonicotinoid and fipronil insecticides used in agricultural and urban settings. Here, we focus on ecosystem services provided by terrestrial soil ecosystem functions, freshwater ecosystem functions, fisheries, biological pest control, and pollination, in addition to reviewing the overall threats of these systemic insecticides to food security.

Terrestrial soil ecosystem functions

Soil ecosystem services and biodiversity

Terrestrial ecosystems are known to provide a complex range of essential ecosystem services involving both physical and biological processes regulated by soils. Soils support physical processes related to water quality and availability such as soil structure and composition (e.g., porosity) to facilitate movement of water to plants, to groundwater aquifers, and to surface water supplies. Water quality is improved by filtration through clean soils that can remove contaminants and fine sediments. As water flows through soils, it interacts with various soil matrices absorbing and transporting dissolved and particulate materials including nutrients and other life-supporting elements to plants and microorganisms. Soils further provide stream flow regulation and flood control by absorbing and releasing excess water.

Many of the soil ecosystem services are biologically mediated, including regulation and cycling of water and nutrients, the facilitation of nutrient transfer and translocation, the renewal of nutrients through organic and waste matter breakdown, elemental transformations, soil formation processes, and the retention and delivery of nutrients to plants (Swift et al. 2004; Dominati et al. 2010; Robinson et al. 2013). Plants, in turn, provide food, wood, and fiber to support human infrastructure and natural habitats, while improving soil retention and erosion control. Over the long term, they also provide raw materials for consumption such as peat for fuel and horticultural substrates and ornamental plants and flowers for decoration. Further services include the biological control of pests and diseases through provision of soil conditions and habitats for beneficial species and natural enemies of pests, the sequestration and storage of carbon through plant growth and biomass retention, and the

detoxification of contaminants through sorption, immobilization, and degradation processes.

Many of the biologically mediated soil ecosystem services listed above require the inputs and activities of interacting diverse and functional biological communities (Swift et al. 2004; Lavelle et al. 2006; Barrios 2007). Biodiversity conservation itself can be considered as an important ecosystem service (Dale and Polasky 2007; Eigenbrod et al. 2010), following on the earlier concept that biodiversity serves as a form of insurance against the loss of certain species and their ecological function through species redundancy (Naeem and Li 1997; Yachi and Loreau 1999). Biodiversity has been shown to be positively related to ecological functions that support ecological services (Benayas et al. 2009). The stability of soil ecosystems has been linked to biodiversity and especially the relative abundances of keystone species or functional groups that underpin the soil food web structure or that facilitate specialized soil processes (de Ruiter et al. 1995; Brussaard et al. 2007; Nielsen et al. 2011).

Natural soils are a reservoir of diverse and complex biological communities. Organisms range from body sizes in millimeters (macrofauna, macroflora) to cell or body sizes in micrometers (mesofauna, microfauna, microflora). Key taxa include macroarthropods (e.g., ground beetles, ants, termites), earthworms, mites, collembolans, protozoans, nematodes, bacteria, and fungi. The activity of these biota and interactions among them condition ecosystem processes on which many ecosystem services depend (Barrios 2007). For example, earthworms have a large impact on organic matter dynamics, nutrient cycling, and soil properties. Earthworms break down plant litter into nutrient-rich organic matter for other consumers and contribute to the mixing of organic matter in soils. They produce casts, mucilages, and other nutrient-rich excretions that contribute to soil fertility and biogeochemical cycling (Beare et al. 1995). Their burrowing activity increases soil porosity and aeration, facilitates water and nutrient transfer, and reduces soil compaction (Edwards and Bohlen 1996). While earthworms play a key role in soil organic matter dynamics, the decomposition and mineralization of organic matter is a complex process that is facilitated by the activities and interactions among diverse biotic communities including other invertebrates, protists, bacteria, and fungi (Swift et al. 2004). These biota-mediated soil processes occur at a scale of centimeters to decimeters by individuals and populations, and the accumulation of these processes over space and time creates a continuous process from which soil properties and services arise to local and regional landscape scales (Lavelle et al. 2006).

A further example of ecosystem services is the biologically mediated nitrogen cycling in soils. Nitrogen (N) is essential for plant growth, and plants convey many of the services derived from soils. Macro- and meso-invertebrates initiate decomposition of soil organic matter by fragmentation, ingestion, and excretion to release organic N which is subsequently mineralized by highly specialized microbial groups to plant-available forms of inorganic N. Available N pools in soils are also greatly

enhanced by nitrogen-fixing microorganisms that convert atmosphere N to plant-available N through root nodule symbioses in plants, especially legumes. Inorganic N can also be taken up by soil microbes, assimilated into biomass, and incorporated into the soil organic N pool (immobilization), which is available for further cycling (Brady and Weil 1996; Brussaard et al. 1997; Barrios 2007). The excess of N is a major cause of soil and water eutrophication with consequences on biodiversity (Vitousek et al. 1997), and therefore, loss of N through denitrification is another valuable ecosystem service provided by wetlands and floodplain forest soils (Shrestha et al. 2012).

Impacts of neonicotinoid insecticides on soil ecosystem services

Given that many of the ecosystem services of soils are biologically mediated, and pesticides can cause depletion or disruption of nontarget biotic communities in soils, it follows that pesticides can pose risks to soil ecosystem processes and services. Effects of pesticides in soils can range from direct acute and chronic toxicity in organisms to many sublethal or indirect effects on behavior, functional roles, predator-prey relationships, and food web dynamics. Any or all of these can occur at the organism, population, or community levels and, therefore, may impact soil biodiversity or ecosystem stability (Edwards 2002). Since soil biodiversity is related to ecological functions that support ecological services (Benayas et al. 2009), pesticide-induced disruptions to biodiversity and ecological function could impair ecosystem services derived from soils (Goulson 2013). Impacts on soil biodiversity and their implications for ecosystem function have been demonstrated for other pesticides affecting microbial (Johnsen et al. 2001) and invertebrate (Jansch et al. 2006) communities, and the same risks are likely to arise from neonicotinoid insecticides in soils. Neonicotinoids can persist in soils for several years (Goulson 2013; Bonmatin et al. 2014, this issue) and can cause significant adverse effects on key soil organisms at environmentally realistic concentrations (Pisa et al. 2014, this issue) and, therefore, have the potential to pose a risk to soil ecosystem services.

While the link between adverse effects on organisms and ecological function or services in soils is theoretically sound, empirical evidence of effects on soil ecosystem services from neonicotinoid insecticides is sparse, partly because its large-scale use started only a decade ago. In our review of the literature, we found only a few studies that reported the effects of neonicotinoids on soil organism function with implications for ecosystem services. Peck (2009a, b) assessed the impacts of the neonicotinoid, imidacloprid, applied to turfgrass for scarab beetle control and found direct and indirect long-term effects on some arthropods and suggested negative implications (although not empirically tested) for soil nutrient cycling and natural regulation of pests. In laboratory microcosms,

Kreutzweiser et al. (2008a, 2009) tested the effects of imidacloprid in the leaves from systemically treated trees on the breakdown of autumn-shed leaves by litter dwelling earthworms over a 35-day exposure period. At realistic field concentrations, the leaf-borne residues of imidacloprid were not directly toxic to earthworms, but did cause feeding inhibition that resulted in a significant reduction in leaf litter breakdown. They further demonstrated that this effect was due to sublethal toxic effects, not avoidance behavior (Kreutzweiser et al. 2009). When imidacloprid was added directly to terrestrial microcosms to simulate a soil injection method for treating trees, a similar effect was detected with significantly reduced breakdown of leaf litter by earthworms at ambient litter concentrations of 7 mg/kg and higher (Kreutzweiser et al. 2008b). Taken together, these studies demonstrated that when imidacloprid is applied as a systemic insecticide for the control of wood-boring insects in trees, residual imidacloprid in autumn-shed leaves poses risk of reduced leaf litter breakdown through a feeding inhibition effect on earthworms, and this has negative implications for organic matter dynamics in soils. A similar effect would presumably occur in the breakdown of other imidacloprid-bearing plant litter in other soils, including agricultural but, to our knowledge, this has not been tested directly. Other effects of neonicotinoids on earthworm behavior that may further influence ecological processes in soils (e.g., burrowing behavior) are reviewed in Pisa et al. (2014, this issue).

Soil microbial communities have also been affected by imidacloprid, which can affect leaf litter decomposition. Although imidacloprid did not inhibit microbial decomposition of autumn-shed leaves of ash trees (*Fraxinus* spp.) (Kreutzweiser et al. 2008b), microbial decomposition of leaves from maple (*Acer saccharum*) trees was significantly inhibited at concentrations expected from systemic treatments to control wood-boring insects (Kreutzweiser et al. 2008a). The authors offer suggestions for observed differences in effects among tree species. Regardless of differences between studies, the data indicate that imidacloprid residues in leaf material have the potential to interfere with microbial decomposition of leaf litter, with implications for organic matter breakdown and nutrient cycling.

Others have assessed the effects of imidacloprid on microbial activity in agricultural soils after treated seed applications. Singh and Singh (2005a) measured microbial enzyme activity as an indicator of population level effects and found that imidacloprid in soils after seed treatment had stimulatory effects on microbial enzyme activity for up to 60 days. In the same set of experiments, they also measured available N in soils and reported increased available N (Singh and Singh 2005b). In a further study at the same site, Singh and Singh (2006) found increased nitrate-N but decreased ammonium, nitrite-N, and nitrate reductase enzyme activity in soils in which imidacloprid-coated seeds had been planted. Tu (1995) added imidacloprid to sandy soils and reported decreased fungal abundance and short-term decreases in

phosphatase activity but no measurable effects on nitrification or denitrification rates. Ingram et al. (2005) reported no inhibition of microbial urease activity by imidacloprid in turfgrass soil or sod. Similarly, Jaffer-Mohiddin et al. (2010) found no inhibition, and some stimulation, of amylase and cellulase activity in soils under laboratory conditions. Ahemad and Khan (2012) measured decreased activity and plant growth promoting traits of a N-fixing bacterium, *Rhizobium* sp., isolated from pea nodules of plants exposed to imidacloprid in soils, but only at three times the recommended application rate (no significant effects at the recommended rate). Overall, these studies demonstrate that neonicotinoids can induce measurable changes in soil microbial activity but the effects are often stimulatory, short-term, and of little or no measurable consequence to soil nutrient cycling. The reported microbial responses have been attributed to inductive adaptation as microbes assimilate or mineralize components of the imidacloprid molecule (Singh and Singh 2005a), essentially a biodegradation process (Anhalt et al. 2007; Liu et al. 2011; Zhou et al. 2013; Wang et al. 2013).

By contrast, at least two other studies have reported adverse or negative effects of neonicotinoids on soil microbial communities and their function. Yao et al. (2006) reported significantly inhibited soil respiration at field realistic concentrations of acetamiprid. Cycon et al. (2013) found measurable changes in soil community structure and diversity, and that these were generally found in conjunction with reduced soil metabolic activity at or near realistic field rates of imidacloprid. It is possible that community level changes associated with the neonicotinoid exposure may facilitate the adaptive responses in functional parameters listed above.

Conclusions on soils as ecosystem services

Given that many soil ecosystem services are dependent on soil organisms, that neonicotinoid insecticides often occur and can persist in soils, and that their residues pose a risk of harm to several key soil invertebrates, neonicotinoids have the potential to cause adverse effects on ecosystem services of soils. From a theoretical perspective and based on findings from studies of better-studied pesticides, the potential for neonicotinoid impacts on soil ecosystem services appears to be high but there are few empirical studies that have tested these effects. From the few studies available, it appears that invertebrate-mediated soil processes are at greater risk of adverse effects from neonicotinoid residues than are microbial-mediated processes.

One issue that remains elusive is the degree to which soil biological communities can absorb pesticide impacts before ecosystem function, and ultimately, the delivery of services is measurably impaired at a local or regional scale. Studies are conflicting with regard to the degree of functional redundancy and resilience inherent in soil and other biological communities that are rich in diversity. Swift et al. (2004) review the impacts of agricultural practices, including the use of pesticides, on the

relationship between biodiversity and ecosystem function and show that some changes in biological communities can be harmful to ecosystem function while others are functionally neutral. They suggest that microbial communities have a high degree of functional redundancy and resilience to impacts on their functional role in soil organic matter processing. On the other hand, reductions in highly specialized taxa with unique or critical roles in an important ecosystem function such as decomposition and nutrient cycling can measurably impact the delivery of ecosystem services (Barrios 2007). Earthworms could be categorized as such, and since adverse effects on earthworms have been reported at realistic concentrations of neonicotinoids in soils and leaf litter, this provides reasonable evidence that some soil ecosystem services can be impaired by the use of neonicotinoid insecticides. Further empirical studies coupled with ecological modeling to test the likelihood and extent of these effects are warranted.

Freshwater ecosystem functions

Nutrient cycling and water quality

Pollution by pesticides is widely recognized to be a major threat to freshwater ecosystems worldwide (Gleick et al. 2001; MEA 2005). Freshwater ecosystems provide an important array of ecosystem services, ranging from clean drinking water and irrigation water to industrial water, water storage, water recreation, and an environment for organisms that support fish and other important foods. Invertebrates make up a large proportion of the biodiversity in freshwater food chains and are a critical link for transfer of energy and nutrients from primary producers to higher trophic levels both in the aquatic and terrestrial ecosystems. Thus, alteration of invertebrate abundance, physiology, and life history by insecticides can have a serious impact on services provided by freshwater ecosystems. Equally, their role in decomposition of organic matter and nutrient cycling offers an essential purification service of water used for human consumption or to support aquatic life.

Peters et al. (2013) conducted a review of the effect of toxicants on freshwater ecosystem functions, namely leaf litter breakdown, primary production, and community respiration. For the review, 46 studies met their empirical specifications (for example, effect size and control treatment available). An important outcome of their review is that in over a third of the observations, reduction in ecosystem functions was occurring at concentrations below the lower limits set by regulatory bodies to protect these ecosystems. These lower limits were often set using LC₅₀ values for common test species like *Daphnia magna*, with risk assessment procedures not including more sensitive species or consideration of species that have critical roles in maintaining ecosystem function. A key

shortcoming of the review of Peters et al. (2013) is that a large number of the included studies involved effects of organophosphates, pyrethroids, and carbamates, but no information is given for the newer insecticide classes such as neonicotinoids or fipronil.

Relatively few studies have formally tested the effects of neonicotinoids or fipronil on ecosystem services in freshwater systems. A recent study by Agatz et al. (2014) did consider the effect of the neonicotinoid, imidacloprid, on the feeding activity of *Gammarus pulex*, a common freshwater amphipod that plays an important role in leaf litter breakdown. Prolonged inhibition of feeding after exposure was found at concentrations of imidacloprid (0.8 to 30 µg/L) that are within the range of those measured in several aquatic environments. Reduced leaf feeding and altered predator-prey interactions of a similar shredder species, *Gammarus fossarum*, have been reported at thiacloprid concentrations of 1–4 µg/L (Englert et al. 2012). Similar findings have been shown for other shredder species, stonefly (*Pteronarcyidae*) and crane fly (Tipulidae) larvae, exposed to imidacloprid in leaves and in water exhibiting mortality at 130 µg/L and feeding inhibition at 12 µg/L when applied directly to water but were more tolerant when exposed through the leaves (Kreutzweiser et al. 2008a). In a second study, the authors were able to determine that the effects on feeding inhibition were important in reducing leaf litter decomposition rates at concentrations of 18 to 30 µg/L (Kreutzweiser et al. 2009).

Prolonged exposure, or exposure to multiple compounds, might affect this and other shredder populations. Although not widely measured, inhibition of this functional feeding group has the potential to negatively affect the conversion of coarse terrestrial material into fine particulates that can be more readily consumed by other species. This in turn is expected to alter the aquatic invertebrate community, decomposition rates, and nutrient cycling, ultimately influencing water quality and the support of biodiversity which is an important ecosystem service. It should be noted that *G. pulex* is more sensitive to imidacloprid than *Daphnia* species and that both are crustacea and not insects. Several insects tend to be much more sensitive than *G. pulex* to imidacloprid so the risk to decomposition processes might be larger than has been assessed by studies with *G. pulex*, depending on the affected species role in the function of ecosystems and the amount of functional redundancy in the community (Beketov and Liess 2008; Ashauer et al. 2011).

Aquatic food chain effects

Ecosystem services related to decomposition and nutrient cycling are important for water quality; however, there is an additional concern for potential indirect effects of insecticides in reducing important invertebrate prey. This may be critical for many freshwater species that are valued for food (e.g., fish

and crayfish) and for ecological reasons (amphibians and aquatic birds). While rarely studied, indirect food chain effects have been reported in freshwater systems. For example, Hayasaka et al. (2012a) performed an experimental rice paddy mesocosm study using the systemic insecticides imidacloprid and fipronil, applied at recommended rates. Zooplankton, benthic, and neuston communities in the imidacloprid-treated field had significantly lower species abundance than those from control. Hayasaka et al. (2012a, b) further found that two annual applications of imidacloprid and fipronil were important in reducing benthic arthropod prey which led to reductions in growth of medaka fish (*Oryzias latipes*). Sánchez-Bayo and Goka (2005, 2006) also studied the ecological changes in experimental paddies treated with imidacloprid throughout a cultivation period. A total of 88 species were observed, with 54 of them aquatic. They reported plankton, neuston, benthic, and terrestrial communities from imidacloprid-treated fields had significantly lower abundance of organisms compared with control. Our knowledge about how aquatic communities react to, and recover from, pesticides, particularly in relation to the water residues, is deficient (Sánchez-Bayo and Goka 2005, 2006).

While not conclusively proven, many of the insectivorous bird species declines are also coincident with agricultural areas using these pesticides and speculation about recent population declines through reductions in emergent invertebrate prey from insecticide use seems plausible given the correlative evidence (Benton et al. 2002; Boatman et al. 2004; Mason et al. 2012). Neonicotinoids are the latest generation of pesticides that have the ability to enter freshwater bodies and negatively affect invertebrate populations which in turn can reduce emergent insects that numerous water-dependent birds and other wildlife depend on. A recent study by Hallmann et al. (2014) is the first to demonstrate the potential cascading effect of low neonicotinoid concentrations in water to insectivorous birds. Future studies should consider the importance of pesticide effects at the community level considering the intricate interaction among species in the trophic chain and the indirect effects on species deemed important for human consumption, recreation, or esthetic value.

Conclusions on freshwater ecosystem functions

Many aquatic species are directly exposed to neonicotinoid and fipronil insecticides in water, often over prolonged periods. Data from long-term and large-scale field monitoring by Van Dijk et al. (2013) have demonstrated the negative effects of imidacloprid on invertebrate life. Such negative impacts have the potential to adversely alter the base of the aquatic food web given that this group is a critical link for the transfer of nutrients and energy from primary producers to consumers. Reductions in survival, growth, and reproduction of freshwater organisms, particularly aquatic insects and crustaceans, can alter ecosystem functions related to decomposition and

nutrient cycling. These processes are central to providing ecosystem services such as clean freshwater and the support of biodiversity. Equally important are the effects on the trophic structure, which can influence the stability, resilience, and food web dynamics in aquatic ecosystems, but also terrestrial ecosystems given that many aquatic insects have adult life stages out of the water.

Fisheries and aquaculture

Sustainably managed fisheries and aquaculture can offer solutions to a growing demand for aquatic animal protein sources. In Africa, Asia, and Latin America, freshwater inland fisheries are providing food to tens of millions of people (Dugan et al. 2010) while ensuring employment, especially to women (BNP 2008). Pesticide use could hamper the successful expansion of global fisheries as well as small-scale inland fisheries, aquaculture, and combined rice-fish farming systems, if those pesticides are negatively affecting fisheries.

Neonicotinoid use has been increasing in fish farming and aquaculture environments because of their relatively low acute toxicity to fish and their effectiveness against sucking parasites and pests. For example, imidacloprid (neonicotinoid) is replacing older pesticides, such as pyrethroids to control rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) infestations in rice-crayfish (*Procambarus clarkii*) rotations (Barbee and Stout 2009) and carbamates (carbaryl) for controlling indigenous burrowing shrimp on commercial oyster beds in Washington (USA) (Felsot and Ruppert 2002). In both of these cases, nontarget effects of imidacloprid to the main fishery have been demonstrated. The degradation of water quality by neonicotinoid pesticides and the resulting ecotoxicological impacts on aquatic organisms are among those risks considered here.

Threats to cultured fish stocks

The majority of insecticides can affect cultured fish production and other nontarget animals in rice paddy systems. Several wild fish species inhabit the paddy and adjacent drains (Heckman 1979) and can be subjected to the effects of pesticides applied routinely. Fish may be affected indirectly by reductions in food resources, particularly aquatic invertebrates (Sánchez-Bayo and Goka 2005, 2006; Hayasaka et al. 2012a, b). Although known to have higher lethal tolerance to neonicotinoids, fish can be exposed to sublethal concentrations and their accompanying surfactants, which can cause adverse effects. Imidacloprid was shown to cause a stress syndrome in juvenile Japanese rice fish (medaka). As often happens with stressed fish, a massive infestation by a parasite, *Trichodina ectoparasite*, was observed in medaka fish in imidacloprid-treated fields (Sánchez-Bayo and Goka 2005). In a recent study, Desai and Parikh (2013)

exposed freshwater teleosts, *Oreochromis mossambicus* and *Labeo rohita*, to sublethal concentration ($LC_{50/10}$ and $LC_{50/20}$) of imidacloprid for 21 days and found significant alterations in several biochemical parameters (ALT, AST, ALP, and GDH). Increased enzyme activity in tissues indicated liver damage, which the authors concluded, was linked to imidacloprid exposure.

While acute mortality of fish from the neonicotinoid insecticides is rare, Rajput et al. (2012) reported that imidacloprid was toxic to freshwater catfish, *Clarias batrachus*, when exposed for 21 days, but only at high doses. Protein loss was reported when exposed to high concentrations that later caused lethality. Although this catfish has the potential to become a particularly harmful invasive species in some areas, it is also considered to be one of the most important catfish species in aquaculture given its economic value as food for human populations throughout most of India.

Shellfish aquaculture

Studies of shellfish aquaculture where neonicotinoids and fipronil are in use are rare. Dondero et al. (2010) reported negative sublethal effects of imidacloprid and thiacloprid at the transcriptomic and proteomic levels in the marine mussel, *Mytilus galloprovincialis*. In the Willapa Bay (Washington State, USA), imidacloprid is applied directly to exposed sediments, when the tide is out, to control native species of burrowing shrimp (*Callinassa* sp.; *Upogebia* sp.) that can negatively affect oyster production, but its effects on nontarget organisms are unknown. According to Felsot and Ruppert (2002), there was a rapid dissipation of imidacloprid from water and it was hypothesized that this could be due to extensive dilution by the tide. However, it was noted that there is a lack of studies concerning its behavior in the wider estuary ecosystem. Environmental monitoring programs are needed to evaluate exposure to salmonids following the treatment of oyster beds. Potential for adverse effects from exposure to nontarget species residing in the bay, such as juvenile Chinook (*Oncorhynchus tshawytscha*) and cutthroat trout (*Oncorhynchus clarki*), is unknown. Neonicotinoids are frequently detected in estuaries among the pollutants found in estuarine areas where oyster farms are located. Although few reports are available, anecdotal data suggest that neonicotinoids are present in estuary environments and might exert effects on cultured shellfish species or the wider ecosystem, but overall, studies to determine impacts are lacking.

Neonicotinoids in fish-rice ecosystems

The development of rice-fish farming systems has been viewed as a sustainable option for rural development, food security, and poverty alleviation. Rice-fish farming systems still frequently rely on insecticides to protect rice crops against

sucking insect pests, although Integrated Pest Management (IPM) practices are recommended to reduce the use of insecticides and their potential negative effects on fish populations. Imidacloprid is known to persist in treated rice paddy waters, demonstrating that it does not completely degrade in this aquatic environment, and in fact, Tišler et al. (2009) report that imidacloprid concentrations are increasing in rice paddies. Pesticides can move from treated rice field water to natural water bodies (Heong et al. 1995; Scientific & Technical Review Panel 2012). A study by Elfmann et al. (2011) in the Philippines showed that pesticides are frequently found in downstream rivers (Scientific & Technical Review Panel 2012). Given their persistent nature, it is likely that neonicotinoid insecticides used in rice paddies will also move to natural waters and downstream reaches.

Conclusion on risks to cultured fisheries

The nutritional benefits of fish consumption have a positive link to increased food security and decreased poverty rates in developing countries. Reducing access to fish for consumption could have particular impact on human populations living in less developed countries, where there is limited access to sufficient food. In some countries, high protein meat produced by fisheries can become an important low-cost nonstaple food source.

As with many other contaminants that have threatened natural and managed aquatic ecosystems, neonicotinoids and fipronil may offer an additional threat to cultured fish production. To ensure long-term sustainability and food security from fisheries (Pauly et al. 2002, 2005), the use of persistent and toxic insecticides in or near fish culture systems should be minimized if those insecticides have been shown to pose risk of harm to fish and their prey species. Although fish appear to have a relatively high toxicity threshold to neonicotinoids, indirect and sublethal effects have been observed from exposure to environmentally relevant concentrations of fipronil, imidacloprid, and thiacloprid. While intensive fish farming can provide important food sources, there is potential for combined or synergistic toxicological effects of diverse contaminants, including neonicotinoids, to threaten fish farm species and other aquaculture commodities.

Biological pest control

Predators as natural pest control

Invertebrate predator-prey relationships are an important part of many natural and agricultural ecosystems. Diversity and interdependence of species strongly influence shape and complexity of food webs. Food web complexity and especially the presence of predators are important for humans when

considering the natural regulation of invertebrate “pests.” Predation (including parasitism) of invertebrate pests by a diverse array of invertebrate and vertebrate predators can be considered an important ecosystem service, often called “biological control” in agricultural systems (Schlapfer et al. 1999; Wilby and Thomas 2002; Bradley et al. 2003).

Although only pest species are targeted by the insecticide, both the pest and natural predators can be affected. Often, the pest, however, exhibits life history strategies that allow their populations to recover faster than their predators. Many of the pest predators are insects and, thus, are also sensitive to neonicotinoid insecticides. In Pisa et al. (2014, this issue), several examples of affected predatory insect species are given but that review is by no means complete. A growing number of studies indicate that predator species and their ecosystem service are at risk when neonicotinoids are used (see reviews by Desneux et al. 2007 and Hopwood et al. 2013). Hopwood et al. (2013) conclude on the basis of more than 40 toxicity studies across a range of biological pest control species that the widespread use of neonicotinoids negatively impacts predatory and parasitoid species that provide much needed biological control of crop pests. Losey and Vaughan (2006) estimated that the value of natural control agents to control native North American pests is about 13.6 billion dollars, which includes pest predators, but also weather and pathogens.

Pollination

Pollination as an ecosystem service

Pollination is considered one of the most essential regulating as well as supporting ecosystem services (Kremen et al. 2007; De Groot et al. 2010; Vanbergen and the Insect Pollinator Initiative, 2013) and may be considered as a cultural ecosystem service as well (esthetics). Biologically mediated pollination is the active or passive transfer of pollen within or between flowers via invertebrate, mammalian, or avian vectors. It is a critical service for fruit, vegetables, nuts, cotton, and seed crop production among many others for agricultural crops and supports reproduction of wild plant communities (Allen-Wardell et al. 1998; Aguilar et al. 2006; UNEP 2010; Ollerton et al. 2011; Lautenbach et al. 2012; Vanbergen and the Insect Pollinator Initiative, 2013).

Without pollination, the fecundity of plants is affected, potentially leading to yield losses in cultivated crops and genetic diversity loss or local extinction in wild plants. Crops can be animal-pollinated, wind-pollinated, self-pollinated, or a combination. In many crops that constitute the human diet, pollination is essential for the setting of fruits and seeds; in others, it promotes these processes in varying gradations. Consequently, the measure of yield increase due to

pollination in crops varies greatly; some crops not showing a yield increase, while others do not produce fruits or seeds unless pollinated (Richards 2001; Klein et al. 2007).

There is a growing concern worldwide about the fate of insect-pollinating species and pollinating services (Potts et al. 2010; Van der Sluijs et al. 2013; Vanbergen and the Insect Pollinator Initiative, 2013; Pisa et al. 2014, this issue). A range of environmental changes that are currently taking place worldwide affect populations of wild and managed pollinating species. These include exposure to toxic chemicals, habitat loss and fragmentation, climate change, pathogens, land-use intensification, parasites, and the spread of invasive species and diseases (Steffan-Dewenter et al. 2002; Tylianakis et al. 2005; Biesmeijer et al. 2006; Kuldna et al. 2009; Potts et al. 2010; Vanbergen and the Insect Pollinator Initiative, 2013).

Sánchez-Bayo and Goka (2014) demonstrated that field realistic residues of neonicotinoid insecticides in pollen pose high risk to honeybees and bumblebees, while in the field synergisms with ergosterol inhibiting fungicides will further amplify these risks. They found that imidacloprid poses the highest risk to bumblebees (31.8–49 %, probability to reach the median lethal cumulative dose after 2 days of feeding on field realistic dose in pollen) and thiamethoxam the highest risk to honeybees (3.7–29.6 %). Other pollinators were not included in their risk assessment. An increase in AChE activity in honeybees was related to in-field exposure to corn pollen in neonicotinoid seed-treated fields (Boily et al. 2013). Because of the persistence of neonicotinoids in soil and water and their use as systemics, which facilitate uptake by wild plants and agricultural crops, all pollinators can be exposed to these insecticides at lethal or sublethal concentrations through multiple exposure routes (Van der Sluijs et al. 2013). Neonicotinoids and fipronil have known lethal and sublethal effects on domestic and wild insect pollinator populations at extremely low concentrations, often reported in the parts per trillion range (Pisa et al. 2014, this issue).

Pollination of crops

Pollinating services are provided by managed honeybees (*Apis mellifera*), but also by wild species such as solitary, stingless bees and bumblebees. In addition, flies, butterflies, wasps, moths, beetles, and other invertebrates and, in some cases vertebrates (such as bats, squirrels, birds and some primates), are also known to pollinate natural plants and crops (Buchmann 1997; Klein et al. 2007; De Luca and Vallejo-Marín 2013; Ghanem and Voigt 2012; Vanbergen and the Insect Pollinator Initiative, 2013). Over 25,000 species of bees have been identified (FAO 2013a), which are responsible for a large portion of pollination services worldwide (Danforth et al. 2006; Breeze et al. 2011). In Europe alone, more than 2,500 species of bees are known pollinators (Vaissiere et al. 2005).

Contrary to popular belief, estimates for the UK indicate that managed honeybees (*A. mellifera*) pollinate approximately one third of the crops, at most (Breeze et al. 2011). Although debated, there is evidence that numerous wild bee species also contribute substantially to the quality and reliability of pollination of a broad range of crops (e.g., Chagnon et al. 1993; Bosch et al. 2006; Greenleaf and Kremen 2006; Hoehn et al. 2008; Lye et al. 2011). Wild insect pollinator species are regarded as the most effective pollinators on fruit crops and seem to be more sensitive to pesticides than honeybees (Cresswell et al. 2012; Laycock et al. 2012). Economic gain from insect pollination on crops increases significantly with increasing numbers of wild bee species in the European Union (Leonhardt et al. 2013). In addition, bumblebees (*Bombus* spp.) are the predominant or exclusive pollinators of many wild plant species (Goulson 2003).

Pollination of wild plants

In addition to pollinating crops, which make up <0.1 % of all flowering plants worldwide, between 60 and 85 % of wild angiosperms (flowering plants) require animal pollinators (Kearns and Inouye 1997; Ashman et al. 2004). Ollerton et al. (2011) estimated that 299,200 species (85 %) of angiosperms depend on pollinators worldwide. However, this estimate does not account for the mean proportion of angiosperms per latitude, varying from 78 % of species in temperate zones up to 94 % in tropical regions. Vanbergen and the Insect Pollinator Initiative, (2013) estimated that insects enable reproduction globally for up to 94 % of wild flowering plants. Pollination of wild plants contributes to human welfare indirectly, of which some examples are esthetics of the landscape, the pleasure of looking at foraging bumblebees in richly flowering meadows, and providing forage for wildlife (Jacobs et al. 2009). Pollination is also instrumental in increasing the genetic diversity in plant species (Benadi et al. 2013).

The impact of insect pollinator loss on ecosystem function is not well understood, although a few cases have been described. An example of a subtle but important interaction is the one between wild species and honeybees. Greenleaf and Kremen (2006) studied pollinator efficiency of honeybees on sunflowers and discovered a fivefold increase in efficiency in the presence of wild bees. Such phenomena are likely to occur in natural environments as well, meaning that the loss of one species can radically alter pollination dynamics of wild plants in affected communities. Furthermore, knowing that the survival of certain host plants is directly linked to the survival of their pollinating species (Kim 1993), this can have a knock-on effect in the biotic community. For instance, Kearns and Inouye (1997) describe how keystone species such as fig trees, one of the 750 species often dependent on a distinct and unique wasp species for pollination, provide the staple food for many species of vertebrate wildlife in tropical

communities. The loss of these wasps has the potential to lead to a complete shift in biotic community structure of these areas. The same goes for other areas with specialized pollinator-plant interactions, such as South Africa (Ollerton et al. 2011).

Although wild plants are often dependent on multiple pollinators or may be able to use wind pollination, it is important to realize that pollinating insects fulfill a crucial role in the ecological food webs. Loss of pollinating species can also affect other networks, thus leading to impairment in ecosystem functioning as a whole (Bartomeus et al. 2013; Burkle et al. 2013; Labar et al. 2013).

Conclusions on ecosystem services from pollinators and other beneficial insects

The role of insects as consumers, predators, pollinators, and decomposers in ecosystems is critical for ecosystem function. High sensitivity of many key pollinating and predating insect species to neonicotinoids, combined with the high risk of exposure, raises concerns about the (long-term) impact of these substances. Adverse impacts of wide-scale insect pollinator and predator loss include cascade effects in biotic communities that can ultimately affect human populations. In human dimensions, the ecosystem services pollination and biological control together represent an estimated global value of about US\$215 billion in 2005 (Vanbergen and the Insect Pollinator Initiative, 2013). The global loss of bee species, as bioindicators of environmental health, is an early warning that global biodiversity and ultimately, human welfare, may be threatened.

Food security

Pollinator-dependent crops

Although the estimated percentage of human food that depends on bee-pollinated crops is relatively small, 15–30 % (O'Toole 1993, in Kearns and Inouye 1997; Greenleaf and Kremen 2006), important components of food production, diversity, security, and stability rely on animal pollinators (Steffan-Dewenter et al. 2002, 2005). Of the 124 major commodity crops directly used for human consumption, 87 (70 %) are dependent on pollination for enhanced seed, fruit, or vegetable production. These 87 crops are essential to our quality of life providing the quality and diversity of the vegetables and fruits we eat and amount to 23×10^8 megatons (35 %) of global food production volume, although only part of this amount is directly attributable to pollination (Klein et al. 2007).

Roubik (1995, in Klein et al. 2007) provided a list of 1,330 tropical crops, of which ca. 70 % have one or more varieties

that show improved production after animal pollination. More specifically, for the European situation, 84 % of crop species produced depend on pollination (Williams 1994), with a total of 12 % of the total cropland area dependent on pollination (Schulp et al. 2014).

The relative importance of crop pollination as an ecosystem service is increasing worldwide. In 2006, pollinator-dependent crops contributed 16.7 and 9.4 % more to total agricultural production in the developed and developing world, respectively, than in 1961 (Aizen et al. 2008; Aizen and Harder 2009). Since then, the continued and foreseen increase in the production of pollinator-dependent crops such as oil palm, sunflower, and canola (FAO 2013b; Schulp et al. 2014) indicates a further rise in these percentages.

The economic value of pollination

The economic value of pollination services can be considered to be the marginal increase in plant production due to pollination (Kremen et al. 2007), for those plants that have a market or subsistence value to humans. Examples are crops used for food or feed, timber, or fiber. Therefore, the loss of insect pollinators has large potential consequences on human food production directly through reduced crop yields. Richards (2001) provides a good overview of impacts on crop yield through inadequate pollinator service. Although pollinator decline was not documented to affect crop yield on a global scale in 2008 (Aizen et al. 2008), there is evidence on a local scale that declines in pollinator (diversity) affect fruit set and seed production (Brittain et al. 2013). The absence of pollinators thus would translate into a 7 % drop in crop production in the EU (Schulp et al. 2014). These crops are nonetheless those that bring our diversity of food in civilized societies and quality of life (Klein et al. 2007).

A second impact of pollinator loss is the reduced production of crops that become less valued by the consumer and are therefore sometimes nonsaleable. Some examples are cucumbers and apples, of which the fruits do not grow according to market standards without proper pollination. Lack of pollination will reduce their value or render them worthless (e.g., curled cucumbers, lopsided apples) (Morse and Calderone 2000).

Increased production costs are a third potential impact of pollinator loss. Almond farmers in the USA, which are completely dependent on commercial pollination services, have experienced a sharp increase in the price for crop pollination services since 2005, due to pollinator scarcity (Sumner and Boriss 2006; Carman 2011).

Many animal-pollinated crops are locally important for the economy of the region. Some examples are olives, sunflowers, and cotton that are not wholly dependent on pollinators, but production is enhanced. Several crops that are completely dependent on pollination are often specialty

products that are not sold on a large scale, such as vanilla (Richards 2001), but are nonetheless an essential resource to specific regions.

Several national studies (e.g., USA: Morse and Calderone 2000; Losey and Vaughan 2006) have applied dependence ratios per crop type, calculating the actual impact on crop production in the absence of pollinators. Although a potentially useful tool, the ratios that were used varied widely between studies and regions. Gallai et al. (2009) therefore aimed to provide an economic valuation of complete world insect pollinator loss, including economic vulnerability per region. The authors calculated a value of €153 billion, 9.5 % of the total value of crops produced globally for direct human consumption in 2005. In the EU, pollinator-dependent crops currently represent 31 % of the EU income from crop production. The total monetary value for insect-pollinating services therein is between 10 and 12 % (Leonhardt et al. 2013; Schulp et al. 2014).

Food supply and food quality

With the expected population growth in the coming decades, meeting the increasing food supply needs in a sustainable way will become a major challenge. The environmental consequence of the intensification of agricultural systems may pose a threat to the future accessibility to an adequate food supply (Matson et al. 1997). But beyond securing access to sufficient food for all people, the need to provide a supply of safe and nutritionally high-quality food to achieve a balanced diet has become an important consideration in order to avoid health impacts such as intellectual and physical disabilities. Access to a large diversity of fruit and vegetables also contributes to the enjoyment of quality foodstuff and food culture that contributes to overall social and cultural identity.

The capability of responding to the current human nutrient requirements is crucial, according to the World Health Organization (WHO 2006). Many people are affected by vitamin and mineral deficiencies, especially in developing countries where one out of three persons suffer from chronic undernourishment in energy and in micronutrients (vitamins and minerals). Eilers et al. (2011) studied the proportion of nutrients derived from more than 150 global leading crops and found that although minerals seem to be fairly evenly distributed over crop types, certain vitamins are scarcer in pollinator-independent crops. An example is the carotenoid group, in which 99.33 and 100 % of β -cryptoxanthin and lycopene, respectively, are provided by pollinator-dependent crops.

In contrast, the developments in agriculture worldwide have largely increased the production of staple foods such as potato, cassava, corn, rice, and wheat over the last 25 years (FAO 2013b). These staple crops are mostly wind- or self-pollinated or propagate otherwise, so do not depend on pollination services. Although these crops provide the required

caloric intake, they contain relatively low levels of most micronutrients. Globally, more than two billion people are affected by “hidden hunger,” a micronutrient deficiency caused by poor diet diversity (Welch and Graham 1999; Muthayya et al. 2013). Pollinator losses leading to reduced diet diversity, especially from plants that provide a larger array of micronutrients, may exacerbate the negative impact on health and economic development in certain regions.

Seed security and seed treatments

Seed security is seen as a key driver of food security (Sperling and McGuire 2012). Food production agronomic traits such as yield, early maturity, resistance to specific stresses, and also nutritional traits should be among the diverse goals of seed security (Sperling and McGuire 2012). Agroecosystems of even the poorest societies have the potential through ecological agriculture and IPM to meet or even exceed conventional yields produced by conventional methods and supply regional and international markets across the developing country regions (IAASTD 2009).

The increased and often prophylactic use of neonicotinoid seed-coated hybrids cannot be viewed as a sustainable way to protect crops from insect damage given the risks described to pollinators, soil organisms, and aquatic invertebrates. Seed treatments offer an easy incentive to farmers to act as a form of crop protection insurance by applying a treatment in anticipation of the pest problem. However, in order for this technique to be ecologically, economically, and socially viable, substantial gains must be seen in yields to offset risks to ecosystem health. In Britain, as elsewhere, agricultural practices have seen rapid increases in the use of neonicotinoid-treated seeds over the past decade. However, little or no gains have been observed in crop yields over the same period or those gains were not great enough to offset the cost of the seed treatment (Goulson 2013). For example, in Canada’s Prairie region, canola (oilseed rape) crops cover 8.5 million hectares of cropland, and 95 % of the canola seeded is coated with neonicotinoids (Main et al. 2014). The authors conservatively estimated that neonicotinoid use in that region of Canada amounted to 44 % of the cropland in a single year or 215,000 kg. Systemic seed treatments have facilitated the extended and widespread use of neonicotinoid insecticides in modern agriculture and represent a threat to agrobiodiversity and food security.

Insecticide resistance

Several crop pests have begun to develop pesticide resistance to neonicotinoids (Jeschke et al. 2011). Examples are imidacloprid and acetamiprid resistance in cotton aphids (*Aphis gossypii*) (Herron and Wilson 2011). Other crop pests that show neonicotinoid resistance are the Greenhouse

whitefly (*Trialeurodes vaporariorum*) (Karatolos et al. 2010) and the Colorado potato beetle (*Leptinotarsa decemlineata*) (Szendrei et al. 2012).

The development of insecticide resistance has also been reported for the brown planthopper (*Nilaparvata lugens*) in East Asian countries such as Vietnam, China, and Japan (Wang et al. 2008). Planthopper resistance to imidacloprid was reconfirmed in more recent studies (Azzam et al. 2011). Zhang et al. (2014) studied nine field populations of the brown planthopper (*N. lugens*) from Central China, East China, and South China, and resistance to insecticides was monitored from 2009 to 2012. All nine field populations collected in 2012 had developed extremely high resistance to imidacloprid, with resistance ratios ranging from 209.3 to 616.6. Resistance to neonicotinoids was much higher in 2012 than in 2009. The resistance ratio of thiamethoxam varied from 17.4 to 47.1, and the resistance ratio of nitenpyram varied from 1.4 to 3.7 in 2012. Of the nine field populations, six populations showed higher resistance to nitenpyram in 2012 than in 2011. Taken together, these reports demonstrate that the widespread use of neonicotinoids increases the rate of the development of target pest resistance. Insect resistance, in turn, usually results in increased application rates or frequency of an insecticide, leading to greater economic and environmental costs.

Conclusions on food security

The definition of food security within the United Nations framework includes the physical availability of food and its stability over time (FAO 2008). Quality and diversity of food and the ecological and social sustainability of the food production are also important parts of food security. Agriculture is becoming more pollinator dependent because of an increasing consumption of pollinator-dependent crops (Aizen et al. 2008). Neonicotinoid insecticides are recognized to be a threat to domestic pollinators such as honeybees but also many wild pollinator species. Although theoretically possible, a global decrease in crop yields and diversity of fruit and vegetables due to reductions in pollination has not yet been demonstrated, but evidence exists at regional scales. Widespread use of seed treatments does not necessarily increase crop yields, but appears to be threatening pollinator and soil health as well as promoting insect pest resistance. Extensive and wide-scale use of any single insecticide has the proven potential to become a threat to agrobiodiversity.

Agrobiodiversity can be thought of as the outcome of agricultural practices that produce a variety of crops, including those that provide essential micronutrients. The focus of future agriculture should not be limited to an increase in overall production, but should also consider the maintenance of genetic diversity in crop plants, which provide valued agronomic traits (Sperling and McGuire 2012). The preservation of

agrobiodiversity and seed security will be achieved by promoting varieties of crops already known in the area, making local (traditional) nutritious varieties more accessible. Many of these crops depend on insect pollination and are therefore at risk from widespread and persistent use of insecticides that negatively affect pollinators. In this regard, the use of neonicotinoid insecticides may threaten food security and the development of sustainable agriculture.

Conclusions

In this paper, we examine the potential impact of systemic insecticides, particularly neonicotinoids but also fipronil, on a variety of ecosystem functions and services. The paper explores the role and vulnerability of invertebrates in soil function and food production systems, as well as threats to the aquatic biodiversity that supports cultured fisheries. Clear evidence of the critical role of microbes, insects, and other invertebrates as consumers, predators, pollinators, and decomposers for the maintenance of healthy ecosystem functions and food production is presented. In exploring the indispensability of these organisms, their vulnerability to systemic insecticides has been highlighted. Most neonicotinoid insecticides are persistent in soil and water and can be found in dust particles during sowing of dressed seeds and are therefore likely to encounter and potentially affect a broad range of biological organisms that provide ecosystem services.

Neonicotinoid and fipronil pesticides are bioavailable in the environment at levels that are known to cause lethal and sublethal effects on a wide range of terrestrial, aquatic, and soil beneficial microorganisms, invertebrates, and vertebrates. These beneficial organisms possess a diversity of traits (e.g., nitrogen fixers, pollinators, and nutrient recyclers) that are key to healthy ecosystem functioning and services (Perrings et al. 2010). There is increasing evidence that the widespread use of neonicotinoids and fipronil is causing harm to these beneficial organisms, and therefore, those impacts have the potential for reducing ecosystem services, either consumptive (e.g., food, fuel) or nonconsumptive (e.g., health).

To help feed the world's population adequately, crop protection methods and products will always be needed to reduce yield losses caused by pests. But sustainable choices should be made while implementing pest control methods and products in order to alleviate potential harm for food security, ecosystem services, and the full functionality of all systems of the environment. Relying on pesticide tolerance and the selection of resistance traits and/or a functional resilience of ecosystems' communities (Köhler and Triebkorn 2013) as justification for the continued widespread and often prophylactic use of neonicotinoid and fipronil insecticides would be a perilous strategy for maintenance of ecosystem services. While the link between nontarget impacts of these systemic insecticides and

their effects on ecosystem services is not always clear in the published literature, their widespread use, persistent nature, and toxicity to a broad range of beneficial organisms are strong indications that ecosystem services dependent on these organisms may be at risk.

Acknowledgments This manuscript benefited from the discussions in the International Task Force on Systemic Pesticides during its plenary meetings in Paris (2010), Bath (2011), Cambridge (2012), Montegrotto-Padua (2012), Louvain-la-Neuve (2013), and Padua-Legnaro (2013). The authors are listed in alphabetic order, except the first who is also the corresponding author. All authors' work for public agencies or universities, except D.A. Noome, whose independent work for the TFSP is financed by the Stichting Triodos Foundation. The work has been funded by the Triodos Foundation's Support Fund for Independent Research on Bee Decline and Systemic Pesticides. This Support Fund has been created from donations by Adessium Foundation (The Netherlands), Act Beyond Trust (Japan), Utrecht University (Netherlands), Stichting Triodos Foundation (The Netherlands), Gesellschaft fuer Schmetterlingsschutz (Germany), M.A.O.C. Gravin van Bylandt Stichting (The Netherlands), Zukunft Stiftung Landwirtschaft (Germany), Study Association Storm (Student Association Environmental Sciences Utrecht University), and citizens. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Ahemad M, Khan MS (2012) Ecological assessment of biotoxicity of pesticides towards plant growth promoting activities of pea (*Pisum sativum*) specific Rhizobium sp. strain MRP1. Emirates J Food Agr 24:334–343
- Agatz A, Ashauer R, Brown CD (2014) Imidacloprid perturbs feeding of *Gammarus pulex* at environmentally relevant concentrations. Environ Toxicol Chem 33:648–653
- Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecol Lett 9:968–980
- Aizen MA, Harder LD (2009) The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr Biol 19:915–918
- Aizen MA, Garibaldi LA, Cunningham SA, Klein AM (2008) Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. Curr Biol 18: 1572–1575
- Allen-Wardell G, Bernhardt P, Bitner R, Burquez A, Buchmann S, Cane J, Cox PA, Dalton V, Feinsinger P, Ingram M, Inouye D, Jones CE, Kennedy K, Kevan P, Koopowitz H, Medellin R, Medellin-Morales S, Nabhan GP, Pavlik B, Tepedino V, Torchio P, Walker S (1998) The Potential Consequences of Pollinator Declines on the Conservation of Biodiversity and Stability of Food Crop Yields. Conserv Biol 12:8–17
- Anhalt JC, Moorman TB, Koskinen WC (2007) Biodegradation of imidacloprid by an isolated soil microorganism. J Environ Sci Health Part B 42:509–514

- Ashauer R, Hintermeister A, Potthoff E, Escher BI (2011) Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action. *Aquat Toxicol* 103: 38–45. doi:10.1016/j.aquatox.2011.02.002
- Ashman T-L, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85: 2408–2421
- Azzam S, Yang F, Wu JC, Geng J, Yang GQ (2011) Imidacloprid-induced transference effect on some elements in rice plants and the brown planthopper *Nilaparvata lugens* (Hemiptera: Delphacidae). *Insect Sci* 18:289–297
- Barbee GC, Stout MJ (2009) Comparative acute toxicity of neonicotinoid and pyrethroid insecticides to non-target crayfish (*Procambarus clarkii*) associated with rice-crayfish crop rotations. *Pest Manag Sci* 65:1250–1256
- Barrios E (2007) Soil biota, ecosystem services and land productivity. *Ecol Econ* 64:269–285
- Bartomeus I, Mia G, Lakso AN (2013) Biodiversity ensures plant–pollinator phenological synchrony against climate change. *Ecol Lett* 16: 1331–1338
- Beare MH, Coleman DC Jr, Crossley DA, Hendrix PF, Odum EP (1995) A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. *Plant Soil* 170:5–22
- Beketov MA, Liess M (2008) Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. *Environ Toxicol Chem* 27:461–470
- Benadi G, Blüthgen N, Hovestadt T, Poethke H-J (2013) When can plant–pollinator interactions promote plant diversity? *Am Nat* 182:131–146
- Benayas JMR, Newton AC, Diaz A, Bullock JM (2009) Enhancement of biodiversity and ecosystem services by ecological restoration: a meta-analysis. *Science* 325:1121–1124
- Benton TG, Bryant DM, Cole L, Crick HQP (2002) Linking agricultural practice to insect and bird populations: a historical study over three decades. *Jour Appl Ecol* 39:673–687
- Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers AP, Potts SG, Kleukers R, Thomas CD, Settele J, Kunin WE (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313: 351–354. doi:10.1126/science.1127863
- BNP (2008) Small-scale capture fisheries. A global overview with emphasis on developing countries. Big Numbers Project, Food and Agriculture Organization of the United Nations.
- Boily M, Sarrasin B, Deblois C, Aras P, Chagnon M (2013) Acetylcholinesterase in honey bees (*Apis mellifera*) exposed to neonicotinoids, atrazine and //glyphosate: laboratory and field experiments. *Environ Sci Pollut Res* 8:5603–5614
- Boatman ND, Brickle NW, Hart JD, Milsom TP, Morris AJ, Murray AWA, Murray KA, Robertson PA (2004) Evidence for the indirect effects of pesticides on farmland birds. *Ibis* 146:131–143
- Bonmatin J-M, Giorio C, Girolami V, Goulson D, Kreutzweiser D, Krupke C, Liess M, Long E, Marzaro M, Mitchell E, Noome D, Simon-Delso N, Tapparo A (2014) Environmental fate and exposure; neonicotinoids and fipronil. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3332-7
- Bosch J, Kemp WP, Trostle GE (2006) Bee population returns and cherry yields in an orchard pollinated with *Osmia lignaria* (Hymenoptera: Megachilidae). *J Econ Entomol* 99:408–413
- Bradley JC, Harvey CT, Gross K, Ives AR (2003) Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecol Lett* 6:857–865. doi:10.1046/j.1461-0248.2003.00508.x
- Brady NC, Weil RR (1996) The nature and properties of soil, 11th edn. Prentice Hall, New Jersey
- Breeze TD, Bailey AP, Balcombe KG, Potts SG (2011) Pollination services in the UK: how important are honeybees? *Agric Ecosyst Environ* 142:137–143. doi:10.1016/j.agee.2011.03.020
- Brittain C, Williams N, Kremen C, Klein A-M (2013) Synergistic effects of non-*Apis* bees and honey bees for pollination services. *Proc R Soc B Biol Sci* 280:20122767. doi:10.1098/rspb.2012.2767
- Brussaard L, Behan-Pelletier VM, Bignell DE, Brown VK, Didden W, Folgarait P, Fragoso C, Freckman DW, Gupta VVSR, Hattori T, Hawksworth DL, Klopatek D, Lavelle P, Malloch DW, Rusek J, Soderstrom B, Tiedje JM, Virginia RA (1997) Biodiversity and ecosystem functioning in soil. *Ambio* 26:563–570
- Brussaard L, de Ruiter PC, Brown GG (2007) Soil biodiversity for agricultural sustainability. *Agric Ecosyst Environ* 121:233–244
- Buchmann SL (1997) The forgotten pollinators. Island Press, Washington, DC, p 312
- Burkle LA, Marlin JC, Knight TM (2013) Plant–pollinator interactions over 120 years: loss of species, co-occurrence and function. *Science* (New York, N.Y.) 1611
- Carman H (2011) The estimated impact of bee colony collapse disorder on almond pollination fees. *ARE Update* 14(5):9–11, University of California Giannini Foundation of Agricultural Economics
- Chagnon M, Gingras J, De Oliveira D (1993) Complementary aspects of strawberry pollination by honey and indigenous bees (Hymenoptera). *J Econ Entomol* 86:416–420
- Costanza R, Arg R, Groot R De, Farberk S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill RV, Paruelo J, Raskin RG, Sutton P, Van den Belt M (1997) The value of the world's ecosystem services and natural capital. *Nature* 387:253–260
- Cresswell JE, Page CJ, Uygun MB, Holmbergh M, Li Y, Wheeler JG, Laycock I, Pook CJ, De Ibarra NH, Smirnoff N, Tyler CR (2012) Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). *Zoology* 115:365–371
- Cycon M, Markowicz A, Borymski S, Wojcik M, Piotrowska-Seget Z (2013) Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities. *J Environ Manag* 131:55–65
- Dale VH, Polasky S (2007) Measures of the effects of agricultural practices on ecosystem services. *Ecol Econ* 64:286–296
- Danforth BN, Sipes S, Fang J, Brady SG (2006) The history of early bee diversification based on five genes plus morphology. *Proc Natl Acad Sci U S A* 103:15118–15123
- De Groot RS, Alkemade R, Braat L, Hein L, Willemen L (2010) Challenges in integrating the concept of ecosystem services and values in landscape planning, management and decision making. *Ecol Complex* 7:260–272. doi:10.1016/j.ecocom.2009.10.006
- De Luca PA, Vallejo-Marín M (2013) What's the “buzz” about? The ecology and evolutionary significance of buzz-pollination. *Curr Opin Plant Biol* 16:429–435
- de Ruiter PC, Neutel AM, Moore JC (1995) Energetics, patterns of interaction strengths, and stability in real ecosystems. *Science* 269: 1257–1260
- Desai B, Parikh P (2013) Biochemical alterations on exposure of imidacloprid and curzate on fresh water fish *Oreochromis mossambicus* and *Labeo rohita*. (*Indian J Forensic Med Toxicol* 7: 87–91
- Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. *Annu Rev Entomol* 52:81–106
- Dominati EJ, Patterson MG, Mackay AD (2010) A framework for classifying and quantifying the natural capital and ecosystem services of soils. *Ecol Econ* 69:1858–1868
- Dondero F, Negri A, Boatti L, Marsano F, Mignone F, Viarengo A (2010) Transcriptomic and proteomic effects of a neonicotinoid insecticide mixture in the marine mussel (*Mytilus galloprovincialis*, Lam.). *Sci Total Environ* 408:3775–3786

- Dugan P, Delaporte A, Andrew N, O'Keefe M, Welcomme R (2010) Blue harvest: inland fisheries as an ecosystem service. WorldFish Center, Penang, Malaysia. United Nations Environment Programme 2010.
- Edwards CA (2002) Assessing the effects of environmental pollutants on soil organisms, communities, processes and ecosystems. *Eur J Soil Biol* 38:225–231
- Edwards CA, Bohlen PJ (1996) Biology and ecology of earthworms, 3rd edn. Chapman & Hall, London
- Elfmann L, Tooke NE, Patring JDM (2011) Detection of pesticides used in rice cultivation in streams on the island of Leyte in the Philippines. *Agricultural Water Management*, 101:81–87
- Eigenbrod F, Armsworth PR, Anderson BJ, Heinemeyer A, Gillings S, Roy DB, Thomas CD, Gaston KJ (2010) The impact of proxy-based methods on mapping the distribution of ecosystem services. *J Appl Ecol* 47:377–385
- Eilers EJ, Kremen C, Smith Greenleaf S, Garber AK, Klein A-M (2011) Contribution of pollinator-mediated crops to nutrients in the human food supply. *PLoS ONE* 6:e21363. doi:10.1371/journal.pone.0021363
- Englert D, Bundschuh M, Schulz R (2012) Thiacloprid affects trophic interaction between gammarids and mayflies. *Environ Pollut* 167: 41–46
- Felsot AS, Ruppert JR (2002) Imidacloprid residues in Willapa Bay (Washington State) water and sediment following application for control of burrowing shrimp. *J Agr Food Chem* 50:4417–4423
- FAO (2008) Food and Agriculture Organization of the United Nations. An introduction to the basic concepts of food security. Rome
- FAO (2013a) Food and Agriculture Organisation of the United Nations. Pollination. Accessible at: <http://www.fao.org/agriculture/crops/core-themes/theme/biodiversity/pollination/en/>
- FAO (2013b) Food and Agriculture Organisation of the United Nations. FAOSTAT database, accessible at: <http://faostat.fao.org/site/291/default.aspx>. Accessed 9 October 2013
- Gallai N, Salles J-M, Settele J, Vaissière BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol Econ* 68:810–821. doi:10.1016/j.ecolecon.2008.06.014
- Ghanem SJ, Voigt CC (2012) Increasing awareness of ecosystem services provided by bats. *Adv Stud Behav* 44:279–302
- Gibbons D, Morrissey C, Mineau P (2014) A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3180-5.
- Gleick PH, Singh A, Shi H (2001) Threats to the world's freshwater resources. Pacific Institute, Oakland
- Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. *J Appl Ecol* 50:977–987
- Goulson D (2003) Bumblebees: their behaviour and ecology. Oxford Univ. Press, Oxford
- Greenleaf S, Kremen C (2006) Wild bees enhance honey bees' pollination of hybrid sunflower. *Proc Natl Acad Sci U S A* 103:13890–13895
- Hallmann CA, Foppen RPB, van Turnhout CAM, de Kroon H, Jongejans E (2014) Declines of insectivorous birds are associated with high neonicotinoid concentrations. *Nature*. doi:10.1038/nature13531
- Hayasaka D, Korenaga T, Suzuki K, Saito F, Sánchez-Bayo F, Goka K (2012a) Cumulative ecological impacts of two successive annual treatments of imidacloprid and fipronil on aquatic communities of paddy mesocosms. *Ecotoxicol Environ Saf* 80:355–362
- Hayasaka D, Korenaga T, Sánchez-Bayo F, Goka K (2012b) Differences in ecological impacts of systemic insecticides with different physicochemical properties on biocenosis of experimental paddy fields. *Ecotoxicology* 21:191–201
- Heckman CW (1979) Rice field ecology in northeastern Thailand—the effect of wet and dry seasons on a cultivated aquatic ecosystem. W. Junk bv Publishers, The Hague
- Heong KL, Escalada MM, Lazaro AA (1995) Misuse of pesticides among rice farmers in Leyte, Philippines. In: Pingali PL, Roger PS (eds) Impact of pesticides on farmer health and the rice environment. Kluwer Acad. Publ, Norwell, pp 97–108
- Herron GA, Wilson LJ (2011) Neonicotinoid resistance in *Aphis gossypii* (Aphididae: Hemiptera) from Australian cotton. *Aust J Entomol* 50: 93–98
- Hoehn P, Tschamtké T, Tylianakis JM, Steffan-Dewenter I (2008) Functional group diversity of bee pollinators increases crop yield. *Proc R Soc B Biol Sci* 275:2283–2291
- Hopwood J, Black SH, Vaughan M, Lee-Maeder E (2013) Beyond the bees and the birds. Effects of neonicotinoid insecticides on agriculturally important beneficial invertebrates. The Xerxes Society for Invertebrate Conservation, Portland, p 32
- Ingram CW, Coyne MS, Williams DW (2005) Effects of commercial diazinon and imidacloprid on microbial urease activity in soil and sod. *J Environ Qual* 34:1573–1580
- IAASTD (International Assessment of Agricultural Knowledge Science and Technology for Development); United Nations Environment Programme (UNEP) (2009) Agriculture at a crossroads. Synthesis report. p 106
- Jacobs JH, Clark SJ, Denholm I, Goulson D, Stoate C, Osborne JL (2009) Pollination biology of fruit-bearing hedgerow plants and the role of flower-visiting insects in fruit-set. *Ann Bot* 104:1397–1404. doi:10.1093/aob/mcp236
- Jaffer-Mohiddin G, Srinivasulu M, Madakka M, Rangaswamy V (2010) Influence of insecticides on the activity of amylase and cellulase in groundnut soils. *Ecol Environ Conservat* 16:383–388
- Jansch S, Frampton GK, Rombke J, van den Brink PJ, Scott-Fordsmand JJ (2006) Effects of pesticides on soil invertebrates in model ecosystem and field studies: a review and comparison with laboratory toxicity data. *Environ Toxicol Chem* 25:2490–2501
- Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* 59: 2897–2908
- Johnsen K, Jacobsen CS, Torsvik V, Sørensen J (2001) Pesticide effects on bacterial diversity in agricultural soils—a review. *Biol Fertil Soils* 33:443–453
- Karatolos N, Denholm I, Williamson M, Nauen R, Gorman K (2010) Incidence and characterisation of resistance to neonicotinoid insecticides and pymetrozine in the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae). *Pest Manag Sci* 66:1304–1307
- Kearns CA, Inouye DW (1997) Pollinators, flowering plants, and conservation biology. *Bioscience* 47:297–307
- Kim KC (1993) Biodiversity, conservation and inventory: why insects matter. *Biodivers Conserv* 2:191–214
- Klein A-M, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tschamtké T (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings. Biological sciences / The Royal Society*, 274:303–13. doi:10.1098/rspb.2006.3721
- Köhler H-R, Triebkorn R (2013) Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? *Science* 341:759–765. doi:10.1126/science.1237591
- Kremen C, Williams NM, Aizen MA, Gemmill-Herren B, LeBuhn G, Minckley R, Packer L, Potts SG, Roulston T, Steffan-Dewenter I, Vázquez DP, Winfree R, Adams L, Crone EE, Greenleaf SS, Keitt TH, Klein A-M, Regetz J, Ricketts TH (2007) Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecol Lett* 10:299–314
- Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA, Thompson DG (2008a) Are leaves that fall from imidacloprid-treated maple trees to control Asian longhorned beetles toxic to non-target decomposer organisms? *J Environ Qual* 37:639–646

- Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA, Holmes SB, Thompson DG (2008b) Effects on litter-dwelling earthworms and microbial decomposition of soil-applied imidacloprid for control of wood-boring insects. *Pest Manag Sci* 64:112–118
- Kreutzweiser DP, Thompson DG, Scarr TA (2009) Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates. *Ecotoxicol Environ Saf* 72:1053–1057
- Kuldna K, Peterson K, Poltimäe H, Luig J (2009). An application of DPSIR framework to identify issues of pollinator loss. *Ecol Econ* 69: 32–42. doi:10.1016/j.ecolecon.2009.01.005
- Labar T, Campbell C, Yang S, Albert R, Shea K (2013) Global versus local extinction in a network model of plant–pollinator communities. *Theor Ecol* 6:495–503
- Lautenbach S, Seppelt R, Liebscher J, Dormann CF (2012) Spatial and temporal trends of global pollination benefit. *PLoS ONE* 7:e35954. doi:10.1371/journal.pone.0035954
- Lavelle P, Decaens T, Aubert M, Barot S, Blouin M, Bureau F, Margerie P, Mora P, Rossi J-P (2006) Soil invertebrates and ecosystem services. *Eur J Soil Biol* 42:S3–S15
- Laycock I, Lenthall KM, Barratt AT, Cresswell JE (2012) Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*). *Ecotoxicology* 21: 1937–1945
- Leonhardt SD, Gallai N, Garibaldi LA, Kuhlmann M, Klein A-M (2013) Economic gain, stability of pollination and bee diversity decrease from southern to northern Europe. *Basic Appl Ecol* 14:461–471
- Liu Z, Dai Y, Huang G, Gu Y, Ni J, Wei H, Yuan S (2011) Soil microbial degradation of neonicotinoid insecticides imidacloprid, acetamiprid, thiacloprid and imidaclothiz and its effects on the persistence of bioefficacy against horsebean aphid *Aphis craccivora* Koch after soil application. *Pest Manag Sci* 67:1245–1252
- Losey JE, Vaughan M (2006) The economic value of ecological services provided by insects. *Bioscience* 56:311–323
- Lye GC, Jennings SN, Osborne JL, Goulson D (2011) Impacts of the use of non-native commercial bumble bees for pollinator supplementation in raspberry. *J Econ Entomol* 104:107–114
- Mason R, Tennekes H, Sánchez-Bayo F, Uhd Jepsen P (2012) Immune suppression by neonicotinoid insecticides at the root of global wildlife declines. *J Environ Immun Toxicol* 1:3–12
- Main AR, Headley JV, Peru KM, Cessna AJ, Michael NL, Morrissey CA (2014) Widespread use and frequent detection of neonicotinoid insecticides in wetlands of Canada's Prairie Pothole Region. *PLoS ONE* 9:e92821. doi:10.1371/journal.pone.0092821
- Matson PA, Parton WG, Power AG, Swift MJ (1997) Agricultural intensification and ecosystem properties. *Science* 227:504–509
- MEA (2003) Ecosystems and human well-being: a framework for assessment. Island Press, Washington, DC
- MEA (2005) Ecosystems and human well-being: synthesis. Island Press, Washington DC
- Morse RA, Calderone NW (2000) The value of honey bees as pollinators of U.S. crops in 2000. *Bee Culture*, 15
- Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE (2013) The global hidden hunger indices and maps: an advocacy tool for action. *PLoS ONE* 8:e67860. doi:10.1371/journal.pone.0067860
- Naeem S, Li S (1997) Biodiversity enhances ecosystem reliability. *Nature* 390:507–509
- Nielsen UN, Ayres E, Wall DH, Bardgett RD (2011) Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity-function relationships. *Eur J Soil Sci* 62: 105–116
- Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? *Oikos* 120:321–326
- Pauly D, Christensen V, Guénette S, Pitcher TJ, Sumaila UR, Walters CJ, Watson R, Zeller D (2002) Towards sustainability in world fisheries. *Nature* 418:689–695
- Pauly D, Watson R, Alder J (2005) Global trends in world fisheries: impacts on marine ecosystems and food security. *Philos Trans R Soc B* 360:5–12
- Peck DC (2009a) Long-term effects of imidacloprid on the abundance of surface- and soil-active nontarget fauna in turf. *Agric For Entomol* 11:405–419
- Peck DC (2009b) Comparative impacts of white grub (*Coleoptera: Scarabaeidae*) control products on the abundance of non-target soil-active arthropods in turfgrass. *Pedobiologia* 52:287–299
- Perrings C, Naeem S, Ahrestani F, Bunker DE, Burkill P, Canziani G, Elmqvist T, Ferrati R, Fuhman J, Jaksic F, Kawabata Z, Kinzig A, Mace GM, Milano F, Mooney H, Prieur-Richard A-H, Tschirhart J, Weisser W (2010) Ecosystem services for 2020. *Science* 330:323–324. doi:10.1126/science.1196431
- Peters K, Bundschuh M, Schaefer RB (2013) Review on the effects of toxicants on freshwater ecosystem function. *Environ Pollut* 180: 324–329
- Pisa L, Amaral-Rogers V, Belzunces LP, Bonmatin J-M, Downs C, Goulson D, Kreutzweiser D, Krupke C, Liess M, McField M, Morrissey C, Noome DA, Settele J, Simon-Delso N, Stark J, van der Sluijs, van Dyck H, Wiemers M. (2014) Effects of neonicotinoids and fipronil on non-target invertebrates. *Environmental Science and Pollution Research* (this issue)
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 25:345–353. doi:10.1016/j.tree.2010.01.007
- Rajput V, Singh SK, Arpita K, Abhishek (2012) Comparative toxicity of butachlor, imidacloprid and sodium fluoride on protein profile of the walking cat fish *Clarias batrachus*. *J Appl Pharmaceut Sci* 2:121–124. doi:10.7324/JAPS.2012.2629
- Richards AJ (2001) Does low biodiversity resulting from modern agricultural practice affect crop pollination and yield? *Ann Bot* 88:165–172
- Robinson DA, Hockley N, Cooper DM, Emmett BA, Keith AM, Lebron I, Reynolds B, Tipping E, Tye AM, Watts CW, Whalley WR, Black HJ, Warren GP, Robinson JS (2013) Natural capital and ecosystem services, developing an appropriate soils framework as a basis for valuation. *Soil Biol Biochem* 57:1023–1033
- Sánchez-Bayo F, Goka K (2005) Unexpected effects of zinc pyrethrin and imidacloprid on Japanese medaka fish (*Oryzias latipes*). *Aquat Toxicol* 74:285–293
- Sánchez-Bayo F, Goka K (2006) Ecological effects of the insecticide imidacloprid and a pollutant from antidandruff shampoo in experimental rice fields. *Environ Toxicol Chem* 25:1677–1687
- Sánchez-Bayo F, Goka K (2014) Pesticide residues and bees—a risk assessment. *PLoS ONE* 9:e94482. doi:10.1371/journal.pone.0094482
- Schlapfer F, Schmid B, Seidl L (1999) Expert estimates about effects of biodiversity on ecosystem processes and services. *Oikos* 84:346–352
- Scientific & Technical Review Panel (2012) Agriculture-wetland interactions: background information concerning rice paddy and pesticide usage. (COP11 DR15). 11th Meeting of the Conference of the Parties to the Convention on Wetlands (Ramsar, Iran, 1971)
- Schulp CJE, Lautenbach S, Verburg PH (2014) Quantifying and mapping ecosystem services: demand and supply of pollination in the European Union. *Ecol Indic* 36:131–141
- Shrestha J, Niklaus PA, Frossard E, Samaritani E, Huber B, Barnard RL, Schleppe P, Tockner K, Luster J (2012) Soil nitrogen dynamics in a river floodplain mosaic. *J Environ Qual* 41:2033–2045
- Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, van Dyck H,

- van Praagh J, van der Sluijs JP, Whitehorn PR and Wiemers M (2014) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research* (this issue)
- Singh J, Singh DK (2005a) Dehydrogenase and phosphomonoesterase activities in groundnut (*Arachis hypogaea* L.) field after diazinon, imidacloprid and lindane treatments. *Chemosphere* 60:32–42
- Singh J, Singh DK (2005b) Available nitrogen and arginine deaminase activity in groundnut (*Arachis hypogaea* L.) fields after imidacloprid, diazinon, and lindane treatments. *J Agric Food Chem* 53:363–368
- Singh J, Singh DK (2006) Ammonium, nitrate and nitrite nitrogen and nitrate reductase enzyme activity in groundnut (*Arachis hypogaea* L.) fields after diazinon, imidacloprid, and lindane treatments. *J Environ Sci Health B* 41:1305–1318
- Spangenberg JH, Görg C, Thanh Truong D, Tekken V, Bustamante JV, Settele J (2014a) Provision of ecosystem services is determined by human agency, not ecosystem functions. Four case studies. *Int J Biodiversity Sci Ecosyst Serv Manag* 10:40–53
- Spangenberg JH, von Haaren C, Settele J (2014b) The ecosystem service cascade: further developing the metaphor. Integrating societal processes to accommodate social processes and planning, and the case of bioenergy. *Ecol Econ* 104:22–32
- Sperling L, McGuire S (2012) Fatal gaps in seed security strategy. *Food Secur* 4:569–579
- Steffan-Dewenter I, Munzenberg U, Burger C, Thies C, Tschamtkke T (2002) Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* 83:1421–1432
- Steffan-Dewenter I, Potts SG, Packer L (2005) Pollinator diversity and crop pollination services are at risk. *Trends Ecol Evol* 20:651–652. doi:10.1016/j.tree.2005.09.004
- Sumner DA, Boriss H (2006) Bee-economics and the leap in pollination fees. *Giannini Foundation Agr Econ* 9:9–11
- Swift MJ, Izac A-MN, van Moordwijk M (2004) Biodiversity and ecosystem services in agricultural landscapes—are we asking the right questions? *Agric Ecosyst Environ* 104:113–134
- Szendrei Z, Grafius E, Byrne A, Ziegler A (2012) Resistance to neonicotinoid insecticides in field populations of the Colorado potato beetle (Coleoptera: Chrysomelidae). *Pest Manag Sci* 68:941–946
- Tišler T, Jemec A, Mozetič B, Trebše P (2009) Hazard identification of imidacloprid to aquatic environment. *Chemosphere* 76:907–914
- Tu CM (1995) Effect of five insecticides on microbial and enzymatic activities in sandy soil. *J Environ Sci Health B* 30:289–306
- Tylianakis JM, Klein AM, Tschamtkke T (2005) Spatiotemporal variation in the diversity of Hymenoptera across a tropical habitat gradient. *Ecology* 86:3296–3302
- UNEP (2003) Ecosystems and human well-being: a framework for assessment. 2. Ecosystems and their services, pp. 49–70.
- UNEP (2010) UNEP emerging issues: global honey bee colony disorder and other threats to insect pollinators. p 16
- Van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin J-M, Belzunces LP (2013) Neonicotinoids, bee disorders and the sustainability of pollinator services. *Curr Opin Environ Sustain* 5:293–305. doi:10.1016/j.cosust.2013.05.007
- Van Dijk TC, Van Staalduinen MA, Van der Sluijs JP (2013) Macro-invertebrate decline in surface water polluted with imidacloprid. *PLoS ONE* 8:e62374. doi:10.1371/journal.pone.0062374
- Vaissiere B, Morison N, Carre G (2005) Abeilles, pollinisation et biodiversité. *Abeilles Cie* 3:10–14
- Vanbergen AJ, the Insect Pollinator Initiative, (2013) Threats to an ecosystem service: pressures on pollinators. *Front Ecol Environ* 11:251–259
- Wang G, Yue W, Liu U, Li F, Xiong M, Zhang H (2013) Biodegradation of the neonicotinoid insecticide acetamiprid by bacterium *Pigmentiphaga* sp. strain AAP-1 isolated from soil. *Bioresour Technol* 138:359–368
- Wang Y, Chen J, Zhu YC, Ma C, Huang Y, Shen J (2008) Susceptibility to neonicotinoids and risk of resistance development in the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae). *Pest Manag Sci* 64:1278–1284
- Williams IH (1994) The dependences of crop production within the European Union on pollination by honey bees. *Agr Zool Rev* 6: 229–257
- Welch RM, Graham RD (1999) A new paradigm for world agriculture: meeting human needs—productive, sustainable, nutritious. *Field Crop Res* 60:1–10
- WHO (2006) Guidelines for drinking water quality, first addendum to third ed., recommendations, vol. 1 World Health Organization, Geneva, Switzerland.
- Wilby A, Thomas MB (2002) Natural enemy diversity and pest control: patterns of pest emergence with agricultural intensification. *Ecol Lett* 5:353–360
- Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecol Appl* 7:737–750
- Yao X, Min H, Lu Z, Yuan H (2006) Influence of acetamiprid on soil enzymatic activities and respiration. *Eur J Soil Biol* 42:120–126
- Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc Natl Acad Sci* 96:1463–1468
- Zhang X, Liu X, Zhu F, Li J, You H, Lu P (2014) Field evolution of insecticide resistance in the brown planthopper (*Nilaparvata lugens* Stål) in China. *Crop Prot* 58:61–66. doi:10.1016/j.cropro.2013.12.026
- Zhou GC, Wang Y, Zhai S, Ge F, Liu ZH, Dai YJ, Yuan S, Hou JY (2013) Biodegradation of the neonicotinoid insecticide thiamethoxam by the nitrogen-fixing and plant-growth-promoting rhizobacterium *Ensifer adhaerens* strain TMX-23. *Appl Microbiol Biotechnol* 97: 4065–4074

Alternatives to neonicotinoid insecticides for pest control: case studies in agriculture and forestry

Lorenzo Furlan · David Kreutzweiser

Received: 14 April 2014 / Accepted: 16 September 2014 / Published online: 3 October 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Abstract Neonicotinoid insecticides are widely used for control of insect pests around the world and are especially pervasive in agricultural pest management. There is a growing body of evidence indicating that the broad-scale and prophylactic uses of neonicotinoids pose serious risks of harm to beneficial organisms and their ecological function. This provides the impetus for exploring alternatives to neonicotinoid insecticides for controlling insect pests. We draw from examples of alternative pest control options in Italian maize production and Canadian forestry to illustrate the principles of applying alternatives to neonicotinoids under an integrated pest management (IPM) strategy. An IPM approach considers all relevant and available information to make informed management decisions, providing pest control options based on actual need. We explore the benefits and challenges of several options for management of three insect pests in maize crops and an invasive insect pest in forests, including diversifying crop rotations, altering the timing of planting, tillage and irrigation, using less sensitive crops in infested areas, applying biological control agents, and turning to alternative reduced risk insecticides. Continued research into alternatives is warranted, but equally pressing is the need for information transfer and training for farmers and pest managers and the need for policies and regulations to encourage the adoption of IPM strategies and their alternative pest control options.

Keywords Neonicotinoid · Integrated pest management · Agriculture · Maize pests · Forestry

Responsible editor: Philippe Garrigues

L. Furlan (✉)
Veneto Agricoltura, Legnaro, PD, Italy
e-mail: lorenzo.furlan@venetoagricoltura.org

D. Kreutzweiser
Canadian Forest Service, Natural Resources Canada, 1219 Queen
Street East, Sault Ste Marie, ON P6A 2E5, Canada

Introduction

Systemic neonicotinoid insecticides are used to protect a wide variety of crops. Based on their efficacy to control many insect pests and their systemic activity, they are used extensively in agriculture so that by 2008, neonicotinoids accounted for one quarter of the global insecticide market (Jeschke et al. 2011), and this rate is increasing (Simon-Delso et al. 2014). The extensive use of neonicotinoids in agriculture has undoubtedly met technical and commercial goals, i.e. simplification of agricultural systems and large pesticide applications for pest prevention to maximize efficiencies and profits. However, increasing evidence indicates that this large-scale use results in high broad-spectrum insecticidal activity of the neonicotinoids even at very low dosages, and this has led to serious risk of environmental impact (Henry et al. 2012; Goulson 2013; van der Sluijs et al. 2013, 2014; Whitehorn et al. 2012). The large-scale, often prophylactic use (Goulson 2013) of neonicotinoid insecticides contrasts with the main principle of an integrated pest management (IPM) approach which includes an assessment of economically important pest populations in order to determine if an insecticide treatment is required. The principles of IPM, derived from dozens of years of field experiments and scientific research (Baur et al. 2011), are summarized and made compulsory in the European Union by Directive 2009/128/CE. For an agricultural setting, the procedure is the following:

1. Before taking any decision on pest control, harmful organisms must be monitored by adequate methods and tools, where available; tools should include observations in the field as well as scientifically sound warning, forecasting, and early diagnosis systems;
2. Treatments may then be carried out only where and when the assessment has found that levels are above predetermined economic thresholds for crop protection;

3. If economic thresholds are exceeded, agronomic solutions, mainly rotation, should be considered to avoid damage to maize crops including the interference of newly established pest populations with tillage timing and other modifications, choice and modification of sowing dates, and alterations of rotation sequences;
4. If economic thresholds are exceeded and no agronomic solutions are available, biological control or physical treatment or any other non-chemical pest control method should be considered as a replacement for chemical treatment;
5. If economic thresholds are exceeded and no agronomic solutions, biological control or physical treatments or any other non-chemical pest control methods are available, chemical treatments should be selected among those that pose the lowest risk to environment and human health, and they should be used in a way that minimizes the risk of pest resistance by limiting their use over space and time.

In order to show that alternatives to neonicotinoids for pest control are available and can be feasible, two case studies will be described: (i) treatment of maize crops, in which it was shown that there was a link between neonicotinoids and negative effects on honeybees (Girolami et al. 2012) and (ii) treatment of trees to control an invasive insect pest. The agricultural case study is significant because it concerns cultivation and pest control methods made on large land bases in Italy (thousands of hectares spanning a 25-year period (Furlan 1989; Furlan et al. 2002, 2007b, 2009a, 2011; Ferro and Furlan 2012)) with potential for side effects on the environment. The forestry case study is significant because it presents a unique pest problem in Canada with environmental issues and solutions of its own.

Case studies of alternative pest management in maize

By 2010, neonicotinoids accounted for 27 % of the world's total insecticide use (Casida and Durkin 2013), and their application to pest management in maize is among the highest use of the insecticides in agriculture. For example, over 18 million ha of maize (corn) was treated with a neonicotinoid insecticide between 2009 and 2011 in the USA (Brassard 2012). This included over 810 t of clothianidin and 570 t of thiamethoxam applied in 1 year in the USA, most of it in maize crops (Simon-Delso et al. 2014). Production of maize for food, feed, and biofuel is the single largest use of arable land in the USA, and almost all seeds used in maize production are coated with neonicotinoid insecticides (USDA-NASS 2013). Maize production in the European Union is about 14 million ha per year, with France, Romania, Germany, Hungary, and Italy each producing more than 1 million ha

per year (Meissle et al. 2010). Neonicotinoid insecticides are applied to maize crops primarily by seed coating and are designed to protect maize seeds, seedlings, and young plants in the early growing season. The increasing use of neonicotinoids, including the use in maize, has been implicated in significant environmental exposure and impacts, including bee disorders and colony collapse, thereby affecting pollination and other ecological services (Goulson 2013; van der Sluijs et al. 2013, 2014; Bonmatin et al. 2014; Chagnon et al. 2014; Pisa et al. 2014).

The first way of reducing insecticide use in Europe in general, and neonicotinoids in particular, is the proper implementation of the IPM strategies proposed by the European Directive 128/2009/EC on the Sustainable Use of Pesticides. This Directive made it compulsory to apply IPM to all crops in the European Union since January 2014. Although IPM strategies are commonly used on plantations such as orchards and vineyards (Baur et al. 2011), they have not been widely introduced for maize and other arable crops in Europe (Furlan et al. 2013). As arable farming often has limited resources in terms of income, labour, and technology, a special effort is needed to ensure that the directive is successful. This means that if IPM is to be introduced for arable crops, there is a need for (a) low-cost strategies, (b) time-effective tools, and (c) economically and environmentally sustainable pesticides or other pest control methods. One way to achieve these goals is to initiate a modern advisory system that can provide online information on crop treatment options and explain technical criteria. This has been demonstrated in Italy by the new *Bollettino delle Colture Erbacee* ("Annual Crops Bulletin") (<http://www.venetoagricoltura.org/subindex.php?IDSX=120>). This advisory bulletin is based on a low-cost area-wide pest and disease monitoring system that establishes when and where pest populations pose an economic risk to arable land. Where the risk actually occurs, it advises how the field evaluation should be carried out. Area-wide monitoring is low-cost since it is based on: (a) pheromone traps, which are user-friendly and inexpensive; (b) pest population models using meteorological information (e.g. the Black Cutworm Monitoring and Forecasting programme (Furlan et al. 2001c) and the Davis model for Western corn rootworm egg hatching, Davis et al. 1996); (c) spatial analysis based on GIS mapping (e.g. geostatistics, De Luigi et al. 2011); and (d) agronomic information from a number of areas. In order to ensure that IPM can be applied to arable crops reliably and affordably, the monitoring and assessment must be conducted at both regional and local farm levels where needed.

At the local farm level, the monitoring procedure requires on-the-ground samples to be taken when areas at risk of significant crop damage from a given insect are identified at regional levels (Furlan et al. 2013). Monitoring crop development may also reveal different susceptibility levels and therefore methods of intervention must be adjusted accordingly.

Farmers and other practitioners are informed in a timely manner about these issues and trained in how to use the information correctly in a successful IPM plan where production costs are competitive and environmental impacts are limited. The following is a brief description of some IPM options for managing some common insect pests on maize crops in Italy (and applicable to other parts of Europe) without relying on the prophylactic use of neonicotinoids.

Controlling wireworms (*Agriotes* spp.)

Long-term data suggest that the majority of maize farmland in Italy does not need to be protected with insecticides at sowing (Furlan 1989; Furlan et al. 2002, 2007b, 2009a, 2011, 2014; Ferro and Furlan 2012). Indeed, the percentage of land with high populations of wireworms (a key soil pest in maize farmland) is often very low (e.g. less than 5 % in the Veneto region (Furlan 1989; Furlan et al. 2002, 2007b, 2009a, 2011; Ferro and Furlan 2012), an area with large-scale maize production). At the European level, similar results are coming from the European project PURE (VII Framework). After the first 3 years of monitoring, no significant wireworm damage in the experimental fields of France, Hungary, Slovenia, Germany, and other Italian regions was detected (Furlan, unpublished data). Hundreds of plots have been examined in studies from Italy, and in the large majority of the experiments, there were no statistically significant differences, in terms of yield and crop stand, between maize treated with neonicotinoids and non-treated plots because of low wireworm damage and/or the compensation capacity of the crops (Balconi et al. 2011; Boicelli 2007; Ferro and Furlan 2012; Furlan et al. 2002, 2007b, 2009a, 2011).

These data demonstrate that insecticides are often not needed and may not always contribute effectively to yield gain (Goulson 2013). In these situations, low pest populations determined by monitoring and field assessments may provide information for successful IPM implementation. Because of this general low-risk level, a crop insurance programme where growers may purchase insurance, instead of soil insecticides, to provide financial compensation when yield losses can be attributed to pests would be more feasible than prophylactic protection. The total cost of damage to maize (need of re-sowing and loss of yield due to delayed sowing or reduced stand) is often lower than the total cost of the prophylactic protection of all planted fields (Furlan et al. 2014), and this does not include any consideration of environmental side effects of neonicotinoids (van der Sluijs et al. 2014).

Accurate wireworm population monitoring and damage prediction

An effective and sustainable maize production strategy is to plant sensitive crops in areas free of harmful wireworm

populations. Currently, some wireworm population levels can be predicted reliably and cost effectively with pheromone traps (Furlan et al. 2001a; Gomboc et al. 2001; Karabatsas et al. 2001; Tóth et al. 2001, 2003), which are suitable for monitoring all of Europe's main *Agriotes* species (*Agriotes sordidus* Illiger, *Agriotes brevis* Candèze, *Agriotes lineatus* L., *Agriotes sputator* L., *Agriotes obscurus* L., *Agriotes rufipalpis* Brullè, *Agriotes proximus* Schwarz, *Agriotes litiginosus* Rossi, and *Agriotes ustulatus* Schüller). In the last few years, research has provided useful information about the biological significance of pheromone trap catches and has demonstrated their range of attraction (Sufyan et al. 2011). Captured adults (click beetles) in pheromone traps may be correlated with the presence of larvae of the same species in soils, at least for the three main species of southern Europe, namely *A. sordidus* Illiger, *A. brevis* Candèze, and *A. ustulatus* Schüller (Burgio et al. 2005, 2012; Furlan et al. 2001b, 2007a; Pozzati et al. 2006). However, this relationship is less certain for other important European species, such as *A. obscurus* L., *A. lineatus* L., and *A. sputator* L. (Benefer et al. 2012; Blackshaw and Hicks 2013). Spatial models (e.g. geostatistical analyses) are available in Italy, providing predictions of *Agriotes* population dynamics at different spatial scales (i.e. large farms, provinces) which are then interfaced with agronomic and geographic variables, leading to improved analysis of risk and optimization of monitoring costs (Burgio et al. 2005).

The information obtained by pheromone trap monitoring can improve the prediction of population levels and the actual risk of crop damage based on the evaluation of a field's agronomic and climatic characteristics along with the biological and ecological information of each species (Furlan 1996, 1998, 2004; Masler 1982; Rusek 1972; Kosmacevskij 1955). The two main risk factors are (i) more than 5 % organic matter content of the soil (Furlan 1989, 2005, unpublished data; Furlan et al. 2011) and (ii) continuous plant cover of the soil with meadow or double crops (such as barley and soybean, ryegrass and maize, etc.) in the two previous years (Furlan 1989, 2005, unpublished data; Furlan and Talon 1997; Furlan et al. 2011). If no agronomic risk factors are present, no treatments are needed. When pheromone traps have detected high beetle population densities and/or agronomic risk factors are present, bait traps for larvae (Chabert and Blot 1992; Parker 1994, 1996; Parker et al. 1994) can then be used to pinpoint the areas with wireworm populations that exceed the economic threshold. However, each *Agriotes* species responded differently to bait traps, and consequently, the thresholds for each species must be assessed separately (Furlan 2011). Therefore, species identification is important, and although polymerase chain reaction (PCR) and DNA sequencing are currently available to identify species (Staudacher et al. 2010), other more practical and feasible identification methods should be developed for each region. Data from maize farms in Italy over the last 20 years have

enabled researchers to establish that there is a close correlation between the number of larvae per square metre, or between the average number of larvae per bait trap, and the number of maize plants damaged by *A. brevis*, *A. sordidus*, and *A. ustulatus* (Furlan 2014). When wireworm populations are above threshold values, agronomic and biological treatment options should be considered before resorting to chemical treatments.

Agronomic strategies for controlling wireworm populations

Crop rotation, food resources, climatic and agronomic conditions (mainly organic matter content), as well as other soil characteristics are the main factors that influence larval population densities (Furlan 2005). Generally, the vast majority of non-sensitive or low-sensitive crops (e.g. soybean) can be planted in identified infested fields, while the remaining cultivated soils can be planted with another sensitive crop, including maize (Furlan and Toffanin 1996). Rotation and correct allocation of crops may suffice to prevent economic damage to crops without the use of any specific control tool (Furlan et al. 2011).

Data from studies in Italy indicate that the most important factor in influencing wireworm population levels is crop rotation (Furlan and Talon 1997; Furlan et al. 2000), and this appears to be the situation in other regions (Eastern Europe, Hungary) as well (e.g. Szarukán 1977). This is because meadows and the use of double cropping within the rotation cycle may result in population increases of a species that has the capacity to overwinter as adults (Furlan 2005). Therefore, any modification of these factors may disrupt wireworm population dynamics. Altering rotations, i.e. temporary removal of the most suitable crops for wireworm development, is a key agronomic strategy for population control.

Altering tillage timing, i.e. choosing a crop rotation that allows for soil tillage in the most critical phase of the wireworm life cycle (e.g. when most eggs are laid and the first instar larvae are in the soil), may also reduce wireworm populations (Furlan 1998, 2004). Tillage timing should be modulated in accordance with the life cycle differences among the main *Agriotes* species. Altering irrigation timing to ensure the drying of the topmost soil layer just after eggs are laid can also be an effective means of controlling *Agriotes* populations (Furlan 1998, 2004). Altering planting timing can also be effective, recognizing that a population's capacity to damage sensitive plants varies with the season. For instance, even very high *A. ustulatus* populations do not damage maize because most of the larvae are in a non-feeding phase by late spring (Furlan 1998). Therefore, adjusting planting timing when possible to coincide with low pest populations or with non-damaging life stages can be effective. Another agronomic tool for population control is intercropping in which winter-wheat or other trap-crop plants are included in fields as a control

strategy to draw pests away from the main economic crop (Furlan and Toffanin 1994; Vernon et al. 2000).

Applying biological tools for controlling wireworm populations

A range of other potential options are available for fields infested with damaging wireworm populations when planting the sensitive crop in non-infested fields has been ruled out (Furlan 2007). The mechanisms and effectiveness of some of these various control methods have been accurately assessed under controlled conditions (Furlan and Toffanin 1998; Furlan and Campagna 2002) and currently show that biocidal plants and seed meals are the only practical options (Furlan et al. 2009b, 2010). Their potential can be considered comparable to that of neonicotinoids and other chemical insecticides that can replace neonicotinoids (Ferro and Furlan 2012), especially when they are used to interfere with population development and not simply to reduce wireworm populations just before or during sowing (Furlan et al. 2009b, 2010).

Applying chemical insecticides for controlling wireworm populations

In fields where wireworm populations exceed economic thresholds and the agronomic and biological alternatives are not feasible, alternative insecticides to neonicotinoids, such as pyrethroids and phosphorganics, are available (Wilde et al. 2004; Ferro and Furlan 2012). They should be used sparingly, in accordance with best practices for pesticide applications. The effectiveness of the soil insecticides can be influenced by soil and weather conditions (e.g. heavy rain taking away insecticide active ingredient) that can result in protection failure for either neonicotinoids and their alternative insecticides (Ferro and Furlan 2012; Furlan et al. 2011, 2014). No significant differences in wireworm control between neonicotinoids and several alternative insecticides were reported by Wilde et al. (2004); trials in Italy conducted over a 10-year period suggest that the likelihood of failure is higher for some alternative insecticides (Ferro and Furlan 2012; Furlan et al. 2011, 2014).

*Controlling Western corn rootworm (*Diabrotica virgifera virgifera*)*

Western corn rootworm (WCR) damage to maize in Europe is only a risk where continuous maize cropping is adopted, especially when cropping is prolonged for several years (Furlan et al. 2014; Kiss et al. 2005; Sivčev et al. 2009). However, economic damage only occurs in areas with high WCR populations. Where maize is rotated, WCR populations are usually held below the economically important threshold, and there is little risk of significant crop damage (Kiss et al.

2005; Meinke et al. 2009; Sivčev et al. 2009). Therefore, IPM for WCR should be based on a systematic rotation of crops and supported by information on pest development and population levels as stated by the Directive 2009/128/EC and confirmed by the Commission Recommendation 2014/63/EU (on measures to control *D. virgifera virgifera* Le Conte in Union where its presence is confirmed).

Accurate WCR population monitoring and damage prediction

Baited and non-baited traps are available to monitor WCR population levels (Schaub et al. 2011). The most widely used non-baited traps include yellow sticky traps, and they are readily available from various manufacturers. The most commonly used sticky trap for threshold assessment is Pherocon AM® (PhAM). Both USA and European authors have demonstrated that there is a correlation between the number of adults captured by yellow sticky traps (i.e. PhAM) and plant damage the following year (Blandino et al. 2014; Boriani 2006; Hein and Tollefson 1985; Kos et al. 2014). The US authors stated that economic thresholds would be exceeded when more than 40 beetles/PhAM trap/week (6 beetles/PhAM trap/day) were caught the previous year in one period (ca. 7 days) during the last 3 weeks of August (Hein and Tollefson 1985). In Italy, the threshold was 42 beetles/PhAM trap/day on average over a 6-week period after the beginning of adult flights (Boriani 2006; Blandino et al. 2014). In Croatia, the threshold was estimated at 41 adults/ PhAM trap in week 31 (Kos et al. 2014). Economic thresholds can greatly vary with climatic/agronomic conditions and prices of maize and insecticides (Oleson et al. 2005). Under low stress levels (suitable soil with sufficient water and nutrient supply), maize yield is not likely to be significantly reduced even with WCR population pressures causing a root damage score of 1 on the 0–3 scale (Oleson et al. 2005). In contrast, low root injury rates may cause yield reduction if high stress levels for maize cultivation occur (Oleson et al. 2005). In any case, the likelihood that a yield reduction occurs is negligible when WCR population pressure is very low (<0.3 root injury score on the 0–3 scale, Furlan et al. 2014). Based on trap monitoring network data, innovative statistical tools (De Luigi et al. 2011) can reliably identify or predict the areas where populations are high enough that they lead to reduced yield.

Agronomic strategies for controlling WCR populations

Although WCR arrived more than 6 years ago in southern Veneto (De Luigi et al. 2011), where rotation is dominant, population levels have remained low and economic damage has not been found, even in nearby continuous maize fields (Furlan et al. 2014). In areas of Veneto where crop rotation is not prevalent, average WCR population levels are high and the risk of root damage is considerable. Continuous maize

may be rotated with any type of crop different from maize. Even Gramineae species that are closely related to maize may be used as a first or second crop after a winter crop (e.g. winter wheat + sorghum or ryegrass +sorghum). Maize itself may even be used as a second crop (e.g. winter wheat + maize) to interrupt a WCR cycle, provided that it is sown after the WCR eggs have hatched (Davis et al. 1996).

The aforementioned results suggest that a proper IPM approach would be to monitor long-standing continuous maize fields each year and when WCR population thresholds are exceeded, to rotate the maize with any other crop for only 1 year followed by monitoring in the subsequent maize crops. Periodic crop rotations disrupt the WCR life cycle, keep populations below economic thresholds, and typically preclude the need for insecticides. In practice, maize may be rotated at varying frequencies, even after several years of continuous maize cultivation, and only when monitoring reveals that WCR population levels are increasing. Crop rotations offer other agronomic benefits in addition to insect population management (Furlan et al. 2014; Saladini et al. 2009), thereby increasing incentives for periodic crop rotation.

The success of flexible rotation as an IPM strategy has also been confirmed by area-wide simulations (metamodels). These models have shown that 100 % rotation of maize is not necessary to keep regional WCR populations beneath economic thresholds, as, e.g. the interruption of continuous maize cropping after 3 years reduces the need for rotation to manage successfully WCR to below 60 % of the maize fields (Szalai et al. 2014). The use of variable rotation frequencies and crops may also be important where, such as was demonstrated in the USA, a “WCR variant” has adapted to crop rotations and are able to successfully lay economically significant levels of eggs outside of corn thereby causing damage to maize in a simple corn-soybean rotation (Levine et al. 2002).

In countries where allowed, another important agronomic alternative is transgenic corn that protects against WCR damage because the *Bacillus thuringiensis* protein expressed in the maize is toxic to WCR larvae (Meissle et al. 2011; Vaughn et al. 2005). Its efficacy has been shown to be better than neonicotinoid insecticides (Oleson and Tollefson 2005, 2006). This transgenic corn must be used under insect resistance management strategies (Onstad et al. 2001) and be integrated with other agronomic tactics to keep populations below the economic thresholds for “non transgenic” maize.

Applying biological tools for controlling WCR populations

Although rotation appears to be the most suitable measure for keeping WCR populations below economic thresholds, effective biological control options are also available as alternatives to chemical insecticides, with entomopathogenic nematodes proving to be a highly effective way of suppressing WCR populations under field conditions (Kurtz et al. 2007; Toepfer

et al. 2010, 2013). Conversely, the parasitoid *Celatoria compressa* (Diptera: Tachinidae) does not appear to be viable for practical application at the moment (Toepfer and Kuhlmann 2004; Kuhlmann et al. 2005; Zhang et al. 2003).

Applying chemical insecticides for controlling WCR populations

Studies show that neonicotinoid seed treatments and soil applications used as in-furrow treatments at planting do not interfere significantly with WCR populations (Furlan et al. 2006). In situations where an IPM process is still insufficient to control crop damage and some maize fields require insecticide protection, alternative insecticides to neonicotinoids are available. For example, pyrethroids and phosphorganics can be as effective as neonicotinoids against WCR (Agosti et al. 2011; AA.VV. 2012; Blandino et al. 2013; Furlan et al. 2006; Waldron et al. 2002; Whitworth and Davis 2008) or even more effective (Oleson 2003; Oleson and Tollefson 2005). Protection against WCR by insecticides is less effective than protection by crop rotation, and insecticide effectiveness can be influenced by soil and weather conditions and by WCR population pressure that can result in protection failure (Boriani 2008, Furlan unpublished data).

Foliage insecticide treatments (e.g. with pyrethroids and phosphorganics) against WCR beetles may sometimes (i) protect maize silks from beetle chewing if applied before flowering, but this is needed only with very high WCR populations (Furlan, unpublished data) that should not be the case when IPM strategies are implemented; and (ii) actually reduce WCR population levels and the subsequent oviposition by females. The use of a development model (Nowatzki et al. 2002) may help to identify the period in which foliage insecticide treatments can significantly reduce the oviposition of females. Furthermore, this development model indicates whether treatment to contain corn borers (e.g. *Ostrinia nubilalis*) would also reduce WCR adult numbers leading to non-economic population levels in the following year. However, foliage treatments should be used with caution and only when other options under an IPM approach have not been successful or are not feasible because wide scale use of insecticides can lead to (i) resistance as already demonstrated in WCR larvae (Ball and Weekman 1962) and adult beetles (Meinke et al. 1998), (ii) outbreaks of secondary pests such as red mites, and (iii) possible environmental impacts.

Based on the principles of IPM and the evidence from numerous field trials in Italy described above, there is strong evidence that neonicotinoids are not required for effective management of WCR damage in maize. These principles and alternatives have also been successfully applied in the USA under an Area-Wide Pest Management scheme for rootworm control in corn fields (French et al. 2007).

*Controlling black cutworm (*Agrotis ipsilon*)*

The majority of attacks on maize in Northern Italy are caused by an invasive species, the black cutworm (BCW) *A. ipsilon* Hufnagel (Furlan et al. 2001c). This species normally cannot overwinter in the conditions of Northern Italy and other northern regions (Zangheri et al. 1998), but rather, outbreaks are due to invasions by massive flights from southerly areas. Insecticide applications at the time of sowing are not recommended because BCW cannot be detected at the time of sowing and because many insecticides applied at planting become less effective over time, whereas outbreaks often occur many days after sowing (Furlan et al. 2001c; Zangheri and Ciampolini 1971; Zangheri et al. 1984) resulting in insufficient control (Furlan 1989; Shaw et al. 1998). However, it has been shown in the USA that rescue treatments (post-emergence applications) using non-neonicotinoid insecticides can be very effective (close to 100 % control, Shaw et al. 1998).

An IPM approach to managing BCW is based on a combination of large-scale pheromone trap monitoring to detect population levels, the analysis of southerly winds that may carry flying moths, and a development model (Black Cutworm Alert programme, Furlan et al. 2001c; Showers 1997). More intensive local-level population monitoring (e.g. scouting of farm fields) is performed only when area-wide monitoring has established that there is a risk. When trap monitoring and wind analysis have established whether and where any moths are present, the degree-day accumulation is calculated, preferably with soil temperature (each day: (maximum temperature–minimum temperature)/2–10.4 °C developmental threshold temperature, Luckmann et al. 1976). Once the predicted risk date is reached (176°-day accumulation when the fourth larval instar forms in the fields), at-risk areas should be monitored for BCW larvae so that appropriate reduced risk insecticides can be used post-emergence, should the average amount of affected crops exceed the 5 % threshold. This reduces the overall amount of insecticide required, and this approach has been tested and demonstrated to be successful in USA and Italy for several years (Furlan et al. 2001c; Showers 1997).

There is evidence that some transgenic maize hybrids can potentially protect against BCW because the *B. thuringiensis* protein expressed in the maize is toxic to BCW, but this may not be as effective as rescue treatments with appropriate insecticides (Kullik et al. 2011). In addition, the use of transgenic corn for BCW control, as it was suggested for WCR control, has to be decided when it is not possible to know if a BCW economic threshold population is actually present or developing. This constraint makes the transgenic corn option of limited use in an IPM approach against BCW.

We suggest that the IPM strategies for major insect pests that we illustrate in a European maize production system can

be applicable to maize production in other countries as well, with some adaptations where other minor pests are present. The overall process for the three major pests we discuss can be summarized as follows: no prophylactic chemical treatments at maize sowing, black cutworm control where and if thresholds are exceeded based on Black Cutworm Alert programme supplemented by scouting when and where needed, WCR kept under control mainly by agronomic strategies, and treatments against wireworms restricted to the minor part of fields with populations exceeding the thresholds detected with the monitoring procedure described above. The cost and crop damage risk of an IPM approach can be effectively minimized by a mutual fund system (a special type of crop insurance directly managed by farmer associations) that ensures a guaranteed farm income in all cases.

Case study of alternative pest management in Canadian forests

The emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), is a wood-boring exotic invasive insect pest that is increasingly threatening the health and survival of ash (*Fraxinus* spp.) trees in large regions of eastern North America (Poland and McCullough 2006; McCullough and Siegert 2007). All North American ash species are susceptible to emerald ash borer, and mortality of ash trees occurs rapidly after infestation. Ash is an important urban forest species, but it can also dominate in landscapes associated with water, such as riparian (shoreline) buffers along agricultural runoff streams and ravines, temporary pools and wetlands, and in headwater or source water areas. In this regard, ash can be a keystone forest species that influences or regulates riparian forest and aquatic ecosystem dynamics and nutrient cycling through canopy cover and leaf litter inputs to forest floors and water bodies (Ellison et al. 2005; Gandhi and Herms 2010; Flower et al. 2013). Therefore, the rapid loss of ash from these ecologically sensitive areas can pose a risk to critical habitats, biodiversity, and some important ecosystem services.

As a first step toward managing the damage from emerald ash borer when the pest populations begin to build, three management options have been proposed to slow the spread and infestation by the insect. These are (i) cutting and removing living ash trees in advance of the infestation, (ii) girdling living ash trees on the leading edge of an infestation, and (iii) the application of an effective systemic insecticide (McCullough and Poland 2010; Mercader et al. 2011). Intentionally removing some of the living ash trees before or in early stages of the infestation reduces the phloem available for larval development. This approach also provides opportunities for forest canopy redevelopment by other tree species through natural regeneration or strategic under-planting to

minimize impacts from the sudden loss of ash by the emerald ash borer infestation (Streit et al. 2012). Girdling living ash trees on the leading edge of an infestation causes the stressed tree to act as a trap tree to which egg-laying females are attracted in large numbers, presumably because of increased attractive volatiles and/or visual cues (McCullough et al. 2009). Those trap trees are then destroyed before larval development, thereby concentrating the future cohort of the emerald ash borer to a specific area and reducing the local population.

The third management option to reduce tree mortality and slow the spread of emerald ash borer is the application of a systemic insecticide. A systemic insecticide is well suited for control of this insect pest because the damaging life stage of the pest is the phloem-feeding larvae. Among the systemic insecticides that have been shown to be effective against the emerald ash borer is the neonicotinoid, imidacloprid (Poland et al. 2006). Applications to trees can be made by soil injections around the base of individual trees or by direct stem injections into tree trunks. However, Canadian field and laboratory studies showed that autumn-shed leaves from imidacloprid-treated trees can contain residues that pose risk of harm to aquatic and terrestrial decomposer organisms through sublethal feeding-inhibition effects (Kreutzweiser et al. 2007, 2008a, 2009). They further showed that field-realistic concentrations of imidacloprid in soils and water posed direct risk of adverse effects to earthworms (Kreutzweiser et al. 2008b) and aquatic invertebrates (Kreutzweiser et al. 2008c). These results, coupled with a commitment to adopt an IPM approach to the emerald ash borer problem, prompted an examination of alternatives to imidacloprid for emerald ash borer control.

In a forest insect pest context, an IPM approach examines and applies a combination of management methods using all available information to make informed management decisions. This approach currently being applied to the control of emerald ash borer in Canada includes studies into the pest biology and behaviour to facilitate biological control (Lelito et al. 2013), effective and practical traps for the highly mobile adults to track infestations (Grant et al. 2010; Ryall et al. 2013), improved detection methods for locating early infestations and potential hot spots (Ryall et al. 2011), and alternative pest management strategies. Here, we briefly describe some of the alternatives to imidacloprid being explored for the control of emerald ash borer in Canada.

Exotic parasitic insects

Three species of hymenopterous parasitoids (parasitic wasps) were found to parasitize emerald ash borer larvae or eggs in China, and these are being reared in the USA as potential biological control agents (Lyons 2013). The emphasis on finding, importing, and rearing exotic parasitoids was on

selecting species that show a high degree of host specificity. The three species, Braconidae: *Spathius agrili*, Eulophidae: *Tetrastichus planipennisi*, and Encyrtidae: *Oobius agrili*, have been released annually since 2007 in northeastern USA under biological control regulations (Gould et al. 2012) and their populations are being monitored. Early indications are that at least one species (*T. planipennisi*) has been successful in establishing a measureable population and has the potential for beginning to control emerald ash borer infestations (Duan et al. 2013). *T. planipennisi* was released at two sites in Canada in 2012 and monitoring is ongoing to determine the success of population establishment (B. Lyons, personal communication).

Native parasitic insects

Surveys were conducted in emerald ash borer-infested areas of Canada to determine if native parasitoids were active on, or associated with, the invasive insect pest. Several species of hymenopterous parasitoids were encountered in these surveys and were trapped and reared to determine a parasitism rate for each species on emerald ash borer. Among those, only a few (e.g. Chalcididae: *Phasgonophora sulcata*, Braconidae: *Atanycolus hicoriae*) have shown relatively high rates of parasitism on emerald ash borer and hold some promise as a native biological control agent (Lyons 2010). Efforts are ongoing to determine the potential for native parasitoids to assist biological control strategies using parasitic wasps. This includes developing techniques for rearing and releasing or otherwise augmenting natural populations of promising native parasitoids. The combined use of exotic and native parasitoids as biocontrol agents may eventually be successful in helping to manage emerald ash borer populations, but they are still in the early stages of development.

Native fungal pathogens

The use of native entomopathogenic fungi as biological control agents against emerald ash borer is being explored in Canada. Screening of prepupal and adult cadavers from established emerald ash borer populations indicated that the most prominent natural pathogenic fungus on emerald ash borer was *Beauveria* spp. (Kyei-Poku and Johny 2013). These were subsequently isolated and characterized, and it was determined that the L49-1AA isolate of *Beauveria bassiana* was the most promising in terms of virulence against emerald ash borer (Johny et al. 2012). An effective entomopathogenic fungus requires an efficient dissemination system to spread the fungus among susceptible hosts of the pest population. Lyons et al. (2012) developed an autocontamination trap system for emerald ash borer in which adults are contaminated with *B. bassiana*, and they found

evidence that this system facilitated horizontal transmission among adults.

Entomopathogens show some promise as biological control agents and some methods for their screening, characterization, and dissemination have been developed. However, there are still some limitations of this approach for broad-scale control of emerald ash borer. Entomopathogens in general do not appear to be significant factors that regulate emerald ash borer populations (Liu et al. 2003), and the pest's biology and behaviour do not lend themselves to efficient fungal transmission. Moreover, many entomopathogens, including *B. bassiana*, are not particularly host-specific, and if they are disseminated as biological control agents, they may pose risks to non-target insects.

An alternative, non-persistent systemic insecticide

Several systemic insecticides were screened for efficacy against emerald ash borer, their translocation efficiencies in ash trees, and their environmental safety. The most promising of these was azadirachtin. Azadirachtin is a natural compound extracted from the seeds of the neem tree, *Azadirachta indica*, and has been shown to have antifeedant, antifertility, and growth-regulating insecticidal properties against a range of insect pests (Schmutterer 1990). Previous studies in a Canadian forestry context showed that azadirachtin was not persistent in the environment (water, soils, tree foliage) and did not present significant risk to most non-target invertebrates at expected environmental concentrations (Thompson and Kreutzweiser 2007), and therefore, it was considered a strong candidate for control of emerald ash borer. Azadirachtin was injected into trunks of infested ash trees and shown to be highly effective at inhibiting larval development and adult emergence and, therefore, effective in protecting ash trees from the wood borer (McKenzie et al. 2010). Subsequent field trials confirmed that azadirachtin is readily taken up following stem injection of ash trees, is rapidly translocated throughout the tree and to foliage, and usually dissipates to near limits of detection in autumn-shed leaves (Grimalt et al. 2011). We conducted a suite of non-target tests following protocols of those used to assess the effects of imidacloprid and showed that azadirachtin in autumn-shed leaves poses no measurable risk of harm to terrestrial or aquatic decomposer invertebrates, even after intentionally high application rates (Kreutzweiser et al. 2011).

Conclusions

These case studies in agriculture and forestry provide examples of reasonable and viable alternatives to neonicotinoid insecticides for control of insect pests. In the agricultural

setting, it is becoming increasingly clear that prophylactic insecticide treatments with neonicotinoids are often not needed and result in unnecessary contamination of the environment thereby increasing risks to non-target organisms (van der Sluijs et al. 2014) and may increase the likelihood of developing resistance among insect pests (Szendrei et al. 2012). As an alternative, an IPM approach should consider all relevant and available information to make informed management decisions, providing pest control options based on actual need. When a need is identified, pest control options that preclude the use of neonicotinoid insecticides are varied and may include diversifying and altering crop rotations, planting dates, tillage, and irrigation; using less sensitive crop species in infested areas; applying biological control agents; and turning to alternative reduced risk insecticides. These options are often most effective when applied in combination under an overall IPM strategy.

Widespread adoption of an IPM approach to insect pest management will require education and acceptance by regulators and practitioners. As an example, a particularly promising incentive for IPM implementation in Italy is a yield insurance scheme (mutual fund) for farmers, in which the required insurance premium is usually lower than insecticide costs (Furlan et al. 2014). An initial public contribution to this kind of crop insurance scheme to offset the risks of IPM implementation would encourage wider adoption of IPM strategies.

We recognize that the adoption of alternatives to neonicotinoids and moving agricultural practices to an IPM approach is particularly challenging where large-scale, cost-effective agricultural operations are on the landscape. Over the past two decades, the trend toward large, commercial agricultural operations has focused on scale economies and efficiencies (Morrison Paul et al. 2004), and this has encouraged the use of prophylactic crop protection by neonicotinoids to reduce risks from pests. Shifting agricultural production from a reliance on prophylactic insecticides to an IPM model and the use of alternative pest control options will take some time and will require investments in research and public extension to promote economically competitive and sustainable agricultural systems (Meissle et al. 2010). However, staying the course of widespread and prophylactic use of neonicotinoids increases the risk of serious environmental harm (van der Sluijs et al. 2014) and may ultimately threaten important ecosystem functions and services that support food security (Chagnon et al. 2014). Implementing sustainable agricultural practices at regional scales would benefit from a landscape perspective and the adoption of landscape design principles based on incentives or regulations (Dale et al. 2013).

While some of the options for alternative pest control that we illustrate in these case studies have been successfully demonstrated and field-tested, others are under ongoing development. Continued research into alternatives is warranted,

but equally pressing is the need for transfer and training of IPM technologies for farmers and other practitioners by public agencies and the need for policies and regulations to encourage the adoption of IPM strategies and their alternative pest control options.

Acknowledgments This manuscript benefited from the discussions in the International Task Force on Systemic Pesticides during its plenary meetings in Paris (2010), Bath (2011), Cambridge (2012), Padua (2012), Louvain-la-Neuve (2013), and Legnaro (2013). We thank the members of the TFSP for their advice and encouragement. We thank particularly Dr. Jean-Marc Bonmatin and Dr. Giovanni Burgio for their critical review of the manuscript. The work has been funded by the Triodos Foundation's Support Fund for Independent Research on Bee Decline and Systemic Pesticides. This Support Fund has been created from donations by the Adessium Foundation (The Netherlands), Act Beyond Trust (Japan), Universiteit Utrecht (Netherlands), Stichting Triodos Foundation (The Netherlands), Gesellschaft fuer Schmetterlingsschutz (Germany), M.A.O.C. Gravin van Bylandt Stichting (The Netherlands), Zukunft Stiftung Landwirtschaft (Germany), Study Association Storm (Student Association Environmental Sciences Utrecht University), and citizens. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest The authors declare no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- AA.VV. (2012) Idiam: la risposta della ricerca al problema diabrotica. *L'Informatore Agrario* 4:48–51
- Agosti M, Boriani M, Edwards CR, Michelon L (2011) Concianti e geodisinfestanti alla prova contro la diabrotica. *L'Informatore Agrario* 5:44–46
- Balconi C, Mazzinelli G, Lanzanova C, Torri A, Valoti P, Motto M, Berardo N (2011) Mais: secondo anno di sperimentazione agronomica nell'ambito del progetto Apenet. *Apoidea* 1–2:41–45
- Ball HJ, Weekman GT (1962) Insecticide resistance in the adult western corn rootworm in Nebraska. *J Econ Entomol* 55:439–441
- Baur R, Wijnands F, Malavolta C (2011) Integrated production—objectives, principles and technical guidelines. IOBC/WPRS Bulletin, Special Issue, ISBN 978-92-9067-244-9
- Benefer CM, Knight ME, Ellis JS, Hicks H, Blackshaw RP (2012) Understanding the relationship between adult and larval *Agriotes* distributions: the effect of sampling method, species identification and abiotic variables. *Appl Soil Ecol* 53:39–48
- Blackshaw RP, Hicks H (2013) Distribution of adult stages of soil insect pests across an agricultural landscape. *J Pest Sci* 86:53–62
- Blandino M, Berrino G, Rigamonti I E, Jucker C, Ferracini C, Saladini M A, Busato E (2013) Strategie integrate di difesa per controllare la diabrotica. *L'Informatore Agrario* 8:Supplemento Difesa delle colture 12–15
- Blandino M, Berrino G, Reyneri A, Ferracini C, Saladini M A, Alma A, Rigamonti I E, Jucker C, Agosti M, Amato F, Stassi P (2014) Monitoraggio diabrotica, quali soglie di intervento. *L'Informatore Agrario* 8, Supplemento Difesa delle colture:15–18

- Boicelli V (2007) La ricerca della redditività: 7 anni di esperienze DEKALB nel programma Concept Farm. Inserto “Le Prospettive economiche del Mais”: il mercato e le scelte dell'imprenditore. Terra&Vita 6
- Bonmatin J-M, Giorio C, Girolami V, Goulson D, Kreutzweiser D, Krupke C, Liess M, Long E, Marzaro M, Mitchell E, Noome D, Simon-Delso N, Tapparo A (2014) Environmental fate and exposure: neonicotinoids and fipronil. Environ Sci Pollut Res. doi:10.1007/s11356-014-3332-7
- Boriani M (2006) La lotta alla diabrotica nelle aziende lombarde. L'Informatore Agrar 7:58–59
- Boriani M (2008) Dossier diabrotica: tutto quello che c'è da sapere sulla diabrotica. Lombardia Verde 10:7–10
- Brassard D (2012) Estimated incremental increase in clothianidin usage pending registration (DP404793). US Environmental Protection Agency memorandum, Washington
- Burgio G, Ferrari R, Ragagnoli G, Petacchi R, Pozzati M, Reggiani A, Furlan L, Galassi T (2005) Analisi spaziale delle popolazioni di Elateridi mediante geostatistica e GIS: primi risultati di un'indagine eseguita in Emilia-Romagna. L'Informatore Fitopatologico 4:53–58
- Burgio G, Ragagnoli R, Petacchi R, Ferrari R, Pozzati M, Furlan L (2012) Optimization of *Agriotes sordidus* monitoring in northern Italy rural landscape, using a spatial approach. Bull Insectology 65:123–131
- Casida JE, Durkin KA (2013) Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annu Rev Entomol 58:99–117
- Chabert A, Blot Y (1992) Estimation des populations larvaires de taupins par un piège attractif. Phytoma 436:26–30
- Chagnon M, Kreutzweiser D, Mitchell D, Morrissey C, Noome D, van der Sluijs J (2014) Risks of large scale use of systemic insecticides to ecosystem functioning and services. Environ Sci Pollut Res. doi:10.1007/s11356-014-3277-x
- Dale V, Kline K, Kaffka S, Langeveld J (2013) A landscape perspective on sustainability of agricultural systems. Landsc Ecol 28:1111–1123
- Davis PM, Brenes N, Allee LL (1996) Temperature dependent models to predict regional differences in corn rootworm (Coleoptera: Chrysomelidae) phenology. Environ Entomol 25:767–775
- De Luigi V, Furlan L, Palmieri S, Vettorazzo M, Zanini G, Edwards CR, Burgio G (2011) Results of WCR monitoring plans and evaluation of an eradication programme using GIS and Indicator Kriging. J Appl Entomol 135:38–46
- Duan JJ, Bauer LS, Abell KJ, Lelito JP, van Driesche R (2013) Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in Michigan: potential for success in classical biocontrol of the invasive emerald ash borer. J Econ Entomol 106:1145–1154
- Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, Foster DR, Kloeppel BD, Knoepf JD, Lovett GM, Mohan J, Orwig DA, Rodenhouse NL, Sobczak WV, Stinson KA, Stone JK, Swan CM, Thompson J, Von Holle B, Webster JR (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. Front Ecol Environ 3:479–486
- Ferro G, Furlan L (2012) Mais: strategie a confronto per contenere gli elateridi. L'Informatore Agrar 42(Supplemento Difesa delle Colture):63–67
- Flower CE, Knight KS, Gonzalez-Meler MA (2013) Impacts of the emerald ash borer (*Agrilus planipennis* Fairmaire) induced ash (*Fraxinus* spp.) mortality on forest carbon cycling and successional dynamics in the eastern United States. Biol Invasions 15:931–944
- French BW, Chandler LD, Riedell WE (2007) Effectiveness of corn rootworm (Coleoptera: Chrysomelidae) areawide pest management in South Dakota. J Econ Entomol 100:1542–1554
- Furlan L (1989) Analisi delle possibilità di riduzione dell'impiego di geodisinfestanti nella coltura del mais nel Veneto. L'Informatore Agrar 17:107–115
- Furlan L (1996) The biology of *Agriotes ustulatus* Schaller (Col., Elateridae). I. Adults and oviposition. J Appl Entomol 120:269–274
- Furlan L, Toffanin F (1996) Suscettibilità di alcune colture erbacee agli attacchi di diverse specie del genere *Agriotes* e valutazione dell'efficacia di alcune strategie di protezione agronomica. Atti Giornate Fitopatologiche 1:215–222
- Furlan L (1998) The biology of *Agriotes ustulatus* Schaller (Col., Elateridae). II. Larval development, pupation, whole cycle description and practical implications. J Appl Entomol 122:71–78
- Furlan L (2004) The biology of *Agriotes sordidus* Illiger (Col., Elateridae). J Appl Entomol 128:696–706
- Furlan L (2005) An IPM approach targeted against wireworms: what has been done and what still has to be done. IOBC/wprs Bull 28(2):91–100
- Furlan L (2007) Management and biological control of wireworm populations in Europe: current possibilities and future perspectives. IOBC/wprs Bull 30:11–16
- Furlan L (2011) The importance of the identification of *Agriotes* larvae to implement IPM in arable crops. IOBC/wprs Bull 66:491–494
- Furlan L (2014) IPM thresholds for *Agriotes* wireworm species in maize in Southern Europe. J Pest Sci. doi:10.1007/s10340-014-0583-5
- Furlan L, Campagna G (2002) Study on the efficacy of imidacloprid and fipronil as seed dressing in controlling wireworms. Atti Giornate Fitopatologiche 1:499–504
- Furlan L, Talon G (1997) Aspetti entomologici: influenza dei sistemi colturali sulla evoluzione delle popolazioni dei fitofagi ipogei ed in particolare di *Agriotes sordidus* Illiger in Modelli Agricoli e Impatto Ambientale, valutazioni aziendali e territoriali, Raisa. UNIPRESS, Padova, pp 11–16
- Furlan L, Toffanin F (1994) Valutazione dell'efficacia di differenti strategie di lotta contro le larve di elateridi di due specie diverse (*Agriotes ustulatus* Schaller, *Agriotes brevis* Candèze). Atti Giornate Fitopatologiche 1994(2):187–194
- Furlan L, Toffanin F (1998) Effectiveness of new insecticides used as seed dressing (imidacloprid and fipronil) against wireworms in controlled environment. ATTI Giornate Fitopatologiche 1998:195–200
- Furlan L, Curto G, Ferrari R, Boriani L, Bourlot G, Turchi A (2000) Wireworm species damaging crops in Po valley. Informatore Fitopatologico 5:53–59
- Furlan L, Tóth M, Yatsinin V, Ujvary I (2001a) The project to implement IPM strategies against *Agriotes* species in Europe: what has been done and what is still to be done. Proceedings of XXI IWGO Conference, Legnaro Italy, 27 October – 3 November 2001:253–262
- Furlan L, Di Bernardo A, Maini S, Ferrari R, Boriani L, Boriani M, Nobili P, Bourlot G, Turchi A, Vacante V, Bonsignore C, Giglioli G, Tóth M (2001b) First practical results of click beetle trapping with pheromone traps in Italy. Proceedings of XXI IWGO Conference, Legnaro Italy, 27 October – 3 November 2001:253–262
- Furlan L, Zangheri S, Barbieri S, Lessi S, Delillo I, Barbi A, Bricchese F (2001c) Black cutworm alert programme in Italy. Proceedings of XXI IWGO Conference, Legnaro Italia, 27 ottobre – 3 Novembre 2001:407–412
- Furlan L, Di Bernardo A, Boriani M (2002) Proteggere il seme di mais solo quando serve. L'Informatore Agrar 8:131–140
- Furlan L, Canzi S, Di Bernardo A, Edwards CR (2006) The ineffectiveness of insecticide seed coatings and planting-time soil insecticides as *Diabrotica virgifera virgifera* LeConte population-suppressors. J Appl Ent 130(9–10):485–490
- Furlan L, Tóth M, Cooperators (2007a) Occurrence of click beetle pest spp. (Coleoptera, Elateridae) in Europe as detected by pheromone traps: survey results of 1998–2006. IOBC/WPRS Bull 30:19–25
- Furlan L, Canzi S, Toffoletto R, Di Bernardo A (2007b) Effetti sul mais della concia insetticida del seme. L'Informatore Agrar 5:92–96
- Furlan L, Caciagli P, Causin R, Di Bernardo A (2009a) Il seme di mais va protetto solo quando serve. L'Informatore Agrar 5:36–44

- Furlan L, Bonetto C, Costa B, Finotto A, Lazzeri L (2009b) Observations on natural mortality factors in wireworm populations and evaluation of management options. IOBC/wprs Bull 45:436–439
- Furlan L, Bonetto C, Costa B, Finotto A, Lazzeri L, Malaguti L, Patalano G, Parker W (2010) The efficacy of biofumigant meals and plants to control wireworm populations. Ind Crop Prod 31:245–254
- Furlan L, Cappellari C, Porrini C, Radeghieri P, Ferrari R, Pozzati M, Davanzo M, Canzi S, Saladini MA, Alma A, Balconi C, Stocco M (2011) Difesa integrata del mais: come effettuarla nelle prime fasi. L'Informatore Agrar 7(Supplemento Difesa delle Colture):15–19
- Furlan L, Vasileiadis VP, Sattin M (2013) Difesa integrata per le colture erbacee. L'Informatore Agrar 7(Supplemento Vigneto Frutteto, Guida alla Difesa):12–15
- Furlan L, Benvegnu' I, Cecchin A, Chiarini F, Fracasso F, Sartori A, Manfredi V, Frigimelica G, Davanzo M, Canzi S, Codato F, Bin O, Nadal V, Giacomet D (2014) Difesa integrata del mais: come applicarla in campo. L'Informatore Agrar 9(Supplemento Difesa delle Colture):11–14
- Gandhi KJK, Herms DA (2010) Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. Biol Invasions 12:389–405
- Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A (2012) Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. J Appl Entomol 136:17–26
- Gomboc S, Milevoj L, Furlan L, Tóth M, Bitenc P, A.B. obnar A, Celar F. (2001) Two-years of monitoring click beetles and wireworms in Slovenia. Proceedings of XXI WGO Conference, Legnano Italia, 27 ottobre – 3 Novembre 2001:283–292
- Gould JR, Bauer LS, Lelito JP, Duan JJ (2012) Emerald ash borer, *Agrilus planipennis* (Fairmaire), biological control release and recovery guidelines. USDA-APHIS-ARS-FS, Riverdale
- Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. J Appl Ecol 50:977–987
- Grant GG, Ryall KL, Lyons DB, Abou-Zaid MM (2010) Differential response of male and female emerald ash borer (Col., Buprestidae) to (Z)-3-hexenol and manuka oil. J Appl Ecol 134:26–33
- Grimalt S, Thompson D, Chartrand D, McFarlane J, Helson B, Lyons B, Meating J, Scarr T (2011) Foliar residue dynamics of azadirachtins following direct stem injection into white and green ash trees for control of emerald ash borer. Pest Manag Sci 67:1277–1284
- Hein GL, Tollefson JJ (1985) Use of Pherocon AM trap as a scouting tool for predicting damage by corn rootworm (Coleoptera: Chrysomelidae) larvae. J Econ Entomol 78:200–203
- Henry M, Béguin M, Requier F, Rollin O, Odoux JF, Aupinel P, Aptel J, Tchamitchian S, Decourtye A (2012) A common pesticide decreases foraging success and survival in honey bees. Science 336:348–350
- Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. J Agric Food Chem 59: 2897–2908
- Johnny S, Kyei-Poku G, Gauthier D, van Frankenhuyzen K, Krell P (2012) Characterization and virulence of *Beauveria* spp. recovered from emerald ash borer in Southwestern Ontario, Canada. J Invert Pathol 111:41–49
- Karabatsas K, Tsakiris V, Zarpas K, Tsitsipis JA, Furlan L, Tóth M (2001) Seasonal fluctuation of adult and larvae Agriotes spp. Proceedings of XXI WGO Conference, Legnano Italia, 27 ottobre – 3 Novembre 2001:269–276
- Kiss J, Edwards CR, Berger HK, Cate P, Cean M, Ceek S, Derron J, Festic H, Furlan L, Igrc-Barčić J, Ivanova I, Lammers W, Omelyuta V, Prinzinger G, Reynaud P, Sivčev I, Sivicek P, Urek G, Vahala O (2005) Monitoring of Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) in Europe 1992–2003. In: Vidal S, Kuhlmann U, Edwards CR (eds) Western corn rootworm: ecology and management. CABI Publishing, Wallingford, pp 29–39
- Kos T, Bažok R, Gunjača J, Igrc Barčić J (2014) Western corn rootworm adult captures as a tool for the larval damage prediction in continuous maize. J Appl Entomol 138:173–182
- Kosmacevskij AS (1955) Nekotoryje voprosy biologii i ekologii scelkunov. Uc. zap. Krasnodar gos ped inst 14:3–22
- Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA, Thompson DG (2007) Non-target effects on aquatic decomposer organisms of imidacloprid as a systemic insecticide to control emerald ash borer in riparian trees. Ecotoxicol Environ Saf 68:315–325
- Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA, Thompson DG (2008a) Are leaves that fall from imidacloprid-treated maple trees to control Asian longhorned beetles toxic to non-target decomposer organisms? J Environ Qual 37:639–646
- Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA, Holmes SB, Thompson DG (2008b) Effects on litter-dwelling earthworms and microbial decomposition of soil-applied imidacloprid for control of wood-boring insects. Pest Manag Sci 64:112–118
- Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA, Thompson DG (2008c) Toxicity of the systemic insecticide, imidacloprid, to forest stream insects and microbial communities. Bull Environ Contam Toxicol 80:211–214
- Kreutzweiser DP, Thompson DG, Scarr TA (2009) Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates. Ecotoxicol Environ Saf 72:1053–1057
- Kreutzweiser DP, Thompson DG, Grimalt S, Chartrand D, Good KP, Scarr TA (2011) Environmental safety to decomposer invertebrates of azadirachtin (neem) as a systemic insecticide in trees to control emerald ash borer. Ecotoxicol Environ Saf 74:1734–1741
- Kuhlmann U, Toepfer S, Zhang F (2005) Is classical biological control against Western corn rootworm in Europe a potential sustainable management strategy? In: Vidal S, Kuhlmann U, Edwards R (eds) Western corn rootworm: ecology and management. CABI, Wallingford, pp 263–284
- Kullik SA, Mark Sears MK, Schaafsma AW (2011) Sublethal effects of Cry 1F Bt corn and clothianidin on black cutworm (Lepidoptera: Noctuidae) larval development. J Econ Entomol 104(2):484–493
- Kurtz B, Toepfer S, Ehlers RU, Kuhlmann U (2007) Assessment of establishment and persistence of entomopathogenic nematodes for biological control of western corn rootworm. J Appl Entomol 131: 420–425
- Kyei-Poku G, Johnny S (2013) Evaluation of indigenous *Beauveria* isolates as potential agents for emerald ash borer management and the development of a diagnostic marker to monitor a post-release isolate. IOBC-WPRS Bull 90:119–124
- Lelito J, Lavalley R, Refugio Lomeli J, Lyons B, Marcotte M (2013) Application of biological control of the emerald ash borer in North America. NAPPO Technical Advisory Group on EAB. North American Plant Protection Organization, Ottawa
- Levine E, Spencer JL, Isard SA, Onstad DW, Gray ME (2002) Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. Amer Entomol 48:94–107
- Liu H, Bauer LS, Gao R, Zhao T, Petrice TR, Haack RA (2003) Exploratory survey for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), and its natural enemies in China. Great Lakes Entomol 36:191–204
- Luckmann WH, Shaw JT, Sherrod DW, Ruesink WG (1976) Development rate of BCW. J Econ Entomol 69(3):386
- Lyons DB (2010) Biological control of emerald ash borer biology. In: Lyons DB, Scarr TA (eds) Workshop proceedings: guiding principles for managing the emerald ash borer in urban environments. Ontario Ministry of Natural Resources and Natural Resources Canada, Canadian Forest Service, Sault Ste Marie, pp 29–34
- Lyons DB (2013) *Agrilus planipennis* Fairmore, emerald ash borer (Coleoptera: Buprestidae). In: Mason P, Gillespie D (eds)

- Biological control programs against insects and weeds in Canada 2001–2012. CABI Publishing, New York, pp 62–72
- Lyons DB, Lavalley R, Kyei-Poku G, van Frankenhuyzen K, Johny S, Guertin G, Francese J, Jones G, Blais M (2012) Towards the development of an autocontamination trap system to manage populations of emerald ash borer with the native entomopathogenic fungus, *Beauveria bassiana*. J Econ Entomol 105:1929–1939
- Masler V (1982) Škodlivé druhy kováčikov (Coleoptera, Elateridae) na Slovensku a ochrana proti nim. Polnohospodárska veda 3/82, Bratislava, 126 pp
- McCullough DG, Poland TM (2010) SLAM: a multi-agency pilot project to SLOW Ash Mortality caused by emerald ash borer in outlier sites. News Mich Entomol Soc 55:4–8
- McCullough DG, Siegert NW (2007) Estimating potential emerald ash borer (Coleoptera: Buprestidae) populations using ash inventory data. J Econ Entomol 100:1577–1586
- McCullough DG, Poland TM, Anulewicz AC, Cappaert D (2009) Emerald ash borer (Coleoptera: Buprestidae) attraction to stressed or baited ash trees. Environ Entomol 38:1331–1345
- McKenzie N, Helson B, Thompson D, Otis G, McFarlane J, Buscarini T, Meating J (2010) Azadirachtin: an effective systemic insecticide for control of *Agrilus planipennis* (Coleoptera: Buprestidae). For Entomol 103:708–717
- Meinke LJ, Siegfried BD, Wright RJ, Chandler LD (1998) Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J Econ Entomol 91:594–600
- Meinke LJ, Sappington TW, David W, Onstad DW, Guillemaud T, Miller NJ, Komáromi J, Levay N, Furlan L, Kiss J, Toth F (2009) Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) population dynamics. Agric For Entomol 11:29–46
- Meissle M, Mouron P, Musa T, Bigler F, Pons X, Vasileiadis V, Otto S, Anitchi D, Kiss J, Palinkas Z, Dörner Z, van der Weide R, Groten J, Czembor E, Adamczyk J, Thibord J-B, Melander B, Cordsen Nielsen G, Poulson R, Zimmermann O, Verschwele A, Oldenburg E (2010) Pests, pesticide use and alternative options in European maize production: current status and future prospects. J Appl Entomol 134:357–375
- Meissle M, Romeis J, Bigler F (2011) Bt maize and integrated pest management—a European perspective. Pest Manag Sci 67(9): 1049–1058
- Mercader RJ, Siegert NW, Liebhold AM, McCullough DG (2011) Simulating the effectiveness of three potential management options to slow the spread of emerald ash borer (*Agrilus planipennis*) populations in localized outlier sites. Can J For Res 41:254–264
- Morrison Paul C, Nehring R, Banker D, Somwaru A (2004) Scale economies and efficiency in US agriculture: are traditional farms history? J Product Anal 22:185–205
- Nowatzki TM, Tollefson JJ, Calvin DD (2002) Development and validation of models for predicting the seasonal emergence of corn rootworm (Coleoptera: Chrysomelidae). Environ Entomol 31:864–873
- Oleson JD (2003) Corn rootworm insecticide performance. Iowa State Research Farm Progress Reports. Paper 1417. http://lib.dr.iastate.edu/farms_reports/1417
- Oleson JD, Tollefson JJ (2005) Products evaluated for corn rootworm management. Iowa State Research Farm Progress Reports. Paper 1158. http://lib.dr.iastate.edu/farms_reports/1158
- Oleson JD, Tollefson JJ (2006) Products Evaluated for corn rootworm management. Iowa State Research Farm Progress Reports. Paper 980. http://lib.dr.iastate.edu/farms_reports/980
- Oleson JD, Park YL, Nowatzki TM, Tollefson JJ (2005) Node-injury scale to evaluate root injury by corn rootworms (Coleoptera: Chrysomelidae). J Econ Entomol 98(1):1–8
- Onstad DW, Guse CA, Spencer JL, Levine E, Gray ME (2001) Modeling the dynamics of adaptation to transgenic corn by western corn rootworm (Coleoptera: Chrysomelidae). J Econ Entomol 94:529–540
- Parker WE (1994) Evaluation of the use of food baits for detecting wireworms (*Agriotes* spp., Coleoptera: Elateridae) in fields intended for arable crop production. Crop Prot 13:271–276
- Parker WE (1996) The development of baiting techniques to detect wireworms (*Agriotes* spp., Coleoptera: Elateridae) in the field, and the relationship between bait-trap catches and wireworm damage to potato. Crop Prot 15:521–527
- Parker WE, Cox T, James D (1994) Evaluation of the use of baited traps to assess the risk of wireworm damage to potato. Proceeding of the Brighton Crop Protection Conference Pest and Diseases 1994, pp. 199–204
- Pisa L, Amaral-Rogers V, Belzunces L, Bonmatin J-M, Downs C, Goulson D, Kreutzweiser D, Krupke C, Liess M, McField M, Morrissey C, Noome D, Settele J, Simon-Delso N, Stark J, van der Sluijs J, van Dyck H, Wiemers M (2014) Effects of neonicotinoids and fipronil on non-target invertebrates. Environ Sci Pollut Res. doi:10.1007/s11356-014-3471-x
- Poland TM, McCullough DG (2006) Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. J For 104:118–124
- Poland TM, Haack RA, Petrice TR, Miller DH, Bauer LS (2006) Laboratory evaluation of the toxicity of systemic insecticides for control of *Anoplophora glabripennis* and *Plectrodera scalator* (Coleoptera: Cerambycidae). J Econ Entomol 99:85–93
- Pozzati M, Reggiani A, Ferrari R, Zucchi L, Burgio G, Furlan L (2006) II monitoraggio degli elateridi con trappole a feromoni. L'Informatore Agrar 3:56–59
- Rusek J (1972) *Agriotes brevis* und *Agriotes sordidus* (Coleoptera elateridae)—Sachlinge in N-Italien. Redia 53:321–329
- Ryall KL, Fidgeon JG, Turgeon JJ (2011) Detectability of the emerald ash borer (Coleoptera: Buprestidae) in asymptomatic urban trees by using branch samples. Environ Entomol 40:679–688
- Ryall KL, Fidgeon JG, Silk PJ, Scarr TA (2013) Efficacy of the pheromone (3Z)-lactone and the host kairomone (3Z)-hexenol at detecting early infestation of the emerald ash borer, *Agrilus planipennis*. Entomol Exp Appl 147:126–131
- Saladini MA, Michelatti G, Alma A, Borreani G, Tabacco E (2009) Sorghum in rotation with maize for *Diabrotica* control. L'Informatore Agrar 65:50–52
- Schaub L, Furlan L, Toth M, Steinger T, Carrasco LR, Toepfer S (2011) Efficiency of pheromone traps for monitoring *Diabrotica virgifera virgifera* LeConte. OEPP/EPPO Bull 41:189–194
- Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu Rev Entomol 35:271–297
- Shaw JT, Finger JW, Kevin L, Steffey KL, Michael E, Gray ME (1998) Efficacy of pre-plant, planting-time, pre-emergence, and rescue insecticide treatments for black cutworm control in Illinois. IPM%20%20Publications%20%20%20Insect%20Management%20%20%20Insecticide%20Evaluations,%20Illinois%201998.htm
- Showers WB (1997) Migratory ecology of the black cutworm. Annu Rev Entomol 42:393–425
- Simon-Delso N, Amaral-Rogers V, Belzunces L, Bonmatin J-M, Chagnon M, Downs C, Furlan L, Gibbons D, Giorio C, Girolami V, Goulson D, Kreutzweiser D, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell E, Morrissey C, Noome D, Pisa L, Settele J, Stark J, Tapparo A, van Dyck H, van Praagh J, van der Sluijs J, Whitehorn P, Wiemers M (2014) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. Environ Sci Pollut Res. doi:10.1007/s11356-014-3470-y
- Sivčev I, Stankovic S, Kostic M, Lakic N, Popovic Z (2009) Population density of *Diabrotica virgifera virgifera* LeConte in Serbian first year and continuous maize fields. J Appl Entomol 133:430–437

- Staudacher K, Pitterl P, Furlan L, Cate PC, Traugott M (2010) PCR-based species identification of *Agriotes* larvae. Bull Entomol Res 101: 201–210
- Streit M, Scarr T, Farintosh L (2012) Preparing for emerald ash borer; a landowner's guide to managing ash forests. Leeds-Grenville Stewardship Council and Eastern Ontario Model Forest. http://www.eomf.on.ca/index.php?option=com_k2&view=item&id=574:preparing-for-emerald-ash-borer&Itemid=331
- Sufyan M, Neuhoﬀ D, Furlan L (2011) Assessment of the range of attraction of pheromone traps to *Agriotes lineatus* and *Agriotes obscurus*. Agric For Entomol 13:313–319
- Szalai M, Kiss J, Kövér S, Toepfer S (2014) Simulating crop rotation strategies with a spatiotemporal lattice model to improve legislation for the management of the maize pest *Diabrotica virgifera virgifera*. Agric Syst 124:39–50
- Szarukán I (1977) Pajorok (Melolonthidae) és drótférgek (Elateridae) a kite taggazdaságok talajában 195-ben. Novenyvedelem, XIII, Evfolyam 2:49–54
- Szendrei Z, Grafius E, Byrne A, Ziegler A (2012) Resistance to neonicotinoid insecticides in field populations of the Colorado potato beetle (Coleoptera: Chrysomelidae). Pest Manag Sci 68:941–946
- Thompson DG, Kreutzweiser DP (2007) A review of the environmental fate and effects of natural “reduced-risk” pesticides in Canada. In: Felsot A, Racke K (eds) Certified organic and biologically derived pesticides: environmental, health and efficacy assessment, vol 947, Symposium Series. American Chemical Society, Washington, pp 245–274
- Toepfer S, Kuhlmann U (2004) Survey for natural enemies of the invasive alien chrysomelid, *Diabrotica virgifera virgifera*, in Central Europe. BioControl 49(4):385–395
- Toepfer S, Burger RU, Ehlers P, Peters A, Kuhlmann U (2010) Controlling western corn rootworm larvae with Entomopathogenic nematodes: effect of application techniques on plant-scale efficacy. J Appl Entomol 134:467–480
- Toepfer S, Kuhlman U, Jehle JA, Bazok R, Crickmore N, López Ferber M, Glazer I, Quesada Moraga E, Traugott M (2013) Research and development for a nematode-based biological control solution for Western corn rootworm in maize. IOBC/WPRS Bull 90:277–282
- Tóth M, Imrei Z, Szarukán I, Korosi R, Furlan L (2001) First results of click beetle trapping with pheromone traps in Hungary 1998–2000. Proceedings of XXI IWGO conference, Llegnaro Italy, 27 October – 3 November 2001:263–268
- Tóth M, Furlan L, Yatsynin VG, Ujváry I, Szarukán I, Imrei Z, Tolasch T, Francke W, Jossi W (2003) Identification of pheromones and optimization of bait composition for click beetle pests in Central and Western Europe (Coleoptera: Elateridae). Pest Manag Sci 59:1–9
- USDA-NASS (2013) Crop production 2013 summary. United States Department of Agriculture, National Agricultural Statistics Services online database: <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=10471>
- van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin J-M, Belzunces LP (2013) Neonicotinoids, bee disorders and the sustainability of pollinator services. Curr Opin Environ Sustain. <http://dx.doi.org/10.1016/j.cosust.2013.05.007>
- van der Sluijs J, Amaral-Rogers V, Belzunces L, Bijleveld van Lexmond M, Bonmatin J-M, Chagnon M, Downs C, Furlan L, Gibbons D, Giorio C, Girolami V, Goulson D, Kreutzweiser D, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell E, Morrissey C, Noome D, Pisa L, Settele J, Simon-Delso N, Stark J, Tapparo A, van Dyck H, van Praagh J, Whitehorn P, Wiemers M (2014) Conclusions of the worldwide integrated assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. Environ Sci Pollut Res. doi:10.1007/s11356-014-3229-5
- Vaughn T, Cavato T, Brar G (2005) A method of controlling corn rootworm feeding using *Bacillus thuringiensis* protein expressed in transgenic maize. Crop Sci 45:931–938
- Vernon RS, Kabaluk T, Behringer A (2000) Movement of *Agriotes obscurus* (Coleoptera: Elateridae) in strawberry (Rosaceae) plantings with wheat (Gramineae) as a trap crop. Can Entomol 132:1–11
- Waldron J.K., Shields E.J., Cox W.J., Testa T. (2002) demonstrating new technologies for improved corn rootworm management project. <http://www.nysipm.cornell.edu/grantspgm/projects/proj02/lfc/waldron.pdf>
- Whitehorn PR, O'Connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336:351–352
- Whitworth J, Davis H (2008) Corn rootworm insecticide/traits efficacy trial—Dickinson Co., KS. <http://entomology.k-state.edu/doc/efficacy-trials/2008-crw-insecticide-efficacy-trial2.pdf>
- Wilde G, Roozeboom K, Claassen M, Janssen K, Witt M (2004) Seed treatment for control of early-season pests of corn and its effect on yield. Agric Urban Entomol 21(2):75–85
- Zangheri S, Ciampolini M (1971) Gravi infestazioni di *Scotia ipsilon* Hfn. nell'Italia centro-settentrionale - Atti Giornate Fitopatologiche 1971:543–554
- Zangheri S, Ciampolini M, Suss L (1984) I gravi danni causati al mais dall'*Agrotis ipsilon*. Inform agr Verona XL(11):71–78
- Zangheri S, Furlan L, Sannino L (1998) Observations on overwintering of *Agrotis ipsilon* (Hufnagel) in different Italian regions. Boll Zool Agr Bachic Ser II 30:125–130
- Zhang F, Toepfer S, Kuhlmann U (2003) Basic biology and small-scale rearing of *Celatoria compressa* (Diptera: Tachinidae), a parasitoid of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Bull Entomol Res 93:569–575

Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning

J. P. van der Sluijs · V. Amaral-Rogers · L. P. Belzunces · M. F. I. J. Bijleveld van Lexmond · J.-M. Bonmatin · M. Chagnon · C. A. Downs · L. Furlan · D. W. Gibbons · C. Giorio · V. Girolami · D. Goulson · D. P. Kreutzweiser · C. Krupke · M. Liess · E. Long · M. McField · P. Mineau · E. A. D. Mitchell · C. A. Morrissey · D. A. Noome · L. Pisa · J. Settele · N. Simon-Delso · J. D. Stark · A. Tapparo · H. Van Dyck · J. van Praagh · P. R. Whitehorn · M. Wiemers

Received: 11 June 2014 / Accepted: 17 June 2014 / Published online: 10 October 2014
© The Author(s) 2014. This article is published with open access at Springerlink.com

Introduction

The side effects of the current global use of pesticides on wildlife, particularly at higher levels of biological organization: populations, communities and ecosystems, are poorly understood (Köhler and Triebkorn 2013). Here, we focus on one of the problematic groups of agrochemicals, the systemic insecticides fipronil and those of the neonicotinoid

family. The increasing global reliance on the partly prophylactic use of these persistent and potent neurotoxic systemic insecticides has raised concerns about their impacts on biodiversity, ecosystem functioning and ecosystem services provided by a wide range of affected species and environments. The present scale of use, combined with the properties of these compounds, has resulted in widespread contamination of agricultural soils, freshwater resources, wetlands, non-target

Responsible editor: Philippe Garrigues

J. P. van der Sluijs (✉) · L. Pisa · N. Simon-Delso
Department of Environmental Sciences, Copernicus Institute,
Utrecht University, Heidelberglaan 2, 3584 CS Utrecht,
The Netherlands
e-mail: jeroen@jvds.nl

V. Amaral-Rogers
Buglife, Bug House, Ham Lane, Orton Waterville, Peterborough PE2
5UU, UK

L. P. Belzunces
INRA, UR 406 Abeilles and Environnement, Laboratoire de
Toxicologie Environnementale, Site Agroparc, 84000 Avignon,
France

M. F. I. J. Bijleveld van Lexmond
46 Pertuis-du-Sault, 2000 Neuchâtel, Switzerland

J.-M. Bonmatin
Centre National de la Recherche Scientifique, Centre de Biophysique
Moléculaire, Rue Charles Sadron, 45071 Orléans Cedex 02, France

M. Chagnon
Département des Sciences Biologiques, Université du Québec À
Montréal, Case Postale 8888, Succursale Centre-Ville, Montreal,
Québec, Canada H3C 3P8

C. A. Downs
Haereticus Environmental Laboratory, P.O. Box 92, Clifford,
VA 24533, USA

L. Furlan
Veneto Agricoltura, Legnaro, PD, Italy

D. W. Gibbons
RSPB Centre for Conservation Science, RSPB, The Lodge, Sandy,
Bedfordshire SG19 2DL, UK

C. Giorio
Department of Chemistry, University of Cambridge, Lensfield Road,
CB2 1EW Cambridge, UK

V. Girolami
Dipartimento di Agronomia Animali Alimenti Risorse Naturali e
Ambiente, Università degli Studi di Padova, Agripolis, viale
dell'Università 16, 35020 Legnaro, Padova, Italy

D. Goulson
School of Life Sciences, University of Sussex, Brighton BN1 9RH,
UK

vegetation and estuarine and coastal marine systems, which means that many organisms inhabiting these habitats are being repeatedly and chronically exposed to effective concentrations of these insecticides.

Neonicotinoids and fipronil currently account for approximately one third (in monetary terms in 2010) of the world insecticide market (Simon-Delso et al. 2014). They are applied in many ways, including seed coating, bathing, foliar spray applications, soil drench applications and trunk injection. These compounds are used for insect pest management across hundreds of crops in agriculture, horticulture and forestry. They are also widely used to control insect pests and disease vectors of companion animals, livestock and aquaculture and for urban and household insect pest control and timber conservation (Simon-Delso et al. 2014).

Although the market authorization of these systemic insecticides did undergo routine ecological risk assessments, the regulatory framework has failed to assess the individual and joint ecological risks resulting from the widespread and simultaneous use of multiple products with multiple formulations and multiple modes of action. These applications co-occur across hundreds of cropping systems including all of our major agricultural commodities worldwide and on numerous cattle species, companion animals, etc. Also, the ecological risk assessment did not consider the various interactions with other environmental stressors. Once a market authorization is granted, the authorization poses limits to the dose and

frequency per allowed application, but no limits are set to the total scale of use of the active ingredients leading to a reduced potential for the recovery of impacted ecosystems from effects. In addition, there has been no assessment of successive neonicotinoid exposure typical in watersheds and resulting in culmination of exposure and effects over time (Liess et al. 2013). The potential interactions between neonicotinoids and fipronil and other pesticide active substances have not been considered either, although additivity and synergisms of toxic mechanisms of action have been documented (Satchivi and Schmitzer 2011; Gewehr 2012; Iwasa et al. 2004).

The Worldwide Integrated Assessment (WIA) presented in the papers in this special issue is the first attempt to synthesize the state of knowledge on the risks to biodiversity and ecosystem functioning posed by the widespread global use of neonicotinoids and fipronil. The WIA is based on the results of over 800 peer-reviewed journal articles published over the past two decades. We assessed respectively the trends, uses, mode of action and metabolites (Simon-Delso et al. 2014); the environmental fate and exposure (Bonmatin et al. 2014); effects on non-target invertebrates (Pisa et al. 2014); direct and indirect effects on vertebrate wildlife (Gibbons et al. 2014); and risks to ecosystem functioning and services (Chagnon et al. 2014) and finally explored sustainable pest management practices that can serve as alternatives to the use of neonicotinoids and fipronil (Furlan and Kreutzweiser 2014).

D. P. Kreutzweiser
Canadian Forest Service, Natural Resources Canada, 1219 Queen
Street East, Sault Ste Marie, ON, Canada P6A 2E5

C. Krupke · E. Long
Department of Entomology, Purdue University, West Lafayette,
IN 47907-2089, USA

M. Liess
Department of System Ecotoxicology, Helmholtz Centre for
Environmental Research - UFZ, 04318 Leipzig, Germany

M. McField
Healthy Reefs for Healthy People Initiative, Smithsonian Institution,
Belize City, Belize

P. Mineau
Pierre Mineau Consulting, 124 Creekside Drive, Salt Spring
Island V8K 2E4, Canada

E. A. D. Mitchell
Laboratory of Soil Biology, University of Neuchâtel, Rue Emile
Argand 11, 2000 Neuchâtel, Switzerland

E. A. D. Mitchell
Jardin Botanique de Neuchâtel, Chemin du Perthuis-du-Sault 58,
2000 Neuchâtel, Switzerland

C. A. Morrissey
Department of Biology and School of Environment and
Sustainability, University of Saskatchewan, 112 Science Place,
Saskatoon, SK S7N 5E2, Canada

D. A. Noome
Task Force on Systemic Pesticides, Pertuis-du-Sault,
2000 Neuchâtel, Switzerland

D. A. Noome
Kijani, Kasungu National Park, Private Bag 151, Lilongwe, Malawi

J. Settele · M. Wiemers
Department of Community Ecology, Helmholtz Centre for
Environmental Research - UFZ, Theodor-Lieser-Str. 4, 06120 Halle,
Germany

J. Settele
iDiv, German Centre for Integrative Biodiversity Research,
Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

N. Simon-Delso
Beekeeping Research and Information Centre (CARI), Place Croix
du Sud 4, 1348 Louvain la Neuve, Belgium

Mode of action, environmental fate and exposure

Due to their systemic nature, neonicotinoids and, to a lesser extent, fipronil as well as several of their toxic metabolites are taken up by the roots or leaves and translocated to all parts of the plant, which, in turn, makes the treated plant effectively toxic to insects that are known to have the potential to cause crop damage. Neonicotinoids and fipronil operate by disrupting neural transmission in the central nervous system of organisms. Neonicotinoids bind to the nicotinic acetylcholine receptor, whereas fipronil inhibits the GABA receptor. Both pesticides produce lethal and a wide range of sublethal adverse impacts on invertebrates but also some vertebrates (Simon-Delso et al. 2014 and Gibbons et al. 2014). Most notable is the very high affinity with which neonicotinoid insecticides agonistically bind to the nicotinic acetylcholine receptor (nAChR) such that even low-dose exposure over extended periods of time can culminate into substantial effects (see the literature reviewed by Pisa et al. 2014).

As a result of their extensive use, these substances are found in all environmental media including soil, water and air. Environmental contamination occurs via a number of disparate routes including dust generated during drilling of dressed seeds; contamination and build-up of environmental concentrations after repeated application in arable soils and soil water; run-off into surface and ground waters; uptake of pesticides by non-target plants via their roots followed by translocation to pollen, nectar, guttation fluids, etc.; dust and spray drift deposition on leaves; and wind- and animal-mediated dispersal of contaminated pollen and nectar from treated plants. Persistence in soils, waterways and non-target plants is variable but can be long; for example, the half-lives of neonicotinoids in soils can exceed 1,000 days. Similarly, they can persist in woody plants for

periods exceeding 1 year. Breakdown results in toxic metabolites, though concentrations of these in the environment are rarely measured (Bonmatin et al. 2014).

This combination of persistence (over months or years) and solubility in water has led to large-scale contamination of, and the potential for build-up in, soils and sediments (ppb–ppm range), waterways (ground and surface waters in the ppt–ppb range) and treated and non-treated vegetation (ppb–ppm range). Screening of these matrices for pesticides and their metabolites has not been done in a systematic and appropriate way in order to identify both the long-term exposure to low concentrations and the short-term erratic exposure to high concentrations.

However, where environmental samples have been screened, they were commonly found to contain mixtures of pesticides, including neonicotinoids or fipronil (with their toxic metabolites). In addition, samples taken in ground and surface waters have been found to exceed limits based on regulatory ecological threshold values set in different countries in North America and Europe. Overall, there is strong evidence that soils, waterways and plants in agricultural and urban environments and draining areas are contaminated with highly variable environmental concentrations of mixtures of neonicotinoids or fipronil and their metabolites (Bonmatin et al. 2014).

This fate profile provides multiple routes for chronic and multiple acute exposure of non-target organisms. For example, pollinators (including bees) are exposed through at least direct contact with dust during drilling; consumption of pollen, nectar, guttation drops, extra-floral nectaries and honeydew from seed-treated crops; water; and consumption of contaminated pollen and nectar from wild flowers and trees growing near treated crops or contaminated water bodies. Studies of food stores in honeybee colonies from a range of environments worldwide demonstrate that colonies are routinely and chronically exposed to neonicotinoids, fipronil and their metabolites (generally in the 1–100 ppb range), often in combination with other pesticides in which some are known to act synergistically with neonicotinoids. Other non-target organisms, particularly those inhabiting soils and aquatic habitats or herbivorous insects feeding on non-crop plants in farmland, will also inevitably be exposed, although exposure data are generally lacking for these groups (Bonmatin et al. 2014).

Impacts on non-target organisms

Impacts of systemic pesticides on pollinators are of particular concern, as reflected by the large number of studies in this area. In bees, field-realistic exposures in controlled settings have been shown to adversely affect individual navigation, learning, food collection, longevity, resistance to disease and fecundity. For bumblebees, colony-level effects have been clearly demonstrated, with exposed colonies growing more slowly and producing significantly fewer queens (Whitehorn

J. D. Stark

Puyallup Research and Extension Centre, Washington State University, Puyallup, WA 98371, USA

A. Tapparo

Dipartimento di Scienze Chimiche, Università degli Studi di Padova, via Marzolo 1, 35131 Padova, Italy

H. Van Dyck

Behavioural Ecology and Conservation Group, Biodiversity Research Centre, Université Catholique de Louvain (UCL), Croix du Sud 4-5 bte L7.07.04, 1348 Louvain-la-Neuve, Belgium

J. van Praagh

Scientific Advisor, Hassellstr. 23, 29223 Celle, Germany

P. R. Whitehorn

School of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK

J. P. van der Sluijs

Centre for the Study of the Sciences and the Humanities, University of Bergen, Postboks 7805, N-5020 Bergen, Norway

et al. 2012). Limited field studies with free-living bee colonies have largely been inconsistent and proved difficult to perform, often because control colonies invariably become contaminated with neonicotinoids, or there is a lack of replication in the study design, all of which demonstrates the challenges of conducting such a study in the natural environment (Maxim and Van der Sluijs 2013; Pisa et al. 2014).

Other invertebrate groups have received less attention. For almost all insects, the toxicity of these insecticides is very high including many species that are important in biological control of pests. The sensitivity to the toxic effect is less clear with non-insect species. For annelids such as earthworms, the LC_{50} is in the lower ppm range for many neonicotinoids (LOEC at 10 ppb). Crustaceans are generally less sensitive, although sensitivity is highly dependent on species and developmental stage. For example, blue crab megalopae are an order of magnitude more sensitive than juveniles.

At field-realistic environmental concentrations, neonicotinoids and fipronil can have negative effects on physiology and survival for a wide range of non-target invertebrates in terrestrial, aquatic, wetland, marine and benthic habitats (see the literature reviewed by Pisa et al. 2014). Effects are predominantly reported from laboratory toxicity testing, using a limited number of test species. Such tests typically examine only lethal effects over short time frames (i.e. 48 or 96 h tests), whereas ecologically relevant sublethal effects such as impairment of flight, navigation or foraging ability and growth are less frequently described. It has become clear that many of the tests use insensitive test species (e.g. *Daphnia magna*) and are not sufficiently long to represent chronic exposure and therefore lack environmental relevance. Laboratory testing to establish safe environmental concentration thresholds is hindered by the fact that most pesticide toxicity tests are based on older protocols. Although these systemic pesticide classes possess many novel characteristics, testing methodologies have remained largely unchanged, resulting in flawed conclusions on their ecological safety (Maxim and Van der Sluijs 2013). New and improved methodologies are needed to specifically address the unique toxicology profiles of chemicals, including their possible cumulative and delayed lethal and non-lethal effects for a variety of terrestrial, aquatic and marine organisms. Nevertheless, our review shows a growing body of published evidence that these systemic insecticides pose a serious risk of harm to a broad range of non-target invertebrate taxa often below the expected environmental concentrations. As a result, an impact on the many food chains they support is expected.

We reviewed nearly 150 studies of the direct (toxic) and indirect (e.g. food chain) effects of fipronil and the neonicotinoids imidacloprid and clothianidin on vertebrate wildlife—mammals, birds, fish, amphibians and reptiles. Overall, at concentrations relevant to field exposure scenarios in fields sown with coated seeds, imidacloprid and

clothianidin pose risks to small birds, and ingestion of even a few treated seeds could cause mortality or reproductive impairment to sensitive bird species (see the studies reviewed by Gibbons et al. 2014). Some recorded environmental concentrations of fipronil have been sufficiently high to potentially harm fish (Gibbons et al. 2014). All three insecticides exert sublethal effects, ranging from genotoxic and cytotoxic effects to impaired immune function, reduced growth or reduced reproductive success. Conclusive evidence was described recently, that neonicotinoids impair the immune response at the molecular level, thus enabling damages by covert diseases and parasites (Di Prisco et al. 2013). All these effects often occur at concentrations well below those associated with direct mortality (Gibbons et al. 2014). This is a trend in many taxa reported throughout the reviewed literature: short-term survival is not a relevant predictor neither of mortality measured over the long term nor of an impairment of ecosystem functions and services performed by the impacted organisms.

With the exception of the most extreme cases, the concentrations of imidacloprid and clothianidin that fish and amphibians are exposed to appear to be substantially below thresholds to cause mortality, although sublethal effects have not been sufficiently studied. Despite the lack of research and the difficulty in assigning causation, indirect effects may be as important as direct toxic effects on vertebrates and possibly more important. Neonicotinoids and fipronil are substantially more effective at killing the invertebrate prey of vertebrates than the vertebrates themselves. Indirect effects are rarely considered in risk assessment processes, and there is a paucity of data, despite the potential to exert population-level effects. Two field case studies with reported indirect effects were found in the published literature. In one, reductions in invertebrate prey from both imidacloprid and fipronil uses led to impaired growth in a fish species, and in another, reductions in populations of two lizard species were linked to effects of fipronil on termite prey (see the studies reviewed by Gibbons et al. 2014).

Impacts on ecosystem functioning and ecosystem services

The concept of ecosystem services is widely used in decision-making in the context of valuing the service potentials, benefits and use values that well-functioning ecosystems provide to humans and the biosphere (e.g. Spangenberg et al. 2014) and as an end point (value to be protected) in ecological risk assessment of chemicals. Neonicotinoid insecticides and fipronil are frequently detected in environmental media (soil, water, air) at locations where no pest management benefit is provided or expected. Yet, these media provide essential resources to support biodiversity and are known to be threatened by long-term or repeated contamination. The literature

synthesized in this integrated assessment demonstrates the large-scale bioavailability of these insecticides in the global environment at levels that are known to cause lethal and sublethal effects on a wide range of terrestrial (including soil) and aquatic microorganisms, invertebrates and vertebrates. Population-level impacts have been demonstrated to be likely at observed environmental concentrations in the field for insect pollinators, soil invertebrates and aquatic invertebrates. There is a growing body of evidence that these effects pose risks to ecosystem functioning, resilience and the services and functions provided by terrestrial and aquatic ecosystems. Such services and functions can be provisioning, regulating, cultural or supporting and include amongst others soil formation, soil quality, nutrient cycling, waste treatment and remediation, pollination, food web support, water purification, pest and disease regulation, seed dispersal, herbivory and weed control, food provision (including fish), aesthetics and recreation.

Knowledge gaps

While this assessment is based on a growing body of published evidence, some knowledge gaps remain. These compounds have been subject to regulatory safety tests in a number of countries. However, several potential risks associated with the present global scale of use are still poorly understood. We highlight key knowledge gaps.

- For most countries, there are few or no publicly available data sources on the quantities of systemic pesticides being applied, nor on the locations where these are being applied. Reliable data on the amounts used are a necessary condition for realistic assessments of ecological impacts and risks.
- Screening of neonicotinoid and fipronil residues in environmental media (soils, water, crop tissues, non-target vegetation, sediments, riparian plants, coastal waters and sediments) is extremely limited. Although their water solubility and propensity for movement are known, also, only very scarce data for marine systems exist.
- An even bigger knowledge gap is the environmental fate of a wide range of ecotoxic and persistent metabolites of neonicotinoids and fipronil. Hence, we cannot evaluate with accuracy the likely joint exposure of the vast majority of organisms.
- There is a poor understanding of the environmental fate of these compounds, and how, for example, soil properties affect persistence and whether they accumulate in (usually flowering) woody plants following repeated treatments with the parent compound. The behaviour of degradation products (which can be highly toxic and persistent) in different media (plants, soils, sediments, water, food chains, etc.) is poorly known.
- Long-term toxicity to most susceptible organisms has not been investigated. For instance, toxicity tests have only been carried out on four of the approximately 25,000 globally known species of bees, and there are very few studies of toxicity to other pollinator groups such as hoverflies or butterflies and moths. Similarly, soil organisms (beyond earthworms) have received little attention. Soil organisms play multiple roles in the formation of soil and in the maintenance of soil fertility. Toxicity to vertebrates (such as granivorous mammals and birds which are likely to consume treated seeds) has only been examined in a handful of species.
- Those toxicological studies that have been performed are predominantly focused on acute toxicity tests, whereas the effects of long-term, acute and chronic exposure is less well known, despite being the most environmentally relevant scenario for all organisms in agricultural and aquatic environments. The long-term consequences of exposure under environmentally realistic conditions have not been studied.
- All neonicotinoids bind to the same nAChRs in the nervous system such that cumulative toxicity is expected. At present, no studies have addressed the additive or synergistic effects of simultaneous exposure to multiple compounds of the neonicotinoid family, i.e. imidacloprid, clothianidin, thiamethoxam, dinotefuran, thiacloprid, acetamiprid, sulfoxaflor, nitenpyram, imidaclothiz, paichongding and cycloxaprid, into an aggregated dose of e.g. “imidacloprid equivalents”. Currently, risk assessments are done for each chemical separately, while many non-target species, such as pollinators, are simultaneously being exposed to multiple neonicotinoids as well as other pesticides and stressors. As a consequence, the risks have been systematically underestimated. While quantifying the suite of co-occurring pesticides is largely an intractable problem, a single metric that incorporates all neonicotinoid exposures to representative taxa would be an invaluable starting point.
- Cumulative toxicity of successive and simultaneous exposure has not been studied in the regulatory assessment and governance of chemical risks.
- Sublethal effects that often have lethal consequences in a realistic environmental setting have not been studied in most organisms. However, they are known to be profound in bees, and for those few other species where studies have been performed, sublethal doses of these neurotoxic chemicals have been reported to have adverse impacts on behaviour at doses well below those that cause immediate death.
- Interactions between systemic insecticides and other stressors, such as other pesticides, disease and food stress, have been explored in only a handful of studies (on bees), and these studies have revealed important synergistic

effects. For example, in honeybees, low doses of neonicotinoids greatly increase susceptibility to viral diseases. Interactions between systemic insecticides and other stressors in organisms other than bees are almost entirely unstudied. In field situations, organisms will almost invariably be simultaneously exposed to multiple pesticides as well as other stressors, so our failure to understand the consequences of these interactions (or even to devise suitable means to conduct future studies in this area) is a major knowledge gap.

- Impacts of these systemic insecticides on the delivery of a wide range of ecosystem services are still uncertain. The accumulation in soil and sediments might lead us to predict impacts on soil fauna such as earthworms and springtails (Collembola), which may in turn have consequences for soil health, soil structure and permeability and nutrient cycling. Contamination of field margin vegetation via dust or ground or surface water might lead us to expect impacts on fauna valued for aesthetic reasons (e.g. butterflies) and is likely to impact populations of important beneficial insects that deliver pollination or pest control services (e.g. hoverflies, predatory beetles). The general depletion of farmland and aquatic insect populations is likely to impact insectivorous species such as birds and bats. Contamination of freshwater is hypothesized to reduce invertebrate food for fish and so impact fisheries. The same might apply to coastal marine systems, potentially posing serious threats to coral reefs and salt marsh estuaries. None of these scenarios have been investigated.
- The short- and long-term agronomic benefits provided by neonicotinoids and fipronil are unclear. Given their use rates, the low number of published studies evaluating their benefit for yield or their cost-effectiveness is striking, and some recent studies (see Furlan and Kreutzweiser 2014) suggest that their use provides no net gain or even a net economic loss on some crops. It is not currently known what the impact on farming would be if these systemic pesticides were not applied or applied less (though their recent partial withdrawal in the EU provides an opportunity for this to be examined).

Given these knowledge gaps, it is impossible to properly evaluate the full extent of risks associated with the ongoing use of systemic insecticides, but the evidence reviewed in this special issue suggests that while the risks affect many taxa, the benefits have not been clearly demonstrated in the cropping systems where these compounds are most intensively used.

Conclusions

Overall, the existing literature clearly shows that present-day levels of pollution with neonicotinoids and

fipronil caused by authorized uses (i.e. following label rates and applying compounds as intended) frequently exceed the lowest observed adverse effect concentrations for a wide range of non-target species and are thus likely to have a wide range of negative biological and ecological impacts. The combination of prophylactic use, persistence, mobility, systemic properties and chronic toxicity is predicted to result in substantial impacts on biodiversity and ecosystem functioning. The body of evidence reviewed in this Worldwide Integrated Assessment indicates that the present scale of use of neonicotinoids and fipronil is not a sustainable pest management approach and compromises the actions of numerous stakeholders in maintaining and supporting biodiversity and subsequently the ecological functions and services the diverse organisms perform.

In modern agricultural settings, it is increasingly clear that insecticide treatments with neonicotinoids and fipronil—and most prominently its prophylactic applications—are incompatible with the original mindset that led to the development of the principles of integrated pest management (IPM). Although IPM approaches have always included insecticide tools, there are other approaches that can be effectively incorporated with IPM giving chemicals the position of the last resort in the chain of preferred options that need be applied first. Note that the current practice of seed treatment is the opposite: it applies chemicals as the first applied option instead of the last resort. The preferred options include organic farming, diversifying and altering crops and their rotations, inter-row planting, planting timing, tillage and irrigation, using less sensitive crop species in infested areas, using trap crops, applying biological control agents, and selective use of alternative reduced-risk insecticides. Because of the persistent and systemic nature of fipronil and neonicotinoids (and the legacy effects and environmental loading that come with these properties), these compounds are incompatible with IPM. We accept that IPM approaches are imperfect and constantly being refined. However, there is a rich knowledge base and history of success stories to work from in many systems where pest management is required. In fact, in Europe, the IPM approach has become compulsory for all crops as of the 1st of January 2014 in accordance with EU Directive 2009/128/EC, but most member states still need to operationalize and implement this new regulation, and IPM is sometimes poorly defined.

Recommendations

The authors suggest that regulatory agencies consider applying the principles of prevention and precaution to further tighten regulations on neonicotinoids and fipronil and consider formulating plans for a substantial reduction of the global scale of use. Continued research into

alternatives is warranted, but equally pressing is the need for education for farmers and other practitioners and the need for policies and regulations to encourage the adoption of alternate agricultural strategies to manage pests (e.g. IPM, organic, etc.). In addition, there is a need for research to obtain a better understanding of the institutional and other barriers that hamper large-scale adoption of proven sustainable agricultural practices that can serve as alternatives to the use of neonicotinoids and fipronil—as of many other pesticides as well.

The adequacy of the regulatory process in multiple countries for pesticide approval must be closely considered and be cognizant of past errors. For example, other organochloride insecticides such as DDT were used all over the world before their persistence, bioaccumulation and disruptive impacts on ecosystem functioning were recognized, and they were subsequently banned in most countries. Organophosphates have been largely withdrawn because of belated realization that they posed great risks to human and wildlife health. The systemic insecticides, neonicotinoids and fipronil, represent a new chapter in the apparent shortcomings of the regulatory pesticide review and approval process that do not fully consider the risks posed by large-scale applications of broad-spectrum insecticides.

Acknowledgments This manuscript benefited from the discussions in the International Task Force on Systemic Pesticides during its plenary meetings in Paris (2010), Bath (2011), Cambridge (2012), Montegrotto, Padova (2012), Louvain-la-Neuve (2013) and Legnaro, Padova (2013). The authors are listed in alphabetic order, except the first who is also the corresponding author. All authors work for public agencies or universities, except V. Amaral-Rogers who is employed by Buglife, a UK charity devoted to the conservation of invertebrates, D.W. Gibbons who is employed by the RSPB, a UK wildlife conservation charity, D.A. Noome, whose independent work for the TFSP is financed by the Stichting Triodos Foundation, and N. Simon-Delso working for CARI (association supported by the Belgium government). Contributions of J. Settele and M. Wiemers were part of www.legato-project.net (funded by the BMBF, German Ministry for Education and Research). The work has been funded by the Triodos Foundation's Support Fund for Independent Research on Bee Decline and Systemic Pesticides. This support fund has been created from donations by Adessium Foundation (The Netherlands), Act Beyond Trust (Japan), Utrecht University (Netherlands), Stichting Triodos Foundation (The Netherlands), Gesellschaft fuer Schmetterlingsschutz (Germany), M.A.O.C. Gravin van Bylandt Stichting (The Netherlands), Zukunft Stiftung Landwirtschaft (Germany), Study Association Storm (Student Association Environmental Sciences Utrecht University), Deutscher Berufs- und Erwerbsimkerbund e. V. (Germany), Gemeinschaft der europäischen Buckfastimker e. V. (Germany) and citizens. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Conflict of interest The authors declare no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- Bonmatin J-M, Giorio C, Girolami V, Goulson D, Kreutzweiser D, Krupke C, Liess M, Long E, Marzaro M, Mitchell E, Noome D, Simon-Delso N, Tapparo A (2014) Environmental fate and exposure; neonicotinoids and fipronil. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3332-7
- Chagnon M, Kreutzweiser DP, Mitchell EAD, Morrissey CA, Noome DA, van der Sluijs JP (2014) Risks of large scale use of systemic insecticides to ecosystem functioning and services. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3277-x
- Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, Nazzi F, Gargiulo G, Pennacchio F (2013) Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proc Natl Acad Sci USA* 110:18466–18471. doi:10.1073/pnas.1314923110
- Furlan L and Kreutzweiser DP (2014) Alternatives to neonicotinoid insecticides for pest control: case studies in agriculture and forestry. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3628-7
- Gewehr M (2012) Pesticidal mixtures. *Eur Patent EP 2 481 284 A2*
- Gibbons D, Morrissey C and Mineau P (2014) A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3180-5
- Iwasa T, Motoyama N, Ambrose JT, Roe RM (2004) Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Prot* 23:371–378. doi:10.1016/j.cropro.2003.08.018
- Köhler H-R, Triebkorn R (2013) Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? *Science* 341:759–765. doi:10.1126/science.1237591
- Liess M, Foit K, Becker A, Hassold E, Dolciotti I, Kattwinkel M, Duquesne S (2013) Culmination of low-dose pesticide effects. *Environ Sci Technol* 47:8862–8868
- Maxim L, Van der Sluijs JP (2013) Seed-dressing systemic insecticides and honeybees. Chapter 16, pp. 401–438. In: European Environment Agency (ed) Late lessons from early warnings: science, precaution, innovation. European Environment Agency (EEA) report 1/2013, Copenhagen
- Pisa L, Amaral-Rogers V, Belzunces LP, Bonmatin J-M, Downs C, Goulson D, Kreutzweiser D, Krupke C, Liess M, McField M, Morrissey C, Noome DA, Settele J, Simon-Delso N, Stark J, van der Sluijs, van Dyck H, Wiemers M (2014) Effects of neonicotinoids and fipronil on non-target invertebrates. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3471-x
- Satchivi NM, Schmitzer PR (2011) Synergistic herbicide/insecticide composition containing certain pyridine carboxylic acids and certain insecticides. *US Patent US 2011/0207606*
- Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, van Dyck H, van Praagh J, van der Sluijs JP, Whitehorn PR and Wiemers M (2014) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites. *Environ Sci Pollut Res*. doi:10.1007/s11356-014-3470-y
- Spangenberg JH, Görg C, Thanh Truong D, Tekken V, Bustamante JV, Settele J (2014) Provision of ecosystem services is determined by human agency, not ecosystem functions. Four case studies. *Int J Biodivers Sci Ecosyst Serv Manag* 10:40–53
- Whitehorn PR, O'Connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* 336:351–352

Appendix 1

Neonicotinoids, bee disorders and the sustainability of pollinator services

This TFSP paper provides an annotated literature review in Current Opinion style, see aims and scope of the journal Cosust for details of the approach:

<http://www.journals.elsevier.com/current-opinion-in-environmental-sustainability/>

Neonicotinoids, bee disorders and the sustainability of pollinator services[☆]

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

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

Addresses

¹ Environmental Sciences, Copernicus Institute, Utrecht University, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands

² School of Life Sciences, University of Sussex, UK

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

⁴ Centre de Biophysique Moléculaire, UPR 4301 CNRS affiliated to Orléans University and to INSERM, 45071 Orléans cedex 02, France

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

Corresponding author: van der Sluijs, Jeroen P (j.p.vandersluijs@uu.nl)

[☆] This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

Current Opinion in Environmental Sustainability 2013, 5:293–305

This review comes from a themed issue on **Open issue**

Edited by **Rik Leemans** and **William Solecki**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 6th June 2013

1877-3435/\$ – see front matter, © 2013 The Authors. Published by Elsevier B.V. All rights reserved.

<http://dx.doi.org/10.1016/j.cosust.2013.05.007>

Introduction

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

Neonicotinoids interact with the nicotinic acetylcholine receptors (nAChRs) of the insect central nervous system. They act mainly agonistically on nAChRs on the post-synaptic membrane, mimicking the natural neurotransmitter acetylcholine by binding with high affinity [3–5,6^{••},7,8^{••}]. This induces a neuronal hyper-excitation, which can lead to the insect's death within minutes [6,9]. Some of the major metabolites of neonicotinoids are equally neurotoxic, acting on the same receptors [10–12] thereby prolonging the effectiveness as systemic insecticide. The nAChR binding sites in the vertebrate nervous system are different from those in insects, and in general they have lower numbers of nicotinic receptors with high affinity to neonicotinoids, which are the reasons that neonicotinoids show selective toxicity for insects over vertebrates [9,13].

The main neonicotinoids presently on the market are imidacloprid, thiamethoxam, clothianidin, thiacloprid, dinotefuran, acetamiprid, nitenpyram and sulfoxaflor [12,14,15]. Since their introduction, neonicotinoids have grown to become the most widely used and fastest

growing class of insecticides with a 2010 global market share of 26% of the insecticide market [16] and imidacloprid the second most widely used (2008) agrochemical in the world [17]. The worldwide production of neonicotinoids is still increasing [18]. Large-scale use in Europe and US started around 2004. Neonicotinoids are nowadays authorised in more than 120 countries for more than 1000 uses [19] for the treatments of a wide range of plants including potato, rice, maize, sugar beets, cereals, oil rapeseed, sunflower, fruit, vegetables, soy, ornamental plants, tree nursery, seeds for export, and cotton.

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

Neonicotinoids and their metabolites are highly persistent in soil, aquatic sediments and water. To give an example: Six years after a single soil drench application of imidacloprid, residue levels up to 19 µg kg⁻¹ could be recovered in *Rhododendron* shrub blossoms [31]. Clothianidin has a half-life in soil between 148–6900 days [32], and imidacloprid 40–997 days [33]. Consequently, neonicotinoids exhibit a potential for accumulation in soil following repeated applications [23] and can be taken up by succeeding crops up to at least two years after application [34]. Imidacloprid has been detected in 97% of 33 soil samples from untreated fields on which treated corn seeds were used 1 or 2 years before the sampling [34]. Concentrations in these soil samples ranged from 1.2 to 22 µg kg⁻¹ [34]. Several studies recovered neonicotinoids in wild flowers near treated fields [35,36^{••}]. However, it remains a knowledge gap to what extent the presence in

wild flowers results from systemic uptake from polluted soil and water or from direct contamination of the flowers by contaminated dust from seed drilling.

At their introduction, neonicotinoids were assumed to be more efficient than the organophosphates and carbamates that they replaced [37]. As a seed treatment, they could be used in much lower quantities and they promised to be less polluting to the environment. It is however not the quantity that is relevant but the potency to cause harm, which results from toxicity, persistence and bioavailability to non-target species. Indeed, soon after the introduction of neonicotinoids, exposure to its residues in pollen, nectar, sowing dust etc., of non-target pollinating insects became clear. This led to various harmful effects [10,37,38,39^{••},40,41,42^{••},43^{••}].

Ecosystem services of pollinators

Amongst the wide diversity of pollinating species [44], bees are the most important. Although bee research mostly focuses on the domesticated *Apis mellifera*, over 25,000 different bee species have been identified (FAO: Pollination; URL: <http://www.fao.org/agriculture/crops/core-themes/theme/biodiversity/pollination/en/>). Bees provide a vital ecosystem service, playing a key role in the maintenance of biodiversity and in food and fibre production [45–47,48^{••},49–51]. Pollination comprises an integrated system of interactions that links earth's vegetation, wildlife and human welfare [52]. Of all flowering plants on earth, 87.5% benefits from animal pollination [53]. Globally, 87 of the leading food crops (accounting for 35% of the world food production volume) depend on animal pollination [45]. Pollinator mediated crops are of key importance in providing essential nutrients in the human food supply [54[•]]. The history of apiculture goes back to pre-agricultural times [55,56] and later co-developed with agriculture [57,58]. In addition, wild bees deliver a substantial and often unappreciated portion of pollination services to agriculture and wildflowers [59,60]. Bees and apiary products have a pharmacological [61,62], scientific and technological [63], poetic [64], aesthetic (springs filled with buzzing bumblebees) culinary (e.g., keeping alive traditional cuisine of patisseries with honey) and cultural value.

Global pollinator decline and emerging bee disorders

Long-term declines have been observed in wild bee populations around the world [47,65–70]. Over the past decades, a global trend of increasing honeybee disorders and colony losses has emerged [71–77]. Winter mortality of entire honeybee colonies has risen in many parts of the world [72[•],73,74,75[•]]. When neonicotinoids were first used, beekeepers started describing different disorders and signs ranging from: bees not returning to the hive, disoriented bees, bees gathered close together in small groups on the ground, abnormal foraging behaviour, the

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

Scientific research appears to indicate no single cause explaining the increase in winter colony losses. All viruses and other pathogens that have been linked to colony collapse have been found to be present year-round also in healthy colonies [78]. That colonies remain healthy despite the presence of these infectious agents, supports the theory that colony collapse may be caused by factors working in combination. Farooqui [79^{*}] has analysed the different hypotheses provided by science when searching for an explanation of Colony Collapse Disorder (CCD). Research points in the direction of a combination of reciprocally enhancing causes. Among those, the advance of neonicotinoid insecticides has gained more weight in light of the latest independent scientific results [80,81^{**},82^{**}]. In the present article, we synthesise the state of knowledge on the role of neonicotinoids in pollinator decline and emerging bee disorders.

Multiple ways of exposure

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

Honeybees' exposure to neonicotinoids can occur through ingestion, contact and inhalation (aerosols). Many possible exposure pathways can exist [85^{*}]. Here, we aggregate exposure pathways into: first, intake of food that contain residues; second, nesting material (resin, wax etc.); third, direct contact with spray drift and dust drift during application; fourth, contact with contaminated plants, soil, water; fifth, use of cooling water in the hive; and sixth, inhalation of contaminated air. For bumble bees and other wild bees that nest in soil, contact with contaminated soil is an additional pathway of concern. Leafcutter bees use cut leaf fragments to form nest cells and can thus be exposed to residues in leaves. There are many other conceivable exposure routes, for instance, a bee hive could have been made from timber from trees treated with neonicotinoids and may thus contain residues. However, the best researched exposure pathway is

via intake of food. Food with residues can be subdivided into self-collected raw food (nectar, pollen, water, honeydew, extrafloral nectar, guttation droplets, various other edible substances available in the foraging area etc.), in-hive processed food (honey, beebread, royal jelly, wax etc.), and food supplied by bee keepers (high fructose corn syrup, sugar water, sugar dough, bee candy, pollen, pollen substitutes based on soybean flower and other vegetable protein supplements etc.).

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

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

Little is known about the real exposure to contaminated food for different categories of honeybees in a colony, either in terms of contact with pollen or contact with, and possible consumption of, nectar if needed. For wild bees very few data exist on exposure in the field. The amount that wild bees actually consume in the field has not been

measured. EFSA estimated that worker bees, queens and larvae of bumblebees and adult females and larvae of solitary bees are likely to have the highest oral uptake of residues [85].

In 2002, 69% of pollen samples collected by honeybees at various places in France contained residues of imidacloprid and its metabolites [89]. In a systematic sampling scheme covering 5 locations over 3 years, imidacloprid was found in 40.5% of the pollen samples and in 21.8% of the honey samples [90,91]. On the basis of data from authorisation authorities, neonicotinoid residues in nectar and pollen of treated crop plants are estimated to be in the range of below analytical detection limit ($0.3 \mu\text{g kg}^{-1}$) to $5.4 \mu\text{g kg}^{-1}$ in nectar, the highest value corresponding to clothianidin in oilseed rape nectar, and a range of below detection limit ($0.3 \mu\text{g kg}^{-1}$) to $51 \mu\text{g kg}^{-1}$ in pollen, the highest value corresponding to thiamethoxam in alfalfa pollen [85]. A recent review reports wider ranges for pollen: $0.2\text{--}912 \mu\text{g kg}^{-1}$ for imidacloprid and $1.0\text{--}115 \mu\text{g kg}^{-1}$ for thiacloprid [92]. Residues of imidacloprid, dinotefuran, and thiamethoxam plus metabolites in pumpkin treated with United States label rates reach average levels up to $122 \mu\text{g kg}^{-1}$ in pollen and $17.6 \mu\text{g kg}^{-1}$ in nectar [93]. Up to 346 mg l^{-1} for imidacloprid and 146 mg l^{-1} for thiamethoxam and 102 mg l^{-1} clothianidin and have been found in guttation drops from leaves of plants germinated from neonicotinoid-coated seeds [84,94]. In melon, guttation levels up to 4.1 mg l^{-1} imidacloprid were found 3 days after a top (US) label rate soil application [95]. In a US wide survey of pesticide residues in beeswax, pollen and honeybees during the 2007–2008 growing seasons, high levels of neonicotinoids were found in pollen (included in [92]) but imidacloprid was also found up to $13.6 \mu\text{g kg}^{-1}$ in wax [96]. In Spain, neonicotinoids were found in beeswax samples from apiaries near fruit orchards: 11 out of 30 samples tested positive in ranges from $11 \mu\text{g kg}^{-1}$ (acetamiprid) to $153 \mu\text{g kg}^{-1}$ (thiacloprid) [97].

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

Acute and chronic effects of lethal and sublethal exposure

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

Acute toxicity is expressed as the lethal dose (LD) at which 50% of the exposed honeybees die within 48 hours: abbreviated to 'LD50 (48 hours)'. Neonicotinoids are highly toxic (in the range of ng/bee) to honeybees [98], both when administered orally and by contact. They also

have high acute toxicity to all other bee species so far tested, including various *Bombus* species, *Osmia lignaria* and *Megachile rotundata* [99–102]. *O. lignaria* is more sensitive to both clothianidin and imidacloprid than is *B. impatiens*, with *M. rotundata* more sensitive still [100]. In an acute toxicity test under semi field conditions on the Indian honeybee *Apis cerana indica*, clothianidin showed the highest toxicity, followed by imidacloprid and thiamethoxam [103].

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

Lethal effects from chronic exposure refer to honeybee mortality that occurs after prolonged exposure. In contrast to acute lethal effects, there are no standardised protocols for measuring chronic lethal effects. Therefore, in traditional risk assessment of pesticides they are usually expressed in three ways: LD50: the dose at which 50% of the exposed honeybees die (often, but not always, within 10 days); NOEC (No Observed Effect Concentration): the highest concentration of imidacloprid producing no observed effect; and LOEC (Lowest Observed Effect Concentration): the lowest concentration of imidacloprid producing an observed effect. However, for neonicotinoids and its neurotoxic metabolites, lethal toxicity can increase up to 100,000 times compared to acute toxicity when the exposure is extended in time [10]. There has been some controversy on the findings of that study, which is discussed in detail by Maxim and Van der Sluijs [40,42]. However, the key finding that exposure time amplifies the toxicity of neonicotinoids is consistent with later findings. Micro-colonies of bumblebees fed with imidacloprid showed the same phenomenon [102]: at one tenth of the concentration of the toxin in feed, it took twice as long to produce 100% mortality in a

bumblebee microcolony. At a 100 times lower dose, it took ca. four times longer to produce 100% mortality. The measurable shortening of the life span ceases to occur only when a dose was administered, for which the (extrapolated) chronic intoxication time would be longer than the natural life span of a worker bumblebee. This implies that the standard 10 day chronic toxicity test for bees is far too short for testing neonicotinoids. Indeed, honeybees fed with one tenth of the LC₅₀ of thiamethoxam showed a 41.2% reduction of life span [116]. Recent studies have shown that chronic toxicity of neonicotinoids can more adequately be expressed by time to 50% mortality instead of by the 10 day LD₅₀ [117–120,121*,122]. There is a linear relation between log daily dose and log time to 50% mortality [118,120,121*]. In experiments with honeybee colonies, similar long term chronic effects have indeed been found with typical times of 14–23 weeks to collapse 25–100% of the colonies exposed to imidacloprid-contaminated food at 20 µg kg⁻¹ [123] and 80–120 days for 1 mg kg⁻¹ dinotefuran and 400 µg kg⁻¹ clothianidin [76]. Note that these studies used concentrations that are on the high end of the currently reported ranges of concentrations found in the field. However, such data are sparse and limited to a few crops, so it cannot yet be concluded whether such concentrations are rare or common in the field.

At low concentrations of neonicotinoids, sublethal effects can occur. Sublethal effects involve modifications of honeybee behaviour and physiology (e.g., immune system). They do not directly cause the death of the individual or the collapse of the colony but may become lethal in time and/or may make the colony more sensitive (e.g., more prone to diseases), which may contribute to its collapse. For instance, an individual with memory, orientation or physiological impairments might fail to return to its hive, dying from hunger or cold. This would not be detected in standard pesticide tests, which focus on acute mortality. A distinction can be made between acute and chronic sublethal effects. Acute sublethal effects are assessed by exposing bees only once to the substance (by ingestion or by contact), and observing them for some time (variable from one laboratory to another, from several minutes to four days). Chronic sublethal effects are assessed by exposing honeybees more than once to neonicotinoids during an extended period of time (e.g., every 24 hours, for 10 days). Both acute and chronic sublethal effects are expressed as NOEC and/or LOEC (No or Lowest Observable Effect Concentration, respectively) [42].

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

level, population level and community level impacts on pollinators.

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

Sublethal doses of neonicotinoids impair the olfactory memory and learning capacity of honeybees [127,128,129*,130] and the orientation and foraging activity [131]. The impact of sublethal exposure on the flying behaviour and navigation capacity has been shown through homing flight tests [82,126,132,133]. Exposed to a very low concentration (0.05 µg kg⁻¹) imidacloprid honeybees show an initial slight increase in travel distance. However, with increasing concentration, starting at 0.5 µg kg⁻¹ imidacloprid decreases distance travelled and interaction time between bees, while time in the food zone increases with concentration [134*]. Imidacloprid disrupts honeybee waggle dancing and sucrose responsiveness at doses of 0.21 and 2.16 ng bee⁻¹ [135].

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

Sublethal effects seem to be detected more frequently and at lower concentrations when bumblebees (*Bombus terrestris*) have to travel to gather food, even when the distances are tiny. No observable impacts of imidacloprid at field realistic concentrations on micro-colonies of *B. terrestris* provided with food in the nest were found, but when workers had to walk just 20 cm down a tube to gather food, they exhibited significant sublethal effects on foraging activity, with a median sublethal effect concentration (EC₅₀) of 3.7 µg kg⁻¹ [102]. In queenright bumblebee colonies foraging in a glasshouse where food was 3 m away from their nest, 20 µg kg⁻¹ of imidacloprid caused significant worker mortality, with bees dying at the feeder. Significant mortality was also observed at

10 $\mu\text{g kg}^{-1}$, but not at 2 $\mu\text{g kg}^{-1}$ [102]. Bumblebees exhibit concentration-dependent sublethal responses (declining feeding rate) to imidacloprid starting at 1 $\mu\text{g l}^{-1}$ in syrup, while honeybees seemed unaffected [137].

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

It has also been shown that pesticides like imidacloprid act on the hypopharyngeal glands of honeybee nurses by degenerating the tissues [140,141,142^{••}], which induces a shift from nest to field activities. In the native stingless bee *Melipona quadrfasciata anthidioides*, imidacloprid causes impairment of the mushroom bodies which are involved in learning [143]. Imidacloprid and clothianidin have been shown to be potent neuromodulators of the honeybee brain, causing mushroom body neuronal inactivation in honeybees, which affect honeybee cognition and behaviour at concentrations that are encountered by foraging honeybees and within the hive [8]. Sublethal doses of imidacloprid were also found to have cytotoxic activity in the Malpighian tubules in honeybees that make up the excretory and osmoregulatory system [144]. Exposure to thiamethoxam has also been shown to result in morphological impairment of the bee brain and bee midgut [116].

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

Short-term and mid-term sublethal effects on individuals or age groups result in long-term effects at the colony level, which follow weeks to months after the exposure, such as honeybee colony depopulation and bumblebee colony queen production [76,81^{••},123,138[•]]. As it has recently been acknowledged, the field tests on which the marketing authorisation of the use of neonicotinoids is essentially based were not developed to detect sublethal nor long-term effects on the colony level, and the observation of the

performances of colonies after experimental exposure do not last long enough [85]. Major weaknesses of existing field studies are the small size of the colonies, the very small distance between the hives and the treated field and the very low surface of the test field. As a consequence of these weaknesses, the real exposures of the honey bees during these field tests are highly uncertain and may in reality be much smaller than what has been assumed in these field studies. [85]

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

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

The challenges of field studies became also clear in the debates over the highly contested field study recently conducted by the Food and Environment Research Agency (FERA) which resorts under the UK Department for Environment, Food and Rural Affairs (DEFRA). This study was set up in response to the *Science* publication that showed that a short term exposure of bumblebees to field realistic imidacloprid concentrations causes a long term 85% reduction in queen production [81^{••}]. At three sites 20 bumblebee colonies were exposed to crops grown from untreated, clothianidin-treated or imidacloprid-treated seeds. The agency concluded that ‘no clear consistent relationships’ between pesticide levels and harm to the insects could be found [FERA: URL: <http://www.fera.defra.gov.uk/scienceResearch/scienceCapabilities/chemicalsEnvironment/documents/reportPS2371V4a.pdf>].

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

Given all the major limitations to the reliability of outcomes of field studies, it is recommendable to give more weight in the risk assessment to reproducible results from controlled lab studies and use the ratio between the environmental concentration and the no effect concentration as the main risk indicator [40,42]. It could perhaps be linked to modelling to explore how, and to what the degree, the various well-known sublethal effects on individual bees can weaken the colony [148].

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

Synergistic effects: pesticide–pesticide and pesticide–infectious agents

A synergy occurs when the effect of a combination of stressors is higher than the sum of the effect of each stressor alone. When neonicotinoids are combined with certain fungicides (azoles, such as prochloraz, or anilides, such as metalaxyl) or other agrochemicals that block cytochrome P450 detoxification enzymes, their toxicity increases by factor from 1.52 to 1141 depending on the combination [151,152]. The strongest synergism has been found for triflumizole making thiacloprid 1141 times more acutely toxic to honeybees [151]. This synergistic effect is

the subject of patents by agrochemical companies [152,153].

Synergy has also been demonstrated for neonicotinoids and infectious agents. Prolonged exposure to a non-lethal dose of neonicotinoids renders beehives more susceptible to parasites such as *Nosema ceranae* infections [39^{••},154^{••},155[•],156]. This can be explained either by an alteration of the immune system or by an impairment of grooming and allogrooming that leads to reduced hygiene at the individual level and in the nest, which gives the pathogens more chances to infect the bees. The same mechanism, where the balance between an insect and its natural enemies is disturbed by sublethal exposures to neonicotinoids that impairs grooming, is well known and often used in pest management of target insects [157–161].

Conclusion and prospects

In less than 20 years, neonicotinoids have become the most widely used class of insecticides. Being used in more than 120 countries in more than 1000 different crops and applications, they now account for at least one quarter of the world insecticide market. For pollinators, this has transformed the agrochemical landscape to one in which most flowering crops and an unknown proportion of wild flowers contain varying concentrations of neonicotinoids in their pollen and nectar. Most neonicotinoids are highly persistent in soil, water and sediments and they accumulate in soil after repeated uses. Severe surface water pollution with neonicotinoids is common. Their systemic mode of action inside plants means phloemic and xylemic transport that results in translocation to pollen and nectar. Their wide application, persistence in soil and water and potential for uptake by succeeding crops and wild plants make neonicotinoids bioavailable to pollinators in sublethal concentrations for most of the year. This results in the frequent presence of neonicotinoids in honeybee hives. Neonicotinoids are highly neurotoxic to honeybees and wild pollinators. Their capacity to cross the ion-impermeable barrier surrounding the central nervous system (BBB, blood–brain barrier) [7[•]] and their strong binding to nAChR in the bee's central nervous system are responsible for a unique chronic and sublethal toxicity profile. Neonicotinoid toxicity is reinforced by exposure time. Some studies indicate a non-monotonic [162[•]] dose–response curve at doses far below the LD50. Mass bee dying events in spring from acute intoxication have occurred in Germany, Italy, Slovenia and France during pneumatic sowing of corn seeds coated with neonicotinoids. Bees that forage near corn fields during sowing get exposed to acute lethal doses when crossing the toxic dust cloud created by the sowing machine.

At field realistic exposure levels, neonicotinoids produce a wide range of adverse sublethal effects in honeybee colonies and bumblebee colonies, affecting colony performance through impairment of foraging success, brood

and larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene etc. Neonicotinoids synergistically reinforce infectious agents such as *N. ceranae* and exhibit synergistic toxicity with other agrochemicals. The large impact of short term field realistic exposure of bumblebee colonies on long term bumblebee queen production (85% reduction) could be a key factor contributing to the global trends of bumblebee decline. Only a few studies assessed the toxicity to other wild pollinators, but the available data suggest that they are likely to exhibit similar toxicity to all wild insect pollinators. The worldwide production of neonicotinoids is still increasing. In view of the vital importance of the service insect pollinators provide to both natural ecosystems and farming, they require a high level of protection. Therefore a transition to pollinator-friendly alternatives to neonicotinoids is urgently needed for the sake of the sustainability of pollinator ecosystem services. The recent decision by the European Commission to temporary ban the use of imidacloprid, thiamethoxam and clothianidin in crops attractive to bees is a first step in that direction [163].

Acknowledgements

This manuscript benefited from the discussions in the IUCN International Task Force on Systemic Pesticides during its plenary meetings in Bath (2011), Cambridge (2012), Padua (2012) and Louvain-la-Neuve (2013). Part of the work by authors JvdS and NSD has been funded by a gift by the Triodos Foundation's Support Fund for Independent Research on Bee Decline and Systemic Pesticides. This Support Fund has been created from donations by Adessium Foundation (The Netherlands), Act Beyond Trust (Japan), Universiteit Utrecht (Netherlands), Stichting Triodos Foundation (The Netherlands), Gesellschaft für Schmetterlingsschutz (Germany), M.A.O.C. Gravin van Bylandt Stichting (The Netherlands), Zukunft Stiftung Landwirtschaft (Germany), Beekeepers Union ABTB (Netherlands), Study Association Storm (Student Association Environmental Sciences Utrecht University) and citizens. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References and recommended reading

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

- of special interest
- of outstanding interest

1. Tomizawa M, Casida JE: **Neonicotinoid insecticides: highlights of a symposium on strategic molecular designs.** *J Agric Food Chem* 2011, **59**:2883-2886 <http://dx.doi.org/10.1021/jf103856c>.
2. Bromilow RH, Chamberlain K, Evans AA: **Physicochemical aspects of phloem translocation of herbicides.** *Weed Sci* 1990, **38**:305-314.
3. Buckingham SD, Lapied B, Le Corronc H, Grolleau F, Sattelle DB: **Imidacloprid actions on insect neuronal acetylcholine receptors.** *J Exp Biol* 1997, **200**:2685-2692.
4. Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Sattelle DB: **Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors.** *Trends Pharmacol Sci* 2001, **22**:573-580.
5. Matsuda K, Shimomura M, Ihara M, Akamatsu M, Sattelle DB: **Neonicotinoids show selective and diverse actions on their nicotinic receptor targets: electrophysiology, molecular biology, and receptor modeling studies.** *Biosci Biotechnol Biochem* 2005, **69**:1442-1452.
6. Belzunces LP, Tchamitchian S, Brunet JL: **Neural effects of insecticides in the honey bee.** *Apidologie* 2012, **43**:348-370.
Excellent review of neural impacts of field exposure of honeybees to sublethal residues of neurotoxic insecticides in pollen and nectar. These impair: firstly cognitive functions, including learning and memory, habituation, olfaction and gustation, navigation and orientation; secondly behaviour, including foraging and thirdly physiological functions, including thermoregulation and muscle activity. Time is a key factor in insecticide toxicity. Combination toxicity of joint exposure to multiple pesticides urgently requires attention.
7. Tomizawa M: **Chemical biology of the nicotinic insecticide receptor.** *Adv Insect Physiol* 2013, **44**:63-99.
Introduction into the molecular basis of binding site interaction and explanation of different binding affinity of neonicotinoids with insect and vertebrate nicotinic acetyl choline receptor. Another key factor explaining the high insect toxicity is its capacity (stemming from hydrophobicity) to penetrate the ion-impermeable barrier surrounding the insect nervous system.
8. Palmer MJ, Moffat C, Saranzewa N, Harvey J, Wright GA, Connolly CN: **Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees.** *Nat Commun* 2013, **4**:1634 <http://dx.doi.org/10.1038/ncomms2648>.
Using recordings from mushroom body Kenyon cells in acutely isolated honeybee brain, it is shown that the neonicotinoids imidacloprid and clothianidin, and the organophosphate miticide coumaphos oxon, cause a depolarization-block of neuronal firing and inhibit nicotinic responses. These effects are observed at concentrations that are encountered by foraging honeybees and within the hive, and are additive with combined application. Exposure to multiple pesticides that target cholinergic signalling will cause enhanced toxicity to pollinators.
9. Tomizawa M, Casida JE: **Neonicotinoid insecticide toxicology: mechanism of selective action.** *Annu Rev Pharmacol Toxicol* 2005, **45**:247-268.
10. Suchail S, Guez D, Belzunces LP: **Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*.** *Environ Toxicol Chem* 2001, **20**:2482-2486.
11. Suchail S, Debrauwer L, Belzunces LP: **Metabolism of imidacloprid in *Apis mellifera*.** *Pest Manag Sci* 2004, **60**:291-296.
12. Casida JE: **Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance.** *J Agric Food Chem* 2010, **59**:2923-2931 <http://dx.doi.org/10.1021/jf102438c>.
13. Liu GY, Ju XL, Cheng J: **Selectivity of Imidacloprid for fruit fly versus rat nicotinic acetylcholine receptors by molecular modeling.** *J Mol Model* 2010, **16**:993-1002.
14. Liu Z, Yao X, Zhang Y: **Insect nicotinic acetylcholine receptors (nAChRs): important amino acid residues contributing to neonicotinoid insecticides selectivity and resistance.** *Afr J Biotechnol* 2008, **7**:4935-4939.
15. Cutler P, Slater R, Edmunds JF, Maienfisch P, Hall RG, Earley GP, Pittner T, Pal S, Paul V-L, Goodchild J, Blacker M, Hagmann L, Crossthwaite AJ: **Investigating the mode of action of sulfoxaflor: a fourth-generation neonicotinoid.** *Pest Manag Sci* 2013, **69**:607-619 <http://dx.doi.org/10.1002/ps.3413>.
16. Casida JE, Durkin K: **Neuroactive insecticides: targets, selectivity, resistance, and secondary effects.** *Annu Rev Entomol* 2013, **58**:99-117 <http://dx.doi.org/10.1146/annurev-ento-120811-153645>.
17. Pollak P: *Fine Chemicals: The Industry and the Business.* John Wiley & Sons; 2011.
18. Shao X, Liu Z, Xu X, Li Z, Qian X: **Overall status of neonicotinoid insecticides in China: production, application and innovation.** *J Pest Sci* 2013, **38**:1-9 <http://dx.doi.org/10.1584/jpestics.D12-037>.
19. European Food Safety Authority: **statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe.** *EFSA J* 2012, **10**:2752 <http://dx.doi.org/10.2903/j.efsa.2012.2752>.
20. Sur R, Stork A: **Uptake, translocation and metabolism of imidacloprid in plants.** *Bull Insectol* 2003, **56**:35-40.

21. Van de Meent D, Hollander A, Peijnenburg W, Breure T: **Fate and transport of contaminants.** In *Ecological Impacts of Toxic Chemicals*. Edited by Sánchez-Bayo F, Van den Brink P, Mann RM. Bentham; 2011.
 22. Gupta S, Gajbhiye V, Kalpana T, Agnihotri NP: **Leaching behavior of imidacloprid formulations in soil.** *Bull Environ Contam Toxicol* 2002, **68**:502-508.
 23. Haith DA: **Ecological risk assessment of pesticide runoff from grass surfaces.** *Environ Sci Technol* 2010, **44**:6496-6502.
 24. Selim HM, Jeong CY, Elbana TA: **Transport of imidacloprid in soils: miscible displacement experiments.** *Soil Sci* 2010, **175**:375-381.
 25. Miranda GRB, Raetano CG, Silva E, Daam MA, Cerejeira MA: **Environmental fate of neonicotinoids and classification of their potential risks to hypogean, epygean, and surface water ecosystems in Brazil.** *Hum Ecol Risk Assess* 2011, **17**:981-995.
 26. Kurwadkar ST, Dewinne D, Wheat R, McGahan DG, Mitchell FL: **Time dependent sorption behavior of dinotefuran, imidacloprid and thiamethoxam.** *J Environ Sci Health B* 2013, **48**:237-242 <http://dx.doi.org/10.1080/03601234.2013.742412>.
 27. Van Dijk T, Van Staalduinen M, Van der Sluijs JP: **Macro-invertebrate decline in surface water polluted with imidacloprid.** *PLoS ONE* 2013, **8**:e62374 <http://dx.doi.org/10.1371/journal.pone.0062374>.
 28. Starner K, Goh KS: **Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010–2011.** *Bull Environ Contam Toxicol* 2012, **88**:316-321 <http://dx.doi.org/10.1007/s00128-011-0515-5>.
 29. Kreuger J, Graaf S: *Pesticides in Surface Water in Areas with Open Ground and Greenhouse Horticultural Crops in Sweden* 2008. 2010.
 30. Anderson TA, Salicea CJ, Erickson RA, McMurphy ST, Coxa SB, Smith LM: **Effects of land use and precipitation on pesticides and water quality in playa lakes of the southern high plains.** *Chemosphere* 2013, **92**:84-90 <http://dx.doi.org/10.1016/j.chemosphere.2013.02.054>.
 31. Doering J, Maus C, Schoening R: *Residues of Imidacloprid WG 5 in Blossom Samples of Rhododendron sp (variety Nova Zembla) after Soil Treatment in the Field Application: 2003, Sampling 2003 and 2004, Bayer CropScience AG. Report No G201806/32.* 2004.
 32. Rexrode M, Barrett M, Ellis J, Gabe P, Vaughan A, Felkel J, Melendez J: *EFED Risk Assessment for the Seed Treatment of Clothianidin 600FS on Corn and Canola.* United States Environmental Protection Agency; 20 February 2003.
 33. NPIC (National Pesticide Information Center): *Imidacloprid Technical Fact Sheet.* 2013. <http://www.npic.orst.edu/factsheets/imidacloprid.pdf> (accessed 17.02.13).
 34. Bonmatin JM, Moineau I, Charvet R, Colin ME, Fleche C, Bengsch ER: **Behaviour of imidacloprid in fields. Toxicity for honey bees.** *Environmental Chemistry – Green Chemistry and Pollutants in Ecosystems.* Berlin: Springer; 2005, 483-494.
 35. Greatti M, Sabatini AG, Barbattini R, Rossi S, Stravisi A: **Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results.** *Bull Insectol* 2003, **56**:69-72.
 36. Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K: **Multiple routes of pesticide exposure for honey bees living near agricultural fields.** *PLoS ONE* 2012, **7**:e29268 <http://dx.doi.org/10.1371/journal.pone.0029268>.
- Bees are exposed in many ways to neonicotinoids in agricultural fields throughout the foraging period. During spring, extremely high levels of clothianidin and thiamethoxam were found in planter exhaust material produced during the planting of treated maize seed. Neonicotinoids were found in soil also of unplanted fields. Dandelions visited by foraging bees growing near these fields contained neonicotinoids: 1.1–9.4 µg kg⁻¹ clothianidin and 1.1–2.9 µg kg⁻¹ thiamethoxam.
37. Bonmatin JM, Marchand PA, Charvet R, Moineau I, Bengsch ER, Colin ME: **Quantification of imidacloprid uptake in maize crops.** *J Agric Food Chem* 2005, **53**:5336-5341 <http://dx.doi.org/10.1021/jf0479362>.
 38. Maini S, Medrzycki P, Porrini C: **The puzzle of honey bee losses: a brief review.** *Bull Insectol* 2010, **63**:153-160.
 39. Vidau C, Diogon M, Aufauvre J, Fontbonne R, Viguès B, Brunet J-L, Texier C, Biron DG, Blot N, El Alaoui H et al.: **Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*.** *PLoS ONE* 2011, **6**:e21550 <http://dx.doi.org/10.1371/journal.pone.0021550>.
- Synergism was found between neurotoxic insecticides and *Nosema ceranae* infections: Sublethal doses of fipronil and thiacloprid make honeybees more prone to *Nosema ceranae* infections and induces increased mortality of nosema-infected bees. This effect does not seem to be linked to a decrease of the bee detoxification system. A step forward in understanding the interactions of factors jointly producing colony depopulation.
40. Maxim L, Van der Sluijs JP: **Uncertainty: cause or effect of stakeholders' debates? Analysis of a case study: the risk for honey bees of the insecticide Gaucho®.** *Sci Total Environ* 2007, **376**:1-17.
 41. Suryanarayanan S, Kleinman DL: **Disappearing bees and reluctant regulators.** *Issues Sci Technol* 2011, **27**:33.
 42. Maxim L, Van der Sluijs JP: **Seed-dressing systemic insecticides and Honeybees.** *Late Lessons from Early Warnings: Science, Precaution, Innovation.* European Environment Agency (EEA); 2013. p. 401-438.
- Using the bans of imidacloprid in France for sunflower (1999) and maize (2004) as a case study, the social processes that ultimately lead to application of the precautionary principle are analyzed in a very insightful way. The analysis focuses on the ways in which scientific findings were used by stakeholders and decision-makers to influence policy during the controversy. Eight lessons are drawn about governance of controversies related to chemical risks.
43. Suryanarayanan S, Kleinman D: **Be(e)coming experts the controversy over insecticides in the honey bee colony collapse disorder.** *Soc Stud Sci* 2013, **43**:215-240 <http://dx.doi.org/10.1177/0306312712466186>.
- This paper explores the politics of expertise in an ongoing controversy in the United States over the role of neonicotinoids in colony collapse disorder. A set of research norms and practices from agricultural entomology came to dominate the investigation of the links between pesticides and honey bee health. The epistemological dominance of these norms and practices served to marginalize the knowledge claims and policy positions of beekeepers in the colony collapse disorder controversy.
44. Buchmann SL, Nabhan GP: *The Forgotten Pollinators.* Island Press; 1997.
 45. Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T: **Importance of pollinators in changing landscapes for world crops.** *Proc Biol Sci* 2007, **274**:303-313 <http://dx.doi.org/10.1098/rspb.2006.3721>.
 46. Brussaard L, Caron P, Campbell B, Lipper L, Mainka S, Rabbinge R, Babin D, Pulleman M: **Reconciling biodiversity conservation and food security: scientific challenges for a new agriculture.** *Curr Opin Environ Sustain* 2010, **2**:34-42.
 47. Settele J, Penev L, Georgiev T, Grabau R, Grobelnik V, Hammen V, Klotz S, Kühn I (Eds): *Atlas of Biodiversity Risk.* Sofia: Pensoft; 2010.
 48. Lautenbach S, Seppelt R, Liebscher J, Dormann CF: **Spatial and temporal trends of global pollination benefit.** *PLoS ONE* 2012, **7**:e35954 <http://dx.doi.org/10.1371/journal.pone.0035954>.
- The first spatially explicit map of pollination demands at world level identifies the areas that benefit and depend the most on pollination. On the basis of this maps the dependency of agriculture on pollination is much higher than what has been so far described. Increases in the price of pollination dependent crops were considered as an early warning tool on the abundance of pollinators. Still the effects of international trade of pollination-dependent crops have not been taken into account, in which case the dependency of developed countries would be higher than shown.
49. Gallai N, Salles JM, Settele J, Vaissière BE: **Economic valuation of the vulnerability of world agriculture confronted with pollinator decline.** *Ecol Econ* 2009, **68**:810-821 <http://dx.doi.org/10.1016/j.ecolecon.2008.06.014>.
 50. Winfree R, Gross BJ, Kremen C: **Valuing pollination services to agriculture.** *Ecol Econ* 2011, **71**:80-88.

51. Maes J, Hauck J, Paracchini ML, Ratamäki O, Hutchins M, Termansen M, Furman E, Pérez-Soba M, Braat L, Bidoglio G: **Mainstreaming ecosystem services into EU policy.** *Curr Opin Environ Sustain* 2013, **5**:128-134 <http://dx.doi.org/10.1016/j.cosust.2013.01.002>.
 52. Kevan PG, Menzel R: **The plight of pollination and the interface of neurobiology, ecology and food security.** *Environmentalist* 2012, **32**:300-310 <http://dx.doi.org/10.1007/s10669-012-9394-5>.
 53. Ollerton J, Winfree R, Tarrant S: **How many flowering plants are pollinated by animals?** *Oikos* 2011, **120**:321-326 <http://dx.doi.org/10.1111/j.1600-0706.2010.18644.x>.
 54. Eilers EJ, Kremen C, Smith Greenleaf S, Garber AK, Klein AM: **Contribution of pollinator-mediated crops to nutrients in the human food supply.** *PLoS ONE* 2011, **6**:e21363 <http://dx.doi.org/10.1371/journal.pone.0021363>.
- Pollinator mediated crops account for >90% of vitamin C, 100% of lycopene, almost 100% of the antioxidants β -cryptoxanthin and β -tocopherol, the majority of the lipid, vitamin A and related carotenoids, calcium and fluoride, and a large portion of folic acid. Ongoing pollinator decline may thus put the provision of a nutritionally adequate diet for the global human population at risk.
55. Dams LR: **Bees and honey-hunting scenes in the Mesolithic rock art of eastern Spain.** *Bee World* 1978, **59**:43-53.
 56. Pattinson D: **Pre-modern beekeeping in China: a short history.** *Agric Hist* 2012, **86**:235-255.
 57. Bloch G, Francoy TM, Wachtel I, Panitz-Cohen N, Fuchs S, Mazar A: **Industrial apiculture in the Jordan valley during Biblical times with Anatolian honeybees.** *Proc Natl Acad Sci U S A* 2010, **107**:11240-11244.
 58. Ebert A: **Nectar for the taking: the popularization of scientific bee culture in England, 1609-1809.** *Agric Hist* 2011, **85**:322-343.
 59. Breeze TD, Bailey AP, Balcombe KG, Potts SG: **Pollination services in the UK: How important are honeybees?** *Agric Ecosyst Environ* 2011, **142**:137-143 <http://dx.doi.org/10.1016/j.agee.2011.03.020>.
 60. Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, Bartomeus I, Benjamin F et al.: **Wild pollinators enhance fruit set of crops regardless of honey bee abundance.** *Science* 2013, **339**:1608-1611 <http://dx.doi.org/10.1126/science.1230200>.
 61. Banskota AH, Tezuka Y, Kadota S: **Recent progress in pharmacological research of propolis.** *Phytother Res* 2001, **15**:561-571.
 62. Jull AB, Rodgers A, Walker N: **Honey as a topical treatment for wounds.** *Cochrane Database Syst Rev* 2009, **4** art.no. CD005083.
 63. Srinivasan MV: **Honeybees as a model for the study of visually guided flight, navigation, and biologically inspired robotics.** *Physiol Rev* 2011, **91**:413-460.
 64. Rogers J, Sleight C: **Here is my honey-machine: Sylvia plath and the mereology of the Beehive.** *Rev Engl Stud* 2012, **63**:293-310.
 65. Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers P et al.: **Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands.** *Science* 2006, **313**:351-354 <http://dx.doi.org/10.1126/science.1127863>.
 66. Holden C: **Ecology: report warns of looming pollination crisis in North America.** *Science* 2006, **314**:397 <http://dx.doi.org/10.1126/science.314.5798.397>.
 67. Goulson D, Lye GC, Darvill B: **Decline and conservation of bumblebees.** *Ann Rev Entomol* 2008, **53**:191-208.
 68. Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE: **Global pollinator declines: trends, impacts and drivers.** *Trends Ecol Evol* 2010, **25**:345-353 <http://dx.doi.org/10.1016/j.tree.2010.01.007>.
 69. Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL: **Patterns of widespread decline in North American bumble bees.** *Proc Natl Acad Sci U S A* 2010, **108**:662-667.
 70. Burkle LA, Marlin JC, Knight TM: **Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function.** *Science* 2013, **339**:1611-1615 <http://dx.doi.org/10.1126/science.1232728>.
 71. UNEP: **Global Honey Bee Colony Disorders and Other Threats to Insects.** Agriculture United Nations Environmental Program; 2010
 72. van der Zee R, Pisa L, Andonov S, Brodschneider R, Chlebo R, Coffey MF, Crailsheim K, Dahle B, Gajda A, Gray A et al.: **Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008-9 and 2009-10.** *J Apic Res* 2012, **51**:100-114 <http://dx.doi.org/10.3896/IBRA.1.51.1.12>.
- This publication gathers the to-date most harmonised information on colony losses worldwide. Colony bee losses are voluntarily communicated by beekeepers through a common worldwide questionnaire designed by the scientific network COLOSS.
73. VanEngelsdorp D, Meixner M: **A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them.** *J Invertebr Pathol* 2010, **103**:S80-S95 <http://dx.doi.org/10.1016/j.jip.2009.06.011>.
 74. VanEngelsdorp D, Caron D, Hayes J, Underwood R, Henson M, Spleen A, Andree M, Andree M, Snyder R, Lee K, Roccasecca K, Wilson M, Wilkes J, Lengerich E, Pettis J: **A national survey of managed honey bee 2010-11 winter colony losses in the USA: results from the Bee Informed Partnership.** *J Apic Res* 2012, **51**:115-124 <http://dx.doi.org/10.3896/IBRA.1.51.1.14>.
 75. Taniguchi T, Kita Y, Matsumoto T, Kimura K: **Honeybee colony losses during 2008-2010 caused by pesticide application in Japan.** *J Apic* 2012, **27**:15-27.
- A survey carried out to beekeepers over three consecutive years identified large numbers of bee losses due to acute intoxications to putatively neonicotinoids. The colonies around rice and orange fields were the most affected.
76. Yamada T, Yamada K, Wada N: **Influence of dinotefuran and clothianidin on a bee colony.** *Jpn J Clin Ecol* 2012, **21**:10-23.
 77. Maxim L, Van der Sluijs JP: **Expert explanations of honeybee losses in areas of extensive agriculture in France: Gaucho® compared with other supposed causal factors.** *Environ Res Lett* 2010, **5**:014006 <http://dx.doi.org/10.1088/1748-9326/5/1/014006>.
 78. Runckel C, Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R, DeRisi JL: **Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, nosema, and crithidia.** *PLoS ONE* 2011, **6**:e20656 <http://dx.doi.org/10.1371/journal.pone.0020656>.
 79. Farooqui T: **A potential link among biogenic amines-based pesticides, learning and memory, and colony collapse disorder: a unique hypothesis.** *Neurochem Int* 2012, **62**:122-136 <http://dx.doi.org/10.1016/j.neuint.2012.09.020>.
- An insightful discussion of a hypothetical link among biogenic amines-based pesticides (neonicotinoids and formamidines) and their disruptive effects on biogenic amine signaling causing olfactory dysfunction in honeybees. The hypothesis that chronic exposure disrupts neural cholinergic and octopaminergic signaling in honeybees is supported by the fact that abnormality in biogenic amines-mediated neuronal signaling impairs their olfactory learning and memory. This explains why foragers exposed to neonicotinoids fail to return to their hive — a possible cause of CCD.
80. Stokstad E: **Agriculture Field research on bees raises concern about low-dose pesticides.** *Science* 2012, **335**:1555 <http://dx.doi.org/10.1126/science.335.6076.1555>.
 81. Whitehorn PR, O'Connor S, Wackers FL, Goulson D: **Neonicotinoid pesticide reduces bumble bee colony growth and queen production.** *Science* 2012:351 <http://dx.doi.org/10.1126/science.1215025>.
- Colonies of bumble bees (*Bombus terrestris*) exposed to field relevant doses of imidacloprid showed significantly lower growth rate and a reduction of 85% of new queen production. This means a severe negative impact on the bumblebees' population.
82. Henry M, Béguin M, Requier F, Rollin O, Odoux JF, Aupinel P, Aptel J, Tchamitchian S, Decourtaye A: **A common pesticide decreases foraging success and survival in honey bees.** *Science* 2012, **336**:348-350 <http://dx.doi.org/10.1126/science.1215039>.

Bees exposed to doses of thiamethoxam at 1.34 ng/bee significantly decrease their ability to carry out their homing flight up to levels triggering depopulation dynamics in their colony. RFID tagging technology is used to register the activity of foragers leaving and entering the hive.

83. Stoner KA, Eitzer BD: **Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*)**. *PLoS ONE* 2012, **7**:e39114 <http://dx.doi.org/10.1371/journal.pone.0039114>.
 84. Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Di Bernardo A, Greatti M, Giorio C, Tapparo A: **Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees**. *J Econ Entomol* 2009, **102**:1808-1815.
 85. European Food Safety Authority: **Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)**. *EFSA J* 2012, **10**:2668 <http://dx.doi.org/10.2903/j.efsa.2012.2668>.
- This non-comprehensive, but rather complete review, includes information about many of the following subjects: first, a definition of specific protection goals linked to pollinators; second, exposure paths of bees to pesticides defined in scientific literature; third, toxicological effects observed; fourth, synergistic effects of pesticides and pathologies and among pesticide molecules; fifth, an analysis of the different toxicological tests currently carried out for risk assessment. On the basis of the previous points, a new proposal for pesticide risk assessment on bees is proposed.
86. Beekman M, Ratnieks FLW: **Long-range foraging by the honey-bee, *Apis mellifera* L.** *Funct Ecol* 2000, **14**:490-496.
 87. Goulson D, Lepais O, O'Connor S, Osborne JL, Sanderson RA, Cussans J, Goffe L, Darvill B: **Effects of land use at a landscape scale on bumblebee nest density and survival**. *J Appl Ecol* 2010, **46**:1207-1215.
 88. Rortais A, Arnold G, Halm M-P, Touffet-Briens F: **Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees**. *Apidologie* 2005, **36**:71-83.
 89. Chauzat M-P, Faucon J-P, Martel A-C, Lachaize J, Cougoule N, Aubert M: **A survey of pesticide residues in pollen loads collected by honey bees in France**. *J Econ Entomol* 2006, **99**:253-262.
 90. Chauzat MP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon JP: **Influence of pesticide residues on honey bee (*Hymenoptera: Apidae*) colony health in France**. *Environ Entomol* 2009, **38**:514-523.
 91. Chauzat M-P, Martel A-C, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M et al.: **An assessment of honeybee colony matrices, *Apis mellifera* (*Hymenoptera: Apidae*) to monitor pesticide presence in continental France**. *Environ Toxicol Chem* 2011, **30**:103-111 <http://dx.doi.org/10.1002/etc.361>.
 92. Blacquière T, Smagghe G, Van Gestel CM, Mommaerts V: **Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment**. *Ecotoxicology* 2012, **21**:973-992 <http://dx.doi.org/10.1007/s10646-012-0863-x>.
 93. Dively GP, Kamel A: **Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators**. *J Agric Food Chem* 2012, **60**:4449-4456 <http://dx.doi.org/10.1021/jf205393x>.
 94. Tapparo A, Giorio C, Marzaro M, Marton D, Soldà L, Girolami V: **Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds**. *J Environ Monit* 2011, **13**:1564-1568 <http://dx.doi.org/10.1039/c1em10085h>.
 95. Hoffmann EJ, Castle SJ: **Imidacloprid in melon guttation fluid: a potential mode of exposure for pest and beneficial organisms**. *J Econ Entomol* 2012, **105**:67-71.
 96. Mullin C, Frazier M, Frazier JL, Ashcraft S, Simonds R, Vanengelsdorp D, Pettis JS: **High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health**. *PLoS ONE* 2010, **5**:e9754 <http://dx.doi.org/10.1371/journal.pone.0009754>.
 97. Yáñez KP, Bernal JL, Nozal MJ, Martín MT, Bernal J: **Determination of seven neonicotinoid insecticides in beeswax by liquid chromatography coupled to electrospray-mass spectrometry using a fused-core column**. *J Chromatogr A* 2013, **1285**:110-117 <http://dx.doi.org/10.1016/j.chroma.2013.02.032>.
 98. Laurino D, Porporato M, Patetta A, Manino A: **Toxicity of neonicotinoid insecticides to honey bees: laboratory tests**. *Bull Insectol* 2011, **64**:107-113.
 99. Abbott V, Nadeau JL, Higo H, Winston ML: **Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (*Hymenoptera: Megachilidae*)**. *J Econ Entomol* 2008, **101**:784-796.
 100. Scott-Dupree CD, Conroy L, Harris CR: **Impact of currently used or potentially useful insecticides for canola agroecosystems on *Bombus impatiens* (*Hymenoptera: Apidae*), *Megachile rotundata* (*Hymenoptera: Megachilidae*), and *Osmia lignaria* (*Hymenoptera: Megachilidae*)**. *J Econ Entomol* 2009, **102**:177-182.
 101. Gradish AE, Scott-Dupree CD, Shipp L, Harris CR, Ferguson G: **Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (*Hymenoptera: Apidae*)**. *Pest Manag Sci* 2010, **66**:142-146.
 102. Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G, Smagghe G: **Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior**. *Ecotoxicology* 2010, **19**:207-215 <http://dx.doi.org/10.1007/s10646-009-0406-2>.
 103. Jeyalakshmi T, Shanmugasundaram R, Saravanan M, Geetha S, Mohan SS, Goparaju A, Balakrishna Murthy R: **Comparative toxicity of certain insecticides against *Apis cerana indica* under semi field and laboratory conditions**. *Pestology* 2011, **35**:23-26.
 104. Tapparo A, Marton D, Giorio C, Zanella A, Soldà L, Marzaro M, Vivan L, Girolami V: **Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds**. *Environ Sci Technol* 2012, **46**:2592-2599 <http://dx.doi.org/10.1021/es2035152>.
- Thanks to a quick analytical method, new potential sources of exposure to pesticides (clothianidin and thiamethoxam) in the environment have been proved: significant amounts of coating particles are emitted by pneumatic drilling machines during corn sowing. Coarse particles seem to sediment closer to the sown field, while fine particles cover longer distances owing to the wind. The amount of toxic compound released is toxic to bees, and this toxicity is facilitated by humidity.
105. Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A: **Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication**. *J Appl Entomol* 2012, **136**:17-26 <http://dx.doi.org/10.1111/j.1439-0418.2011.01648.x>.
 106. Girolami V, Marzaro M, Vivan L, Mazzon L, Giorio C, Marton D, Tapparo A: **Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers**. *J Appl Entomol* 2013, **137**:35-44 <http://dx.doi.org/10.1111/j.1439-0418.2012.01718.x>.
 107. ApeNet: **Effects of Coated Maize Seed on Honey Bees – Report Based on Results Obtained from the Second Year (2010) Activity of the APENET Project**. 2012.
 108. Pochi D, Biocca M, Fanigliulo R, Pulcini P, Conte E: **Potential exposure of bees, *Apis mellifera* L., to particulate matter and pesticides derived from seed dressing during maize sowing**. *Bull Environ Contam Toxicol* 2012, **89**:354-361 <http://dx.doi.org/10.1007/s00128-012-0664-1>.
 109. Tapparo A, Giorio C, Soldà L, Bogioli S, Marton D, Marzaro M, Girolami V: **UHPLC-DAD method for the determination of neonicotinoid insecticides in single bees and its relevance in honeybee colony loss investigations**. *Anal Bioanal Chem* 2013, **405**:1007-1014 <http://dx.doi.org/10.1007/s00216-012-6338-3>.
 110. Gross M: **Pesticides linked to bee deaths**. *Curr Biol* 2008, **18**:684.
 111. Sgolastra F, Renzi T, Draghetti S, Medrzycki P, Lodesani M, Maini S, Porrini C: **Effects of neonicotinoid dust from maize seed-dressing on honey bees**. *Bull Insectol* 2012, **65**:273-280.

112. European Commission: **Commission Directive 2010/21/EU amending Annex I to Council Directive 91/414/EEC as regards the specific provisions relating to clothianidin, thiamethoxam, fipronil and imidacloprid.** *Off J* 2010, **L 65**.
113. Biocca M, Conte E, Pulcini P, Marinelli E, Pochi D: **Sowing simulation tests of a pneumatic drill equipped with systems aimed at reducing the emission of abrasion dust from maize dressed seed.** *J Environ Sci Health B: Pest Food Contam Agric Wastes* 2011, **46**:438-448 <http://dx.doi.org/10.1080/03601234.2011.583825>.
114. Marzaro M, Vivan L, Targa A, Mazzon L, Mori N, Greatti M, Petrucco Toffolo E, Di Bernardo A, Giorio C, Marton D et al.: **Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat.** *Bull Insectol* 2011, **64**:119-126.
115. Austria Investigations in the incidence of bee losses in corn and oilseed rape growing areas of Austria and possible correlations with bee diseases and the use of insecticidal plant protection products (MELISA). Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH, Institut für Pflanzenschutzmittel; 2012.
116. Oliveira RA, Roat TC, Carvalho SM, Malaspina O: **Side-effects of thiamethoxam on the brain and midgut of the africanized honeybee *Apis mellifera* (Hymenoptera: Apidae).** *Environ Toxicol* 2013. (in press).
117. Sánchez-Bayo F: **From simple toxicological models to prediction of toxic effects in time.** *Ecotoxicology* 2009, **18**:343-354 <http://dx.doi.org/10.1007/s10646-008-0290-1>.
118. Tennekes HA: **The significance of the Druckrey-Küpfmüller equation for risk assessment – the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time.** *Toxicology* 2010, **276**:1-4 <http://dx.doi.org/10.1016/j.tox.2010.07.005>.
119. Maus C, Nauen R: **Response to the publication: Tennekes, H.A. (2010): the significance of the Druckrey-Küpfmüller equation for risk assessment – the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time.** *Toxicology* 2011, **280**:176-177 <http://dx.doi.org/10.1016/j.tox.2010.11.014>.
120. Tennekes HA: **The significance of the Druckrey-Küpfmüller equation for risk assessment—The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time: Responding to a Letter to the Editor by Drs. C. Maus and R. Nauen of Bayer CropScience AG.** *Toxicology* 2011, **280**:173-175 <http://dx.doi.org/10.1016/j.tox.2010.11.015>.
121. Tennekes HA, Sánchez-Bayo F: **Time-dependent toxicity of neonicotinoids and other toxicants: implications for a new approach to risk assessment.** *J Environ Anal Toxicol* 2011, **S4**:001 <http://dx.doi.org/10.4172/2161-0525.S4-001>.
Neonicotinoids have an irreversible impact on nAChR which implies that a time-to-event model of toxicity is the adequate description of their chronic toxicity profile. Because of time depends of the toxic effect, the standard risk assessment procedures are not valid in case of exposure to sublethal concentrations of neonicotinoids for long periods of time, so LC50 is a misleading indicator for harm.
122. Mason R, Tennekes H, Sánchez-Bayo F, Uhd Jepsen P: **Immune suppression by neonicotinoid insecticides at the root of global wildlife declines.** *J Environ Immunol Toxicol* 2013, **1**:2-12 <http://dx.doi.org/10.7178/jeit.1>.
123. Lu C, Warchol KM, Callahan RA: **In situ replication of honey bee colony collapse disorder.** *Bull Insectol* 2012, **65**:99-106.
124. Desneux N, Decourtye A, Delpuech JM: **The sublethal effects of pesticides on beneficial arthropods.** *Annu Rev Entomol* 2007, **52**:81-106 <http://dx.doi.org/10.1146/annurev.ento.52.110405.091440>.
125. Easton AH, Goulson D: **The neonicotinoid insecticide imidacloprid repels pollinating flies and beetles at field-realistic concentrations.** *PLoS ONE* 2013, **8**:e54819 <http://dx.doi.org/10.1371/journal.pone.0054819>.
126. Bortolotti L, Montanari R, Marcelino J, Medrzycki P, Maini S, Porrini C: **Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees.** *Bull Insectol* 2003, **56**:63-67.
127. Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, Cluzeau S, Pham-Delègue MH: **Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*.** *Arch Environ Contam Toxicol* 2005, **48**:242-250 <http://dx.doi.org/10.1007/s00244-003-0262-7>.
128. Gauthier M: **State of the art on insect nicotinic acetylcholine receptor function in learning and memory.** In *Insect Nicotinic Acetylcholine Receptors*. Edited by Thany SH. Berlin: Springer; 2010:97-115.
129. Yang EC, Chang HC, Wu WY, Chen YW: **Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval stage.** *PLoS ONE* 2012, **7**:e49472 <http://dx.doi.org/10.1371/journal.pone.0049472>.
A short term sublethal dosage of imidacloprid given to honeybee larvae has a long term effect: it renders the olfactory associative behavior of the adult bees impaired. This may affect the survival condition of the entire colony, even though the larvae survive to adulthood. Also, the brood-capped, pupation, and eclosion rates of the larvae decrease significantly with dose.
130. Williamson SM, Wright GA: **Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees.** *J Exp Biol* 2013, **216**:1799-1807 <http://dx.doi.org/10.1242/jeb.083931>.
131. Yang EC, Chuang YC, Chen YL, Chang LH: **Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae).** *J Econ Entomol* 2008, **101**:1743-1748 <http://dx.doi.org/10.1603/0022-0493.101.6.1743>.
132. Decourtye A, Devillers J, Aupinel P, Brun F, Bagnis C, Fourrier J, Gauthier M: **Honeybee tracking with microchips: a new methodology to measure the effects of pesticides.** *Ecotoxicology* 2011, **20**:429-437 <http://dx.doi.org/10.1007/s10646-011-0594-4>.
133. Schneider CW, Tautz J, Grünwald B, Fuchs S: **RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*.** *PLoS ONE* 2012, **7**:e30023 <http://dx.doi.org/10.1371/journal.pone.0030023>.
134. Teeters BS, Johnson RM, Ellis MD, Siegfried BD: **Using video-tracking to assess sublethal effects of pesticides on honey bees (*Apis mellifera* L.).** *Environ Toxicol Chem* 2012, **31**:1349-1354 <http://dx.doi.org/10.1002/etc.1830>.
Video-tracking technology has been used to measure effects on behaviour of two pesticides: tau-fluvalinate (topical) and imidacloprid (oral). While at very low dose (0.05 µg kg⁻¹) imidacloprid showed an activating effect, starting at 0.5 µg kg⁻¹ imidacloprid triggered a dose dependent negative effect: an increase in time spent at feeder, while distance travelled and interaction time between bees decrease with dose.
135. Eiri DM, Nieh JC: **A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing.** *J Exp Biol* 2012, **215**:2022-2029 <http://dx.doi.org/10.1242/jeb.068718>.
136. Medrzycki P, Sgolastra F, Bortolotti L, Bogo G, Tosi S, Padovani E, Porrini C, Sabatini AG: **Influence of brood rearing temperature on honey bee development and susceptibility to intoxication by pesticides.** *J Apic Res* 2010, **49**:52-59.
137. Cresswell JE, Page CJ, Uygun MB, Holmbergh M, Li Y, Wheeler JG, Laycock I, Pook CJ, de Ibarra NH, Smirnov N et al.: **Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid).** *Zoology* 2012, **115**:365-371 <http://dx.doi.org/10.1016/j.zool.2012.05.003>.
138. Gill RJ, Ramos-Rodriguez O, Raine NE: **Combined pesticide exposure severely affects individual- and colony-level traits in bees.** *Nature* 2012, **491**:105-108 <http://dx.doi.org/10.1038/nature11585>.
Chronic exposure of bumble bees to a neonicotinoid (imidacloprid) and a pyrethroid (λ-cyhalothrin) at field-relevant concentrations reduced the foraging performance and increased worker mortality. This leads to a reduction of brood production and colony success. A synergistic effect of both pesticides is clearly observed.
139. Laycock I, Lenthall KM, Barratt AT, Cresswell JE: **Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*).** *Ecotoxicology* 2012, **21**:1937-1945 <http://dx.doi.org/10.1007/s10646-012-0927-y/>.

Environmental relevant concentrations of imidacloprid negatively affects ovary development and fecundity in bumble bees. Dietary imidacloprid at $1 \mu\text{g l}^{-1}$ is capable to reduce brood production by one third. The causal speculations include a feeding reduction linked to toxic exposure and/or alteration of the social communication among bumblebee workers.

140. Smodis Skerl MIS, Gregorc A: **Heat shock proteins and cell death in situ localisation in hypopharyngeal glands of honeybee (*Apis mellifera carnica*) workers after imidacloprid or coumaphos treatment.** *Apidologie* 2010, **41**:73-86.

141. Heylen K, Gobin B, Arckens L, Huybrechts R, Billen J: **The effects of four crop protection products on the morphology and ultrastructure of the hypopharyngeal gland of the European honeybee, *Apis mellifera*.** *Apidologie* 2011, **42**:103-116 <http://dx.doi.org/10.1051/apido/2010043>.

142. Hatjina F, Papaefthimiou C, Charistos L, Dogaroglu T, Bouga M, Emmanouil C, Arnold G: **Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo.** *Apidologie* 2013 <http://dx.doi.org/10.1007/s13592-013-0199-4>.

Imidacloprid administered under laboratory conditions to honeybees at $2 \mu\text{g kg}^{-1}$ in sugar solution and $3 \mu\text{g kg}^{-1}$ in pollen pastry has sublethal effects on the development of the hypopharyngeal glands (HPGs) and respiratory rhythm. The acini, the lobes of the HPGs of imidacloprid-treated honeybees, were 14.5% smaller in diameter in 9-day-old honeybees and 16.3% smaller in 14-day-old honeybees than in the same-aged untreated honeybees. Imidacloprid also significantly affected the bursting pattern of abdominal ventilation movements (AVM) by causing a 59.4% increase in the inter-burst interval and a 56.99% decrease in the mean duration of AVM bursts.

143. van Tormé HV, Martins GF, Lima MAP, Campos LAO, Guedes RNC: **Imidacloprid-induced impairment of mushroom bodies and behavior of the native stingless bee *Melipona quadrifasciata anthidioides*.** *PLoS ONE* 2012, **7**:e38406 <http://dx.doi.org/10.1371/journal.pone.0038406>.

144. De Almeida Rossi C, Roat TC, Tavares DA, Cintra-Socolowski P, Malaspina O: **Effects of sublethal doses of imidacloprid in malpighian tubules of africanized *Apis mellifera* (Hymenoptera, Apidae).** *Microsc Res Tech* 2013, **76**:552-558 <http://dx.doi.org/10.1002/jemt.22199>.

145. Wu JY, Anelli CM, Sheppard WS: **Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity.** *PLoS ONE* 2011, **6**:e14720 <http://dx.doi.org/10.1371/journal.pone.0014720>.

146. Cresswell JE: **A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees.** *Ecotoxicology* 2010, **20**:149-157 <http://dx.doi.org/10.1007/s10646-010-0566-0>.

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

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

148. Becher MA, Thorbek P, Kennedy PJ, Osborne J, Grimm V: **Towards a systems approach for understanding honeybee decline: a stock-taking and synthesis of existing models.** *J Appl Ecol* 2013. (in press).

149. Moritz RFA, Southwick EE: **Bees as Superorganisms: An Evolutionary Reality.** Verlag: Springer; 1992, .

150. Khoury DS, Myerscough MR, Barron AB: **A quantitative model of honey bee colony population dynamics.** *PLoS ONE* 2012, **6**:e18491 <http://dx.doi.org/10.1371/journal.pone.0018491>.

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

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

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

154. Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP, Le Conte Y: **Interactions between *Nosema microspores* and a neonicotinoid weaken honeybees (*Apis mellifera*).** *Environ Microbiol* 2010, **12**:774-782 <http://dx.doi.org/10.1111/j.1462-2920.2009.02123.x>.

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

155. Pettis JS, Vanengelsdorp D, Johnson J, Dively G: **Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*.** *Naturwissenschaften* 2012, **99**:153-158 <http://dx.doi.org/10.1007/s00114-011-0881-1>.

Nosema infection increases significantly whenever bee colonies are exposed chronically to sublethal levels of the neonicotinoid, imidacloprid. 5 and $20 \mu\text{g kg}^{-1}$ imidacloprid were administered to pollen patties and provided to bee colonies over 5 and 8 weeks.

156. Aufauvre J, Biron DG, Vidau C, Fontbonne R, Roudel M, Diogon M, Viguès B, Belzunces LP, Delbac F, Blot N: **Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee.** *Nat Sci Rep* 2012, **2**:326.

157. Paula AR, Carolino AT, Paula CO, Samuels RI: **The combination of the entomopathogenic fungus *Metarhizium anisopliae* with the insecticide Imidacloprid increases virulence against the dengue vector *Aedes aegypti* (Diptera: Culicidae).** *Paras Vec* 2011, **4**:8 <http://dx.doi.org/10.1186/1756-3305-4-8>.

158. Boucias DG, Stokes C, Storey G, Pendland JC: **The effects of imidacloprid on the termite *Reticulitermes flavipes* and its interaction with the mycopathogen *Beauveria bassiana*.** *Pflan Nachr Bayer* 1996, **49**:103-144.

159. Quintella ED, McCoy CW: **Pathogenicity enhancement of *Metarhizium anisopliae* and *Beauveria bassiana* to first instars of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with sublethal doses of imidacloprid.** *Environ Entomol* 1997, **26**:1173-1182.

160. Koppenhöfer AM, Grewal PS, Kaya HK: **Synergism of imidacloprid and entomopathogenic nematodes against white grubs: the mechanism.** *Entomol Exp Appl* 2000, **94**:283-293.

161. James RR, Xu J: **Mechanisms by which pesticides affect insect immunity.** *J Invertebr Pathol* 2012, **109**:175-182 <http://dx.doi.org/10.1016/j.jip.2011.12.005>.

162. Fagin D: **The learning curve.** *Nature* 2012, **490**:5-8.

Researchers say that some chemicals have unexpected and potent effects at very low doses. The complex interplay of receptor binding and gene reprogramming can generate bizarre dose-response relationships, many of which are still being mapped out. Regulators are not convinced.

163. Stokstad E: **Pesticides under fire for risks to pollinators.** *Science* 2013, **340**:674-675.

Appendix 2

IUCN resolution WCC-2012-Res-137: Support for a comprehensive scientific review of the impact on global biodiversity of systemic pesticides by the joint task force of the IUCN Species Survival Commission (SSC) and the IUCN Commission on Ecosystem Management (CEM)

Adopted by the General Assembly of the IUCN in Jeju, Korea, on 15 September 2012.

WCC-2012-Res-137-EN

Support for a comprehensive scientific review of the impact on global biodiversity of systemic pesticides by the joint task force of the IUCN Species Survival Commission (SSC) and the IUCN Commission on Ecosystem Management (CEM)

RECOGNIZING the mission of IUCN in promoting the conservation of biological diversity since its inception;

AWARE that over the past decade neonicotinoid insecticides have rapidly become the most widely used and fastest-growing class of insecticides worldwide following their introduction to the market in the mid-1990s, now with a global market share of about one-third of the world insecticide market, with seed treatment as their major application and having been registered nowadays in more than 120 countries;

REMINDED that neurotoxic neonicotinoid pesticides which are highly persistent and act systemically and cumulatively, entering the plant sap through the roots, making the whole plant permanently toxic to insects, including beneficial pollinators, and being unique in their application and in the way they affect insects and other invertebrates through sub-lethal doses and chronic exposure;

NOTING that neonicotinoid and other systemic pesticides are suspected by many scientists of being a factor in contributing to the worldwide honeybee disorders, to the decline of wild pollinators, and to observed declines of entomofauna at large, and so better insight into the ecological risks associated with the use of these pesticides is urgently needed;

ALARMED at the continuously increasing loss of biodiversity in all its components, including species, ecosystems and genes;

WELCOMING the establishment of a Task Force on Systemic Pesticides (TFSP) under the IUCN Species Survival Commission (SSC) and the IUCN Commission on Ecosystem Management (CEM) in March 2011; and

ALSO WELCOMING the task of the TFSP to carry out a comprehensive, objective, scientific review and assessment of the impact of systemic pesticides on biodiversity, and on the basis of the results of this review to make any recommendations that might be needed with regard to risk management procedures, governmental approval of new pesticides, and any other relevant issues that should be brought to the attention of decision makers, policy developers and society in general;

The World Conservation Congress, at its session in Jeju, Republic of Korea, 6–15 September 2012:

1. CALLS ON all IUCN Members to support the TFSP in its endeavours so that it can complete its review and recommendations in a timely fashion;
2. REQUESTS the Director General to assist SSC and CEM in fundraising for the work of the TFSP so that it can complete its work during the 2013–2016 quadrennial;
3. REQUESTS the Director General, based on the outcomes of the scientific assessment by the TFSP, and in close collaboration with the IUCN Commission on Environmental Law (CEL) and the IUCN Environmental Law Centre (ELC), to provide the necessary assistance to CEM and SSC, and to other stakeholders as appropriate, regarding any

legislative and regulatory consequences that might arise with regard to the implementation of the recommendations from the TFSP; and

4. FURTHER REQUESTS the Director General to write to governments to seek national-level information on the levels and trends of use of systemic pesticides.