Environmental Science Processes & Impacts

ROYAL SOCIETY OF CHEMISTRY

CRITICAL REVIEW

View Article Online
View Journal | View Issue

Cite this: *Environ. Sci.: Processes Impacts*, 2020, **22**, 1315

A critical review on the potential impacts of neonicotinoid insecticide use: current knowledge of environmental fate, toxicity, and implications for human health†

Darrin A. Thompson, *[ab] Hans-Joachim Lehmler, [a] Dana W. Kolpin, [c] Michelle L. Hladik, [d] John D. Vargo, [e] Keith E. Schilling, [f] Gregory H. LeFevre, [g] Tonya L. Peeples, [h] Matthew C. Poch, [a] Lauren E. LaDuca, [a] David M. Cwiertny [bg] and R. William Field *[a]

Neonicotinoid insecticides are widely used in both urban and agricultural settings around the world. Historically, neonicotinoid insecticides have been viewed as ideal replacements for more toxic compounds, like organophosphates, due in part to their perceived limited potential to affect the environment and human health. This critical review investigates the environmental fate and toxicity of neonicotinoids and their metabolites and the potential risks associated with exposure. Neonicotinoids are found to be ubiquitous in the environment, drinking water, and food, with low-level exposure commonly documented below acceptable daily intake standards. Available toxicological data from animal studies indicate possible genotoxicity, cytotoxicity, impaired immune function, and reduced growth and reproductive success at low concentrations, while limited data from ecological or cross-sectional epidemiological studies have identified acute and chronic health effects ranging from acute respiratory, cardiovascular, and neurological symptoms to oxidative genetic damage and birth defects. Due to the heavy use of neonicotinoids and potential for cumulative chronic exposure, these insecticides represent novel risks and necessitate further study to fully understand their risks to humans.

Received 13th December 2019
Accepted 18th March 2020

DOI: 10.1039/c9em00586b

rsc.li/espi

Environmental significance

Given their systemic activity, widespread use and presence in the environment, neonicotinoids represent a unique human exposure and health risk. In this critical review, we examine their environmental fate, transformation, and toxicity, and also review published literature relevant risks posed to human exposure and health.

1. Introduction

Neonicotinoids represent a relatively new class of insecticides that have quickly become the most widely used class[1] in the world for a variety of urban and agricultural uses.[2–4] Industry crop scientists consider the discovery of neonicotinoid insecticides a milestone in agrochemical research that resulted in the most rapidly-growing class of insecticides since the commercialization of pyrethroids.[5] The word neonicotinoid means "new nicotine-like insecticide".[6,7] Historically, neonicotinoid insecticides were viewed as ideal replacements for some insecticides (*e.g.*, organophosphates and carbamates) due in part to both their perceived low risk to the environment and to non-target organisms.[5] Within the agricultural sector, neonicotinoids are preferred over other insecticides for several reasons including their (1) flexibility of application (*e.g.*, spray, injections, or seed treatments);[2,8–13] (2) broad-spectrum insect toxicity; (3) perceived low acute toxicity to non-target aquatic and terrestrial organisms,[14–17] and (4) high potency for insects.[3,8,13,18–22] In the United States, neonicotinoids are commonly

[a]*University of Iowa, College of Public Health, Iowa City, IA, USA. E-mail: Darrin-thompson-1@uiowa.edu*
[b]*University of Iowa, Center for Health Effects of Environmental Contamination, Iowa City, IA, USA*
[c]*U.S. Geological Survey, Central Midwest Water Science Center, Iowa City, IA, USA*
[d]*U.S. Geological Survey, California Water Science Center, Sacramento, CA, USA*
[e]*State Hygienic Laboratory at the University of Iowa, Iowa City, IA, USA*
[f]*Iowa Geological Survey, Iowa City, IA, USA*
[g]*University of Iowa, Department of Civil & Environmental Engineering, Iowa City, IA, USA*
[h]*Department of Chemical Engineering, University Park, PA, USA*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9em00586b

applied as seed treatments[1,23] and neonicotinoid use has become particularly prevalent in the Midwest.[24]

Few studies have characterized human exposure to neonicotinoids or the insecticides' potential adverse human health risks. An editorial entitled, "Catching Up with Popular Pesticides: More Human Health Studies Are Needed on Neonicotinoids",[25] pointed to accumulating evidence that neonicotinoids are "contributing to devastating losses of honeybees" and while there is widespread exposure, "little research had been conducted assessing potential effects on human health." In addition, a systematic literature review on neonicotinoid studies published between 2005 and 2015 identified only eight studies that addressed human health effects of neonicotinoids.[26] Four of these studies focused on acute exposures (*e.g.*, intentional self-poisoning) and four studies reviewed chronic environmental exposures. The findings from the four chronic exposure studies, which primarily used surrogate exposure data, indicated causal associations between chronic low-level neonicotinoid exposure and various adverse developmental outcomes and neurological effects.[26] In 2013, the European Union (EU) identified two neonicotinoids, acetamiprid and imidacloprid, as potential neurodevelopmental toxins.[27] The U.S. Environmental Protection Agency (EPA) is currently finalizing a human health risk assessment for acetamiprid, imidacloprid, clothianidin, thiamethoxam, and dinotefuran.[28–32] Preliminary human health assessments for these compounds were completed and released for comment in 2017 and 2018. The USEPA registration for thiacloprid was voluntarily cancelled by the registrant in 2014.[33]

The purpose of this critical review is to bring together current literature to understand the environmental fate of neonicotinoids and their potential consequences for human health. The review followed a structured approach, starting with a general search of PubMed and Google Scholar databases for a combination of the key words – "neonicotinoids", "neonicotinoids AND health" "neonicotinoids AND nicotine", "neonicotinoids AND metabolism" and "neonicotinoids AND environment". Over 500 publications were reviewed, with over 300 cited here to provide a comprehensive overview of the risks posed to human health.

2. Structure, mode of action & receptor binding mechanisms

Seven synthetic commercially available neonicotinoid insecticides (Fig. 1, Table 1) have been introduced into the marketplace including imidacloprid (Bayer CropScience) in 1991, nitenpyram (Sumitomo Chemical Takeda Agro Co.) and acetamiprid (Nippon Soda) in 1995, thiamethoxam (Syngenta) in 1998, clothianidin (Sumitomo Chemical Takeda Agro Co./Bayer

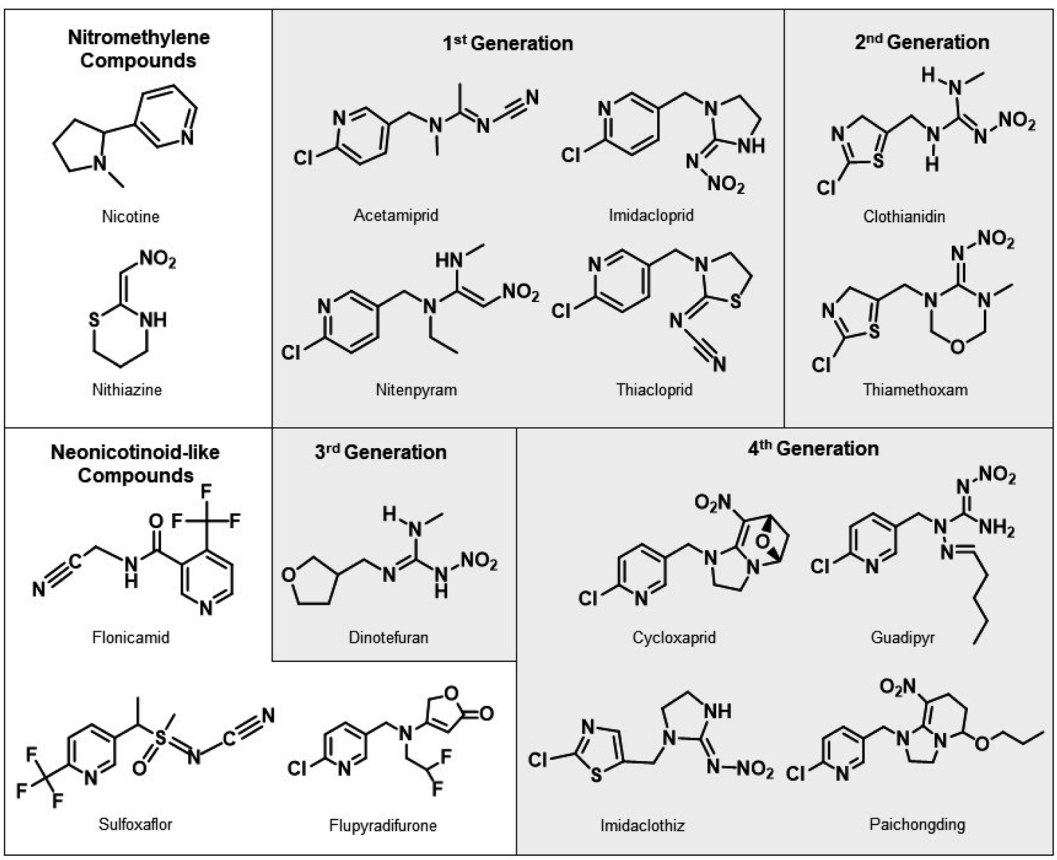

Fig. 1 Chemical structures of common neonicotinoids and related compounds. Figure adapted from C. Giorio, *et al. Environmental Science and Pollution Research*, 2017, 1–33.[35]

Table 1 Neonicotinoid background information[a]

Name	Year introduced	EPA PC code	CAS number	CAS name	Chemical formula	Molar mass
Acetamiprid	1995	099050	135410-20-7	(1*E*)-*N*-[(6-Chloro-3-pyridinyl)methyl]-*N*′-cyano-*N*-methylethanimidamide	$C_{10}H_{11}ClN_4$	222.7
Clothianidin	2001	044309	210880-92-5	(C(*E*))-*N*-[(2-Chloro-5-thiazolyl)methyl)-*N*′-methyl-*N*′-nitroguanidine	$C_6H_8ClN_5O_2S$	249.7
Dinotefuran	2002	044312	165252-70-0	*N*-Methyl-*N*′-nitro-*N*″-((tetrahydro-3-furanyl)methyl)guanidine	$C_7H_{14}N_4O_3$	202.2
Imidacloprid	1991	129099	138261-41-3	(2*E*)-1-[(6-Chloro-3-pyridinyl)methyl)-*N*-nitro-2-imidazolidinimine	$C_9H_{10}ClN_5O_2$	255.7
Nitenpyram	1995	—	150824-47-8	(1*E*)-*N*-[(6-Chloro-3-pyridinyl)methyl]-*N*-ethyl-*N*′-methyl-2-nitro-1,1-ethenediamine	$C_{11}H_{15}ClN_4O_2$	270.7
Thiacloprid	2001	014019	111988-49-9	(*Z*)-[3-((6-Chloro-3-pyridinyl)methyl)-2-thiazolidinylidene)cyanamide	$C_{10}H_9ClN_4S$	252.7
Thiamethoxam	1998	060109	153719-23-4	3-[(2-Chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-*N*-nitro-4*H*-1,3,5-oxadiazin-4-imine	$C_8H_{10}ClN_5O_3S$	291.7

[a] Table adapted from Lewis, *et al.*, *Human and Ecological Risk Assessment*, 2016. **22**(4): pp. 1050–1064;[17] Jeschke, *et al.*, *Journal of Agriculture and Food Chemistry*, 2010. **59**: pp. 2897–2908;[1] Simon-Delso, *et al.*, *Environmental Science and Pollution Research*, 2015. **22**: pp. 5–34;[8] Bass, *et al.*, *Pestic. Biochem. Physiol.*, 2015, 121. pp. 78–87;[34] Abou-Donia, *et al.*, *Toxicology and Environmental Health Impact, Part A*, 2008, 71: pp. 119–130;[35] and U.S. Environmental Protection Agency, 2017.[28–32,57] This article contains CAS Registry Numbers®, which is a Registered Trademark of the American Chemical Society. CAS recommends the verification of the CASRNs through CAS Client Services[SM].

CropScience) and thiacloprid (Bayer CropScience) in 2001, and dinotefuran (Mitsui Chemicals) in 2002.[1,8,24,34–36]

Imidacloprid, nitenpyram, thiacloprid, and acetamiprid share a chloropyridine moeity, thiamethoxam and clothianidin each have a chlorothiazole group, and the structure of dinotefuran includes a tetrahydrofuran functionality. Neonicotinoids can be further classified as either *N*-nitroguanidines (*i.e.*, imidacloprid, thiamethoxam, clothianidin, and dinotefuran), nitromethylenes (*i.e.*, nitenpyram), or *N*-cyanoamidines (*i.e.*, acetamiprid).[1] The electron-rich nitromethylene, nitroimine, or cyanoimine group enables the neonicotinoid to selectively target insects by binding more strongly to insect nicotinic acetylcholine receptors (nAChRs) compared to mammalian nAChRs.[3,18,37]

Several other compounds have been identified as neonicotinoids or neonicotinoid-like, including nithiazine, flonicamid, flupyradifurone, sulfoxaflor, guadipyr, cycloxaprid, paichongding, and imidaclothiz.[5,8,38–40] Nithiazine was one of the first neonicotinoids, but due to its limited efficacy in field trials was not commercialized until 1997 as an active ingredient against flies.[5] Flonicamid, flupyradifurone, and sulfoxaflor are classified as pyridine, butenolid, and sulfoximine compounds respectively.[5,17,39,41] Flupyradifurone and sulfoxaflor are sometimes included as neonicotinoids because they have similar neonicotinoid-like modes of action.[39,42] Flonicamid is often classified as a neonicotinoid, but has a different mode of action compared to other neonicotinoids.[38,41] New neonicotinoid-like compounds are also being produced. In China, over 600 neonicotinoid compounds have been synthesized.[43] Cycloxaprid, guadipyr, paichongding, and imidaclothiz are four compounds registered for use in China, but not in the United States or EU.[44,45] Cycloxaprid and paichongding are *cis*-neonicotinoids (in which the nitro or cyano group is located in a cis orientation instead of the typical trans arrangement),[43] and imidaclothiz is a nitroguanidine thiazole neonicotinoid.[43] This review will primarily focus on the first, second, and third generation neonicotinoids shown in Fig. 1.

Neonicotinoids work systemically in plants. Upon application, the insecticides are absorbed by plants and translocated throughout the roots, leaves, and tissue.[46,47] Once ingested by insects, neonicotinoids bind with nAChRs.[1,3,48,49] This bond is irreversible and triggers nerve signaling in a manner similar to how acetylcholine operates. Acetylcholine esterase, the enzyme that breaks down acetylcholine, is unable to breakdown neonicotinoids, leading to nerve stimulation at low concentrations and receptor blockage, paralysis, and death at higher concentrations.[3,50]

Nicotinic acetylcholine receptors are not exclusive to targeted pests but are expressed broadly in both vertebrates and invertebrates. In humans, nAChRs are found in both the peripheral and central nervous systems.[37] They transmit signals within the nervous system direct to skeletal muscles to contract.[7] In insects, the function of nAChRs is not as clearly understood as humans, but they are also responsible for post-synaptic neurotransmission.[51] The receptors are found in greater numbers in insects than mammals, but are expressed primarily in the insects' central nervous system.[3,37]

All neonicotinoids are structurally similar to nicotine.[3] The binding behavior of neonicotinoids on the insect nAChRs is

similar to those of nicotine and nicotinoids in humans.[1,48] Nicotine and other nicotinoids, are both agonists of nAChRs.[37,52] In humans, nicotine remains at synapses longer than acetylcholine, which is hydrolyzed by acetylcholine esterase. This results in modifications to neural signaling.[53–56] A key difference is that nicotine is protonated at physiological pH whereas the neonicotinoids are not protonated but possess an electronegative nitro- or cyano-functional group.[3,37]

The weaker interaction between the insecticides and vertebrates relative to insects is due to the abundance of nAChRs in insects and differences in nAChR subtypes and binding sites at the molecular level between insects and mammals.[3,19–22] On this basis, neonicotinoids are estimated to be 5 to 10 times more selective for insects *versus* mammals compared to organophosphates, methylcarbamates, and organochlorines.[3]

Neonicotinoids are high affinity agonists of insect nAChRs resulting in paralysis and death in insects.[3,37] Because neonicotinoids do not have a high affinity for mammalian and other non-insect nAChRs,[58–64] they are thought to have limited toxicity in non-target organisms, including humans. For example, the half maximal effective concentration (EC50) values for imidacloprid are 0.86–1 μM against insect nAChRs compared to an EC50 of 70 μM against mammalian nAChRs.[65] Neonicotinoids produce moderate to minimal toxicity in animal studies irrespective of the route of exposure (Lethal Dose, 50% (LD50) values >200 mg kg^{-1} body weight) by mechanisms that may involve nicotinic stimulation or represent non-specific toxic effects.[66–69]

3. Use

Neonicotinoid insecticides have rapidly gained popularity, in the United States and globally, for both urban and agricultural use since their introduction in the 1990s.[1,5,8,24] Neonicotinoids were registered in over 120 counties as insecticides marketed for protection against chewing insects (*e.g.*, plant hoppers, thrips, some micro-Lepidoptera, and other coleopteran pests[1]), capturing 25% of the total global insecticide sales[34] in 2014 and a global market value of approximately $3.7 billion U.S. Dollars (USD).[70] In 2012, thiamethoxam, imidacloprid, and clothianidin represented 85% of all neonicotinoids sales in the world.[34] From a global perspective, imidacloprid trails only the herbicide glyphosate[71] as the second most widely used pesticide in the world.[72] While neonicotinoid use has led to a decrease in some older pesticides, such as pyrethroids and carbamates, overall use of insecticides has not declined.[1,4,8,24,73] Use of neonicotinoids has led to an expansion of treatment on new acres.[74] This increase has not corresponded with an increased risk from target pest species, meaning some applications may be unnecessary.[74]

Neonicotinoids are registered globally for both agricultural and non-agricultural uses.[33] Neonicotinoids can be applied using several application methods including foliar application by aerial or ground spray equipment, soil drench, chemigation, tree injection, and as seed treatments.[2,8–13] In the United States, for example, there are over 1000 EPA primary and supplementary registered neonicotinoid-containing products on the market.[75] They are used in products ranging from oral formulations (*i.e.* tablet) for dogs and cats as a flea adulticide to agricultural products including cucurbit vegetables, fruiting vegetables, grapes, leafy brassica, leafy vegetables, and nut trees (Table S-1†).[33] In 2014, the U.S. Geological Survey (USGS) estimated that over 3.5 million kg of neonicotinoids were applied annually to all crops in the United States.‡[24]

Neonicotinoids have a variety of formulations and application methods that are effective against a wide spectrum of insects.[9] For example, professional application treatment options for the emerald ash borer *Agrilus planipennis* include soil injection or drench using either imidacloprid or dinotefuran (Merit®, Safari™, Transtect™, Xylam® Liquid Systemic Insecticide, and Xytect™), trunk injection using imidacloprid (Imicide®), or systemic basal bark spray using dinotefuran (Safari™, Transtect™, and Xylam® Liquid Systemic Insecticide).[76] Homeowner applications include soil drench with a mixture of dinotefuran and imidacloprid (Bayer Advanced™ Protect and Feed II), soil drench using imidacloprid (Bayer Advanced™ Tree & Shrub Insect Control or Optrol™), and use of dinotefuran containing granules (Ortho Tree and Shrub Insect Control Ready to Use Granules®).[76]

Neonicotinoids are most commonly used as seed treatments.[77] In 2008, the insecticides comprised 80% of the global treated seed market.[1] By 2025, the seed treatment market is expected to grow to as much as $10 billion USD per year, with use increasing as much as 14% in China.[78] North America represents the largest market presently for seed treatments[1] with clothianidin or thiamethoxam coated seeds applied to over 80% of corn (maize) seed grown on the continent.[77] Over the past decade there has been a three-fold increase in the use of treated seeds in the United States[8,24,79] with a particularly rapid increase in use between 2003 and 2011 as a pre-emptive insecticide applied as a seed coating for row crops such as corn (maize), cotton, soybeans, and wheat.[74] Presently, 50% of soybeans (18.2 million hectares),[23] nearly 100% of corn (>36.4 million hectares) and 95% of cotton (15 million hectares) are treated with neonicotinoids.[74,80] By comparison, <50% of corn and <10% of soybean acreage was treated with any type of insecticide prior to their popular use as seed treatments.[81] Seed treatments also contain multiple active ingredients – fungicides, herbicide safeners, nematicides, and plant growth regulators and surfactants/adjuvants.[4] They are also often applied with other pesticides and can co-occur in the environment with fertilizers, metals and pharmaceuticals.[4]

Concerns about the potential detrimental effects of neonicotinoid use have been growing in recent years due to their potential negative effects on pollinators.[82] The European Food Safety Authority (EFSA) stated that neonicotinoids pose an unacceptably high risk to bees.[72] In 2013, the EU halted the use of imidacloprid, clothianidin, and thiamethoxam on flowering field crops such as corn because of evidence that the pesticides harm domesticated honeybees.[83]

‡ County level estimates from the U.S. Geological Survey (USGS) that include seed treatments are only available through 2014. In 2015, the provider of the surveyed pesticide use data stopped making estimates to derive the county-level use estimates of seed treatment use because of complexity and uncertainty.

Table 2 Solubility and half-life of neonicotinoids[d]

Neonicotinoid	Half-life (DT50)[c] in soil (days	Solubility in water at 20 °C, pH = 7 (mg L^{-1})	Leaching potential[a]	Vapor pressure 20 °C (mPa)	Volatility[b]
Acetamiprid	31–450	2950	Low	1.73×10^{-4}	Low
Clothianidin	148–6931	340	High	2.8×10^{-8}	Low
Dinotefuran	75–82	39 830	High	0.0017	Low
Imidacloprid	100–1250	610	High	4.0×10^{-7}	Low
Nitenpyram	8 days	590 000	Moderate	0.0011	Low
Thiacloprid	3.4–1000 days	184	Low	3.00×10^{-7}	Low
Thiamethoxam	7–335 days	4100	High	6.60×10^{-6}	Low

[a] Leaching potential = >2.8 = high leachability 2.8–1.8 = transition state <1.8 = low leachability. [b] Volatility = vapor pressure at 25 °C (mPa) <5.0 = low volatility 5.0–10.0 = moderately volatile >10 = highly volatile. [c] Soil degradation reported at DT50 for aerobic environment at typical for field conditions. [d] Table adapted from Kathleen A. Lewis, *et al.*, *Human and Ecological Risk Assessment*, 2016. **22**(4): pp. 1050–1064;[17] Bonmatin, *Environ. Sci. Pollut. Res. Int.*, 2015, **22**, 35–67;[46] Wood and Goulson, *Environmental Science and Pollution Research*, 2017, **24**, 17 285–17 325;[91] and Goulson, *J. Appl. Ecol.*, 2013, **50**, 977–987.[92]

In 2018, this ban was made permanent and extended to include use on all outdoor field crops.[84] Health Canada's Pest Management Regulatory Agency (PMRA) is planning to phase-out the use of some neonicotinoids over the next 3–5 years,[85–88] while the EPA cancelled 12 products containing thiamethoxam and clothianidin in 2019.[89] The EPA was scheduled to release updated pollinator risk assessments on five neonicotinoids by the end of 2019.[33] As of 2019, six states in the United States have enacted legislation intended to limit neonicotinoids and protect pollinators, and an additional seven states had bills introduced that added restrictions to neonicotinoid usage.[84–90]

4. Occurrence in the environment, water and food

The persistence of neonicotinoids in water, soil, and biota presents a potential environmental health concern that has been previously highlighted by numerous researchers and public health agencies.[4,5,8,40,46,47,91–94] Neonicotinoids have relatively long half-lives in soil, high water solubility, and low sorption in soil (Table 2); these factors contribute to the persistence and transport of these insecticides in the environment.[46,92,93,95–97]

4.1 Soil

Neonicotinoids can persist in soils; the reported half-lives for neonicotinoids in soil range from as little as 1 day to almost 19 years (Table 2).[46,92] Half-lives can also be affected by local soil type, ultraviolet radiation, moisture, temperature, and pH.[46] For example, neonicotinoids typically biodegrade rapidly, but in dry soils with high organic matter content and low temperatures, they can persist and potentially accumulate.[46] These values include results from both field and laboratory studies with different soil types, including clay, elder, loam, and sand and silt soils.[92]

For neonicotinoids used as seed treatments or granules, only 2 to 20% of the active ingredient in the coating is absorbed by the crop.[47] This leaves between 80 to 98% of the active ingredient remaining in the environment and able to accumulate in soil, be lost as dust during planting, or be transported to surface and/or groundwater.[98,99] The risk of dust drift is affected by several factors including: design and setting of the seed drill, mass and size of particles, quality of seed coating, meteorological conditions, and morphological properties of dust particles.[100] Depending on the crop, individual seeds can contain from 1–17 mg kg^{-1} of neonicotinoids.[47] Planting treated seeds results in dust clouds around the operating tractor, with neonicotinoid concentrations as high as 30 μg m^{-3}.[98,101–104] Atmospheric emissions of neonicotinoid seed treatment as particulate matter are also caused by tillage and wind events. With passive and active air samplers, the average clothianidin and thiamethoxam concentrations in total suspended particulates ranged from trace to 1.91 ng m^{-3} during tillage and wind compared to a peak of 16.22 ng m^{-3} during planting.[105]

Several studies have documented that neonicotinoids persist in soils several years after the planting of treated seeds and can accumulate in soils after repeat application.[106,107] A study investigating imidacloprid seed treatments on winter wheat in the United Kingdom documented soil concentrations increasing from 6 to 8 ng g^{-1} after the first year of planting to 18 to 60 ng g^{-1} 6 years later.[92] Randomly sampled soil from 74 farms in France documented that 84% of the fields contained detectable levels of imidacloprid (>0.1 ng g^{-1}) and 59% contained >1 ng g^{-1}; farms with 2 years of imidacloprid treatment had higher soil concentrations than those soils that received only 1 year of treatment.[106] In areas of seed treatment use in North America, clothianidin and thiamethoxam have commonly been detected with average soil concentrations of <10 ng g^{-1}.[108–111] Studies have also shown that soil concentrations increase with repeated applications, plateauing around 4–6 years after repeated use of seed treatments, and may remain in the soil several years after treated seed have stopped being used, with average concentrations below 6 ng g^{-1}.[4,92,106,109–112] Similarly, studies examining spray applications of imidacloprid in Europe have also shown accumulation in soil with concentrations ranging from 6 to 18 ng g^{-1} one year after sowing

compared to 18 to 60 ng g^{-1} after 5 years of repeat application.[92,113]

4.2 Water

As highly water-soluble compounds, neonicotinoids are frequently detected in surface and groundwater around the world.[99,107,111,114–125] Neonicotinoids are resistant to hydrolysis at neutral or acidic pH under anaerobic conditions, but may undergo rapid photodegradation if there is sufficient light penetration.[93,126–128] Increasing turbidity in water can reduce photodegradation.[129] Additionally, differences in light intensity and temperature with latitude affect half-lives by region, with mid and higher latitudes having longer half-lives compared to tropical regions.[46]

Contamination of water can take place through multiple pathways and mechanisms, including overspray, drift, spread of talcum and graphite powder from treated seeds, surface runoff, leaching into groundwater, discharge into wetlands, and *via* snowmelt.[13,108,109,114,117,118,121,124,130–133] In China, six neonicotinoids have been detected in 100% of samples from the Han and Yangtze Rivers, where median concentrations ranged from 13–186 ng L^{-1}.[134] In Canada, imidacloprid, clothianidin, and thiamethoxam were detected in over 90% of streams sampled, with two locations exceeding Canadian freshwater guidelines with concentrations in excess of 230 ng L^{-1} in 75% of samples.[135] In streams within the Midwestern United States, Hladik *et al.* detected clothianidin, thiamethoxam, and imidacloprid in 75%, 47%, and 23%, respectively, of samples during growing season with maximum individual concentrations between 42.7–257 ng L^{-1}.[117] Currently, neonicotinoids are not regulated under the U.S. Safe Drinking Water Act's National Primary Drinking Water Regulations or Canada's Guidelines for Drinking Water Quality.[136,137] In Europe, pesticides are regulated through several directives, with maximum limits set at 100 ng L^{-1} for individual pesticides and their degradates and 500 ng L^{-1} for total pesticide residues.[138–142] For comparison, the maximum contaminant levels established for lindane, methoxychlor and oxamyl, insecticides regulated by the EPA in public drinking water, range between 200 and 200 000 ng L^{-1}.[137] The limits for glyphosate and atrazine, two commonly used herbicides, are 700 000 ng L^{-1} and 3000 ng L^{-1}, respectively. The EPA does not regulate total pesticide residues.[137]

Recent planting of treated seeds and subsequent precipitation events can drive neonicotinoid pulses to streams.[117] In one study, clothianidin and thiamethoxam concentrations were positively correlated with the percentage of the land used for cultivated crop production, while imidacloprid was linked to the percentage of urban area within the basin.[143] Across studies, average concentrations for imidacloprid in surface waters were generally in the tens of ng L^{-1}, with a maximum concentration of 320 000 ng L^{-1} reported in the Netherlands.[99] The EPA estimates peak surface water contamination to be between 40 and 269 000 ng L^{-1} for acetamiprid, clothianidin, dinotefuran, imidacloprid and thiamethoxam.[28–32,144]

Groundwater concentrations as high as 140 ng L^{-1} have been measured beneath areas planted with treated seeds[108,118] and concentrations of up to 10 000 ng L^{-1} with in-furrow applications for potatoes.[124] Groundwater concentrations in fields with coated seeds exhibited less seasonable variability compared to surface water, which tends to have higher concentrations occurring later in the summer.[108] The EPA estimates that the peak concentrations of neonicotinoids in groundwater range from 58 000 to 211 000 ng L^{-1} for acetamiprid, clothianidin, dinotefuran, imidacloprid and thiamethoxam.[28–32,144]

Despite the ubiquity of their occurrence in water sources, limited research has been conducted to assess human exposure risk from neonicotinoids in drinking water. Only six studies, all published within the past 3 years, have focused on municipal systems. A cross-sectional study of 100 sites across the United States detected imidacloprid in untreated and treated water samples from 0.008–0.202 ng L^{-1} between 1999 and 2015, with detection frequency increasing between 2004 and 2011.[145] Detections peaked in 2011, with imidacloprid found in 36.7% of untreated and 29.7% of treated water.[145] In 2016, Klarich *et al.* found clothianidin, imidacloprid, and thiamethoxam at concentrations up to 57 ng L^{-1} in finished water samples at the University of Iowa water treatment facility in Iowa City, Iowa, USA, over a 7 week period following corn and soybean planting.[118] While conventional water treatment processes, like rapid sand filtration, did not remove clothianidin or imidacloprid, thiamethoxam decreased by 40–60% following lime softening due to hydrolysis at the high pH values used for this process. In comparison, treatment using granular activated carbon was able to remove >80% of all three neonicotinoids.[118] A follow-up study detected two metabolites of imidacloprid, desnitro-imidacloprid and imidacloprid-urea, in finished drinking water with concentrations up to 0.60 ng L^{-1}.[146] In China, multiple neonicotinoids have been detected in 100% of tap water samples, with median concentrations between 2.6 and 138 ng L^{-1}.[134]

Water treatment was most effective at removing acetamiprid (40% reduction) and thiacloprid (20%).[134] In Canada, thiamethoxam, clothianidin, and imidacloprid have been detected in water systems.[147,148] In Ontario, the mean concentrations were below the limits of detection but had a peak value of 91.7 ng L^{-1} for thiamethoxam.[147] In Quebec, tap water from four samples had maximum concentrations for these three neonicotinoids ranging from 1.0–10 ng L^{-1}.[148]

No studies were identified to date that looked at private well water as a potential source of exposure. Globally, 435 million people rely on drinking water from unregulated wells and springs.[149] These water sources are generally vulnerable to contamination from a variety of sources with treatment left to individual households.[149–151] The World Health Organization estimates that groundwater constitutes 97% of global freshwater and is one of the most important supplies of drinking water.[152] Groundwater can also be contaminated by chemical hazards from the land surface, with contaminated groundwater sources reported in countries from all levels of economic development.[152] For example, in the United States, a study of 2100 wells nationwide found that 23% of domestic wells contained one or more contaminants at concentrations greater than human-health benchmarks and 73% of wells contained

multiple contaminants greater than human-health benchmarks.[153] Based on these earlier works, it is likely that many rural wells are similarly contaminated with neonicotinoids, and further research is needed to characterize well water contamination with these pesticides. Several studies have hypothesized that long-term consumption of private well-water is associated with increased risks for adverse health effects due to exposure to contaminants.[154–158]

4.3 Food

The small molecular weight and high water solubility of neonicotinoids provide the systemic property to enable the insecticides to enter plant tissues.[159] Several neonicotinoids have been shown to translocate into pollen, vegetables, and fruits and therefore represent a potential human exposure route as washing does not completely remove neonicotinoid insecticides in fruits and vegetables being consumed.[160–166]

Neonicotinoids have been frequently detected in food including rice, tea, honey, and fruit and vegetables.[38,145,167–197] A "market basket" study was conducted to assess levels of neonicotinoids in fresh fruits, vegetables, and honey available in a supermarket in Massachusetts, USA.[38] All foods, except nectarines and tomatoes, tested positive for one or more neonicotinoids. Imidacloprid was detected at the highest singular concentration and most frequently (70% overall). Seventy-two percent of fruits and 45% of vegetables contained multiple neonicotinoids.[38] A review analyzing neonicotinoid pesticides from the U.S. Department of Agriculture Pesticide Data Program's between 1999 and 2015 found the insecticides in both domestic and imported commodities.[145] The study found that neonicotinoids were detected in commonly consumed fruits and vegetables, and trends indicated an increase in use of acetamiprid, clothianidin, and thiamethoxam. Imidacloprid was the most commonly detected neonicotinoid with an overall detection frequency of 12%. The overall detection frequency for neonicotinoids was 5%. Higher detection frequencies were observed for specific foods: cherries (46%), apples (30%), pears (24%), and strawberries (21%) for acetamiprid; and cauliflower (58%), lettuce (46%), spinach (39%), kale (31%), potatoes (31%), cilantro (31%), grapes (29%), cherries (26%), collard greens (25%), and celery (21%) for imidacloprid.[145] In the Pesticide Data Program's 2015 report, clothianidin was detected in 31% of spinach, 23% of potatoes, and 10% of tomato samples.[38,186] Thiamethoxam was detected in 30% of cherries, 22% of lettuce, and 14% of watermelons. Imidacloprid was detected in 46% of potatoes, 43% of spinach, and 35% of cherries.[38,186]

Two cross-sectional studies (the U.S. Congressional Cafeteria Study and Hangzhou China Study) provide further evidence that neonicotinoids have become ubiquitous in the global food supply.[184] The results showed that most of the tested fruits and vegetables contained neonicotinoids. The United States study found at least one neonicotinoid in 79% of fruits and 65% of vegetables, and 3 or more in 62% and 38% of fruits and vegetables, respectively. In comparison, the Hangzhou study detected one or more neonicotinoid in 57% of fruits and 63% of vegetables, and three or more in 30% and 42% of commodities. Thiamethoxam (U.S.-53%; China-51%) and imidacloprid (U.S.-52%; China-66%) were the most frequently detected neonicotinoids in both studies. A 2011 survey conducted by the Japanese Ministry of Agriculture, Forestry, and Fisheries found dinotefuran to be the most frequently detected neonicotinoid in fruits and vegetables with a frequency rate of 63.3%.[187] In Turkey, acetamiprid, imidacloprid, and thiamethoxam have been detected in 71%, 36%, and 7%, respectively, of fruit, and 83%, 67%, and 33%, respectively of vegetables.[176]

Neonicotinoid residues may be reduced, but not eliminated through processing and washing. One study examined processed foods (tomato products) in addition to raw fruits, reporting that food processing altered the concentration of neonicotinoid residuals.[171] This research also reported that the concentrations of imidacloprid were significantly lower (*i.e.*, 7–30%) on washed tomatoes, indicating that at least some portion of neonicotinoids can be mechanically removed. The intake of imidacloprid through direct ingestion of tomatoes has been found to vary between 10^{-2} and 10^{-6} ($kg_{ingested}/kg_{applied}$) depending on whether the tomatoes were unwashed, washed, or washed and peeled.[188]

Neonicotinoids have also been frequently detected in honey.[38,189–197] A global survey found five neonicotinoids present in honey[190] with 75% of all honey samples testing positive for at least one neonicotinoid.[190] North America, Asia, and Europe had the greatest proportion of detections.[190] Results from eight studies report that the detection of individual neonicotinoids ranged from non-detect to as high as 192.8 μg kg^{-1} (clothianidin).[38,191,192–197] Metabolites such as imidacloprid-olefin (5.6 ng g^{-1}) and 5-hydroxy imidacloprid (21.1 ng g^{-1}) have also been detected, indicating that an increased emphasis is warranted on metabolites in terms of potential health risks and exposure through food.[197]

Despite the frequency of detection, estimates of daily intake indicate consumption typically does not exceed established tolerance levels.[38,145,174,183,184,198,199] Maximum residue levels have been established in several countries for tea, grains, fruits, vegetables, dairy, and meat. Values range from 10 to 600 000 μg kg^{-1}.[31,45,200–209] In Japan, it is estimated the average adult consumes from 206 to 1050 μg per day of individual neonicotinoids (reviewed in[198]). Harada *et al.* estimated that the average daily intake of neonicotinoids amongst Japanese adults was between 0.53 and 3.66 μg per day, with a high of 64.5 μg per day for dinotefuran.[198] This peak value was <1% of the acceptable daily intake set by the Japanese government.[198] Studies have estimated the total daily dietary intake of neonicotinoids in the United States and China to be 10.1 and 37.9 μg per day, respectively.[184] These concentrations are below the acceptable daily intake levels established by the World Health Organization.[210] These levels indicate that between 10 and 200 μg per kilogram of body weight of various neonicotinoids can be consumed daily for a lifetime without appreciable risk to health (Table S-2†).[210]

The EPA estimates that exposure to neonicotinoids through acute and chronic dietary exposure is generally not a health concern for the U.S. population. Risk estimates ranged from 2%

to 38% of the acceptable daily intake overall for the general population.[28–32] Children 1–2 years old are considered the most vulnerable, with risk estimates ranging from 8% to 93% for acute and 6% to 52% for chronic population adjusted dose. The EPA considers exposure a concern when risk estimates exceed 100% of the reference dose.[28–32] Similarly, a Chinese study assessed the cumulative risk of total neonicotinoids from fruit and vegetable intake school-aged children between 8 and 12 years old.[199] Although the study detected at least one neonicotinoid in each of the fruits and vegetables tested, the acceptable daily intake was found to be below the chronic reference doses established by the EPA and the Chinese government.[199]

4.4. Knowledge gaps and research needs

Neonicotinoids are known to occur in numerous environmental compartments (*e.g.*, soil, water, food) but to date there has been little emphasis on how these residues relate to human exposure. More information is needed on the exposure of neonicotinoids in matrices directly consumed by humans. This includes more information on the occurrence of neonicotinoids in drinking water sources and potential removal of neonicotinoids during drinking water treatment. Drinking water data are especially needed for rural areas with high-intensity agriculture where neonicotinoid use is particularly prevalent. While current food resides indicate daily consumption rates of neonicotinoids are well below daily intake levels, the addition of metabolites to the analysis of water and food would provide a more comprehensive exposure of humans to neonicotinoids.

5. Transformation pathways

5.1 Transformation products of altered toxicity

Understanding the environmentally relevant transformation products and pathways of neonicotinoids is critical to characterizing potential health risks. Some transformation products are biologically active, sometimes even increasing toxicity to either insects or vertebrate organisms.[211] In most cases, altered bioactivity is due to transformation of the electron-rich nitro- or cyano-functional groups (pharmacophore) on the parent neonicotinoid. The nitro group can transform to the desnitro guanidine metabolite, then to the urea metabolite. The desnitro form is substantially more toxic to vertebrates due to positive charge distribution at the guanidine that favorably interacts with the mammalian nAChRs (Fig. 2).[212] The cyano functional group appears more stable than the nitro group, with more limited reports of transformation.[213,214] Major structural alteration of metabolites likely decreases toxicity to vertebrates. Exceptions to nitro- or cyano-transformation are the formation of imidacloprid olefin, which is significantly more insecticidal[215] and the demethylation of acetamiprid, which results in a less toxic product.[216]

Several reviews[46,94] have summarized physiochemical characteristics of neonicotinoids, reporting degradation and persistence in soil and water matrices, and degradation by abiotic and microbial transformations, as well as plant uptake and sorption to/leaching from soils. Current knowledge is summarized here.

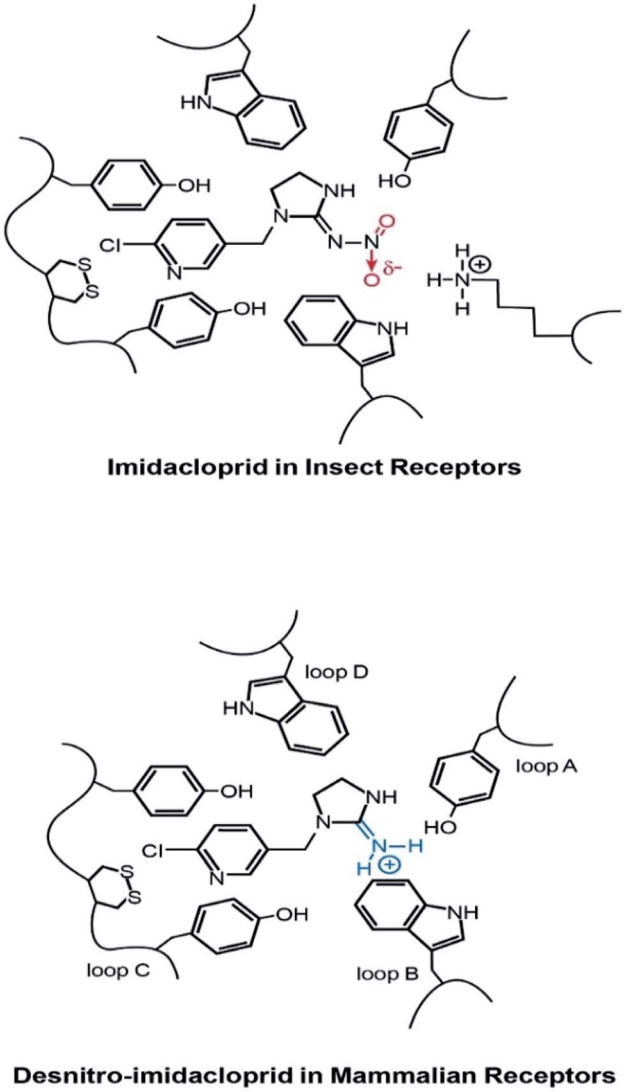

Fig. 2 The electronegative pharmacophore (either nitro- or cyano-group) of the neonicotinoid confers selective binding to the insect nicotinic acetylcholine receptor (nAChR). Insecticidal target specificity is lost when the metabolite desnitro-imidacloprid without the nitro-group binds to the vertebrate nAChR. Republished with permission of Annual Reviews, Inc, from Neonicotinoid Insecticide Toxicology: Mechanisms of Selective Action, Motohiro Tomizawa, John E. Casida, vol 45, 2005; permission conveyed through Copyright Clearance Center, Inc.

5.2 Abiotic transformation mechanisms in the environment

5.2.1 Photolysis. Photolysis studies under environmentally relevant conditions are limited. Nitro-based neonicotinoids thiamethoxam, clothianidin, and imidacloprid can undergo direct photodegradation. The cyano-neonicotinoids acetamiprid and thiacloprid are largely stable in sunlight. Although laboratory reports of direct photolysis half-lives can be on the order of minutes to hours,[217,218] the authors acknowledge that

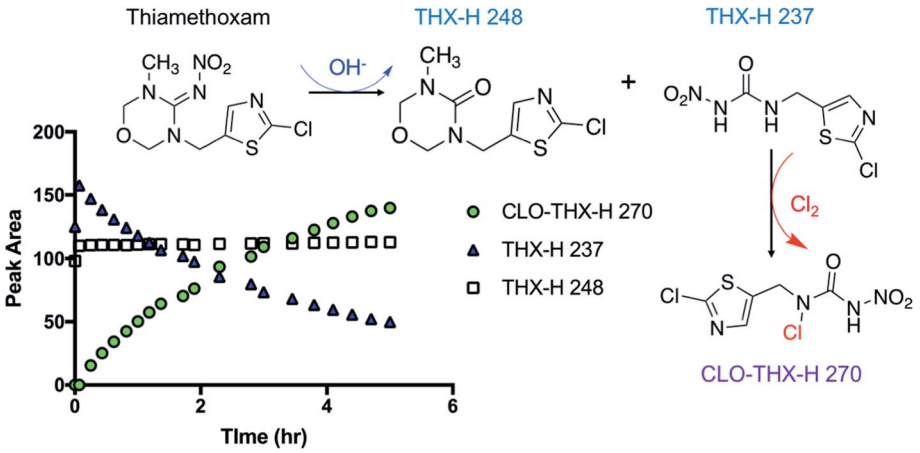

Fig. 3 Chlorination of the hydrolysis products of thiamethoxam (THX-H 237 and THX-H 248) to form novel chlorinated product CLO-THX-H 270. Figure taken with permission from Klarich-Wong, *et al.*, *Environmental Science & Technology Letters*, 2019. **6**(2): pp. 98–105.[146] Copyright 2019 American Chemical Society.

these rates are only applicable under ideal, near-surface conditions; environmental rates are presumed much lower and help explain neonicotinoid persistence.[218] A laboratory study with simulated natural light (>290 nm) estimated neonicotinoid photolysis rate constants and half-lives for direct photolysis during different seasons.[217] Acetamiprid can undergo indirect photolysis with hydroxyl radical, but is not expected to contribute significantly to degradation in the environment.[218] Multiple photoproducts were observed, with limited information on their structures.

Imidacloprid photo transformation on thin films revealed transformation to desnitro-imidacloprid (16%) and imidacloprid urea (84%), indicating a mechanism involving photodissociation of the nitro group.[219] Another study[220] reported the main products of imidacloprid following irradiation at 290 nm were[221] 6-chloronicotinaldehyde, *N*-methulnicotinacidamide, 1-(6-chloronicotinyl)imidazolidone and 6-chloro-3-pyridylmethylethylendiamine.

Maximum absorbance of clothianidin[222] occurs at about 267 nm ($t_{1/2}$ = 3.6 h), yielding eight major products *via* multiple mechanisms (*e.g.*, denitration, nucleophilic substitution, ring opening/closure), including several stable products. Advanced oxidation processes yielded half-lives between 5 hours and 19 days for imidacloprid, thiacloprid, and acetamiprid.[223]

5.2.2 Hydrolysis and chlorination. Under most environmentally relevant conditions and time scales, the only neonicotinoid to meaningfully transform *via* hydrolysis is thiamethoxam.[118] In experiments up to 150 days, other neonicotinoids representing diverse structural groups (*i.e.*, nitenpyram, imidacloprid, acetamiprid, clothianidin) underwent hydrolysis only at pH 10 conditions (not pH 4, 6, 7, 8) and in all cases appears base-catalyzed, forming urea hydrolysis products.[218] A thiamethoxam carbonyl product under alkaline pH soil conditions has been reported,[224] ($t_{1/2}$ = 11–26 days). Indeed, elevated pH conditions can be relevant to human exposure through some drinking water treatment processes. In the lime softening basin of a drinking water treatment plant in Iowa, base-catalyzed hydrolysis occurred.[118] Batch tests using the softening revealed a $t_{1/2}$ of 0.75 day (0.9 d^{-1}).[118] Another study[225] determined thiamethoxam was largely stable at neutral pH ($t_{1/2}$ = 29.2 days) as compared to acidic ($t_{1/2}$ = 13.9 days) or alkaline ($t_{1/2}$ = 2.1 days) conditions. Hydrolysis reaction rates changed with temperature but were similar at environmentally relevant conditions.[226] Base-catalyzed hydrolysis of thiamethoxam yields two products (Fig. 3).[146] Notably, thiamethoxam-H 237 is reactive toward chlorine; chemical disinfection typically occurs subsequent to softening. At a chlorine residual of 5 mg L^{-1} as Cl_2, the half-life of thiamethoxam-H 237 was 4.8 hours, yielding a single species referred to as clothianidin-thiamethoxam-H 270. Neither thiamethoxam nor thiamethoxam-H 248 reacted with chlorine. The proposed products of hydrolysis[146] are shown in Fig. 3. Clothianidin, imidacloprid, and two imidacloprid metabolites (desnitro-imidacloprid and imidacloprid-urea), also react during chlorination,[146] indicating the potential for formation of novel disinfection by-products from neonicotinoids during treatment and distribution (Fig. 4).

5.3 Biotransformation

5.3.1 Microbial processes. Biotransformation is often implicated in neonicotinoid loss in soil degradation studies, even when not specifically examined.[227] Microbial transformation of neonicotinoids generates a suite of metabolites. Nitro-based neonicotinoids most commonly used (*e.g.*, imidacloprid, clothianidin, thiamethoxam) have been best studied in terms of microbial metabolites. For example, isolated soil bacteria were capable of degrading imidacloprid and thiamethoxam when not the sole carbon source.[228,229] Both neonicotinoids transformed to nitrosoguanidine (=N–NO), desnitro (=NH), and urea (=O) metabolites (Fig. 5) in a pathway similar to the metabolization of neonictinoids by the liver in mammals.[228] Soil microbes also transformed imidacloprid to 5-hydoxyl imidacloprid and imidacloprid olefin; the latter is significantly more insecticidal than imidacloprid.[215]

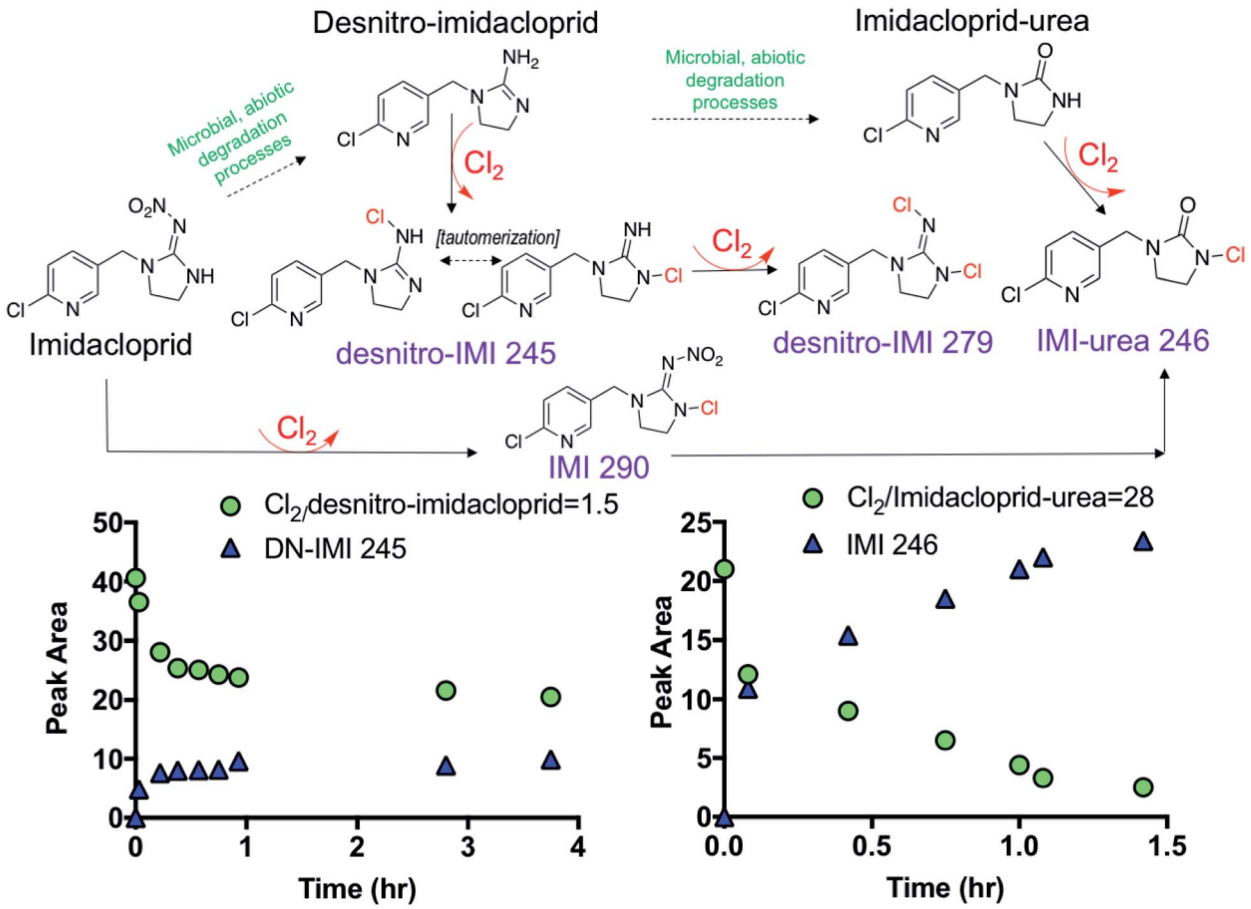

Fig. 4 Chlorination of desnitro-imidacloprid and imidacloprid-urea to form chlorinated products desnitro-IMI 245, desnitro-IMI 279, and IMI-urea 246. Figure taken with permission from Klarich-Wong, *et al.*, *Environmental Science & Technology Letters*, 2019. **6**(2): pp. 98–105.[146] Copyright 2019 American Chemical Society.

Transformation of imidacloprid to 5-hydroxyl imidacloprid occurred with cultures enriched with imidacloprid as the sole nitrogen source, but not sole carbon source; addition of sucrose promoted hydroxylation.[215]

Microbial transformation of cyano-based neonicotinoids results in a different suite of metabolites, mainly the result of demethylation but also formation of amide products. Activated sludge bacteria were capable of dechlorinating and demethylating acetamiprid as the sole carbon source (>99% in 3 days).[230]

Bacteria degraded acetamiprid *via* an N-deacetylation reaction that removes the electronegative functional group.[231],[232] N-demethylation also occurs,[233] with the metabolite retaining the cyano functional group, but with lowered insecticidal activity.

Thiacloprid and acetamiprid undergo similar transformations at the cyano group (Fig. 6). Microbially facilitated hydrolysis of thiacloprid (*via* nitrile hydratase) to thiacloprid amide represents 98% of the final product[214,234] and is presumed the main sink in soils.[213] Similar to thiacloprid, acetamiprid is transformed *via* nitrile hydratase to a *N*-carbamoylimine derivative, impacting the cyano group.[235] This metabolite also had significantly lower insecticidal effects and was further degraded to acetamiprid-NH and acetamiprid-NH_2 products.

Neonicotinoids can alter microbial communities when applied at high levels. For example, imidacloprid treatment altered soil respiration and soil biomass carbon content, the total number/diversity of bacteria, and enzyme activities, with nitrifying and nitrogen fixing bacteria being the most sensitive.[236–238] Over application of neonicotinoids has also been linked to alteration of the biocatalytic capacity of soils, including loss of nitrite and nitrate reductase enzyme activity.[239]

5.3.2 Fungal transformation. There is limited specific information on fungal transformation of neonicotinoids. Fungal biotransformation differs from microbial processes due to the presence of both extra and intracellular enzymes. Extracellular enzymes do not degrade neonicotinoids; rather, cytochrome P450 enzymes are involved.[216,240] One study focused on clothianidin transformation by the white-rot fungi *Phanerochaete sordida*[240] reported 37% degraded in 20 days in a liquid culture incubation. The metabolite *N*-(2-chlorothiazol-5-yl-methyl)-*N*′-methylurea was identified and determined to no longer be neurotoxic to mouse neuroblastoma cells. This product represents the same major transformation pathway as described for bacteria (Fig. 5).

Acetamiprid[216] was transformed *via* a demethylation reaction, yielding in the same metabolite shown in Fig. 6. This

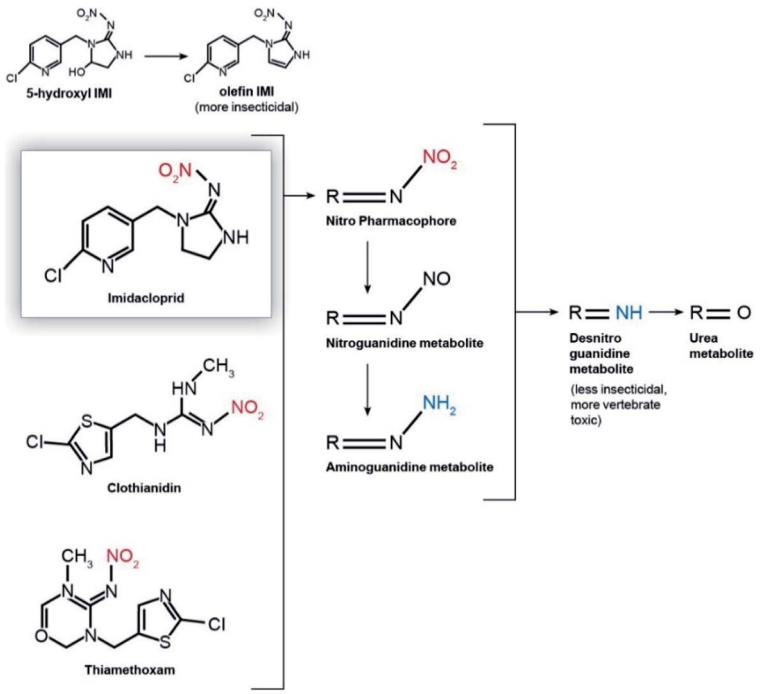

Fig. 5 Main transformation of the nitro-functional group neonicotinoids from the electronegative functional group to the positive guanidine (blue) metabolites of enhanced vertebrate toxicity. Image created by LeFevre; Data and information adapted from Dai *et al.*, *Appl. Microbiol. Biotechnol.*, 2006, **71**, 927–934;[215] Pandey *et al.*, *Biochem. Biophys. Res. Commun.*, 2009, **380**, 710–714;[228] and Zhou *et al.*, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 4065–4074.[229]

product was detected in honeybees, mice, spinach, and soil bacteria,[241–244] and has a 10-fold lower insecticidal activity than the parent compound.[233]

5.3.3 Plant processes. Because neonicotinoids are designed to be applied to plants and act systemically, a substantial amount of research has focused on plant uptake

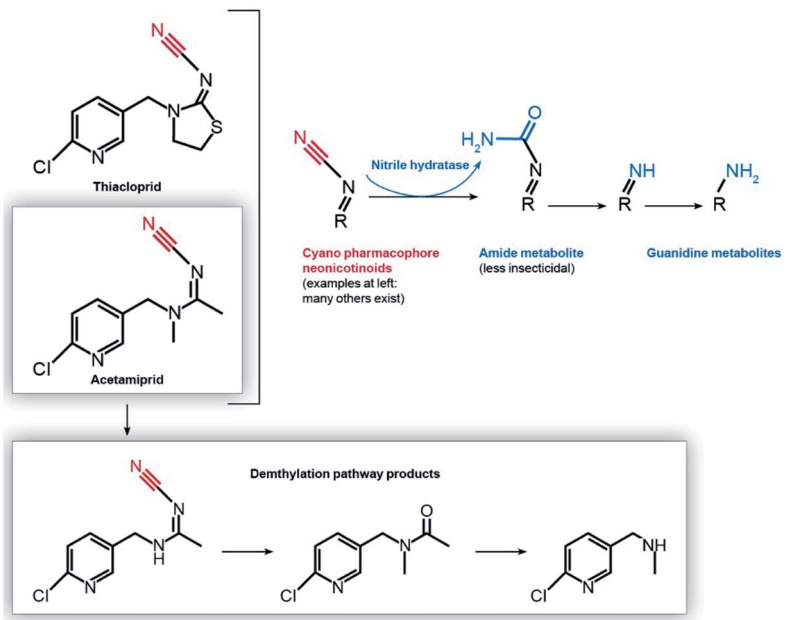

Fig. 6 Microbial transformation of neonicotinoids containing a cyano functional group. The electronegative cyano pharmacophore can be transformed *via* nitrile hydratase enzymes to amide metabolites, with subsequent decomposition to guanidine metabolites. Various products are also formed *via* the demethylation pathway. Image created by LeFevre; Data and information adapted from Chen *et al.*, *Biodegradation*, 2008, 19, 651–658.;[233] Dai *et al.*, *J. Agric. Food Chem.*, 2010, 58, 2419–2425.;[213] Tang *et al.*, *Process Biochem.*, 2012, 47, 1820–1825.;[232] Wang *et al.*, *Bioresour. Technol.*, 2013, 138, 359–368.;[231] Yang *et al.*, *Int. Biodeterior. Biodegrad.* 85, 2013, 95–102.;[230] Zhang *et al.*, *J. Agric. Food Chem.*, 2012, 60, 153–159.;[214] and Zhou *et al.*, *Int. Biodeterior. Biodegrad.*, 2014, 93, 10–17.[235]

and translocation for insecticidal effects and is outside the scope of this review.[5,245] Much less work has focused on the transformation of neonicotinoids by plants to yield novel products of ecological or human exposure significance. One such study[246] examined in substantial detail the plant transformation products and pathways of a suite of neonicotinoids in spinach plants, comparing the products to metabolites discovered in mice. Metabolic reactions occurred including nitro reduction, cyano hydrolysis, demethylation, sulfoxidation, hydroxylation and olefin formation, hydroxylation and ring opening, and dechlorination. The phase I plant metabolites were similar to those observed in other transformation processes, but phase II plant metabolites were different, and included *N*-glucosides and amino acid conjugates. Some plant metabolites remain metabolically active for vertebrates (*e.g.*, desnitro- and decynanoguanidines and olefins) and insects. Specifically, in spinach,[243] the $=N-NO_2$ group was reduced to $=N-NO$ and $=N-NH_2$.

Neonicotinoids also affect plants. Beyond the insecticidal mode of action, imidacloprid and clothianidin increase plant vigor against abiotic stress by inducing salicylic acid-associated responses *via* their *in-planta* metabolites.[247] Neonicotinoids can alter the plant hormone systems, as demonstrated by geno and phenotypical evidence.

The microbial community associated with plants may facilitate neonicotinoid transformation. Duckweed *Lemna* and its microbial community synergistically transformed nitro-based imidacloprid and cyano-based thiacloprid, but neither the duckweed nor bacteria from the duckweed degraded the neonicotinoids on their own.[248] Formation of metabolites with increased toxicity was very small or absent, indicating duckweed-microbial transformation likely decreased overall aquatic toxicity.[248]

5.4 Mammalian species

In mammals, neonicotinoids are metabolized by enzymes in the liver[249,250] (*e.g.*, cytochrome P450 enzymes, aldehyde oxidase), as well as anywhere these enzymes are expressed[250–252] (*i.e.*, including red blood cells, plasma, lung, skin, brain, kidney, spleen, endocrine tissue, adipose tissue, placenta, testis, and ovaries). Metabolism of neonicotinoids in vertebrates occurs *via* several reactions: reduction, demethylation, hydroxylation, and olefin formation.[8,211,243,253] Imidacloprid, nitenpyram, thiacloprid, and acetamiprid share a chloropyridinyl chemical structure that degrades into 6-chloronicotinic acid. Thiamethoxam and clothianidin both have a chlorothiazole structure that is metabolized into 2-chloro-1,3-thiazole-5-carboxylic acid. Dinotefuran is metabolized into 3-furoic acid. Each of these metabolites are then conjugated with either glycine or glucuronic acid and excreted in the urine.[49,242] These metabolites have been observed in both mouse and human urine.[49,187,198,242,253–258]

Imidacloprid and thiamethoxam are metabolized to produce nitrosoguanidine metabolites and later to less toxic urea metabolites. As in bacterial transformation, intermediate formation of desnitro/guanidine intermediates (*i.e.*, desnitro-imidacloprid) occurs. This metabolite is also formed in metabolism studies with human liver microsomes.[259–261] Similar metabolism occurs in other vertebrates, including mice, rats, goats, and hens.[49,228]

Neonicotinoids and their metabolites are primarily excreted in the urine due to their low molecular weights and water solubility.[250] Pharmacokinetic models for acetamiprid, clothianidin, dinotefuran, and imidacloprid describe fates in urine.[198] In this study, 12 healthy adults orally ingested micro doses of deuterated neonicotinoids. Increased urinary concentrations of the labelled neonicotinoids followed dosing, with 64% of the clothianidin and 93% of dinotefuran recovered unchanged in 3 days. In comparison, imidacloprid and acetamiprid were readily metabolized with 13% of imidacloprid and 3% of acetamiprid recovered unchanged. Acetamiprid, mainly (31%) transformed to desmethyl-acetamiprid, which was eliminated more slowly.[198] Imidacloprid, clothianidin, and dinotefuran are also excreted in urine with short biological half-lives in animal studies.[49,242] Indeed, 46% of nitenpyram, 27% of thiamethoxam, and 1% of thiacloprid were recovered unchanged in mice urine within 24 hours.[49,242] Thiacloprid was converted to 6-chloropyridine carboxylic acid (12%) and thiamethoxam was converted into clothianidin (11%).[49,242]

5.5 Knowledge gaps and research needs

Bonmatin *et al.* specifically commented on the lack of information regarding the concentrations and dynamics of neonicotinoid degradation products and metabolites,[46] highlighting this as a research need. This distinct paucity could have profound effects on human and ecosystem health. For example, neonicotinoid metabolites were substantially more reactive towards chlorine than parent compounds,[146] highly relevant to human exposure during drinking water disinfection. Neonicotinoid metabolite toxicity profiles for vertebrates is also distinct from parent pesticides as a result of slight structural alteration to the insecticidal pharmacophore. Additional research and monitoring (including availability of commercial standards) are needed to elucidate novel neonicotinoid metabolites/transformation products in the environment, as well as measure levels in media relevant to human exposure (*i.e.*, plants for food, drinking water).

6. Toxicity in mammals

The ecotoxicology of neonicotinoids has been reviewed previously. For example, imidacloprid and clothianidin exerted sublethal effects in vertebrate wildlife—mammals, birds, fish, amphibians, and reptiles.[262] The effects observed included genotoxicity, cytotoxicity, impaired immune function, reduced growth and reproductive success at concentrations well below those expected to cause mortality.[262,263] A comparison of the toxicology data in rats, birds, and fish shows that most of the neonicotinoids have a moderate to high acute oral LD50 (ref. 17). Comparable insecticides like chlorpyrifos, diazinon, and malathion (organophosphates) and carbofuran and aldicarb (carbamates) typically have higher acute toxicity in mammals

Table 3 Summary of toxicity data for neonicotinoids[a]

Health Issue	Acetamiprid	Clothianidin	Dinotefuran	Imidacloprid	Nitenpyram	Thiacloprid	Thiamethoxam
Carcinogen	No	No	No	No	No Data	Possible	No
Genotoxic							
Chromosome aberration	No data	No data	No data	Negative	No data	Negative	Negative
DNA Damage/ repair	No data	No data	No data	Negative	No data	No data	Negative
Gene mutation	No data	No data	No data	Negative	No data	No data	Negative
Genome mutation	No data	No data	No data	Negative	No data	No data	No data
Unspecified genotoxicity	Negative	Negative	No data	Mixed	No data	Negative	No data
Endocrine disruption	No data	Possible	No data	No data	No data	Yes	No
Reproduction/ development effects	No	Possible	Possible	Yes	No	Yes	No
Cholinesterase inhibition	No	No	No	No	No data	No	No
Neurotoxicant	No	Yes	No	Possible	No data	Yes	No
Respiratory tract irritant	No	No	No data	No	Yes	No	No
Skin irritant	Yes	No	Possible	Possible	No	Yes	Possible
Skin sensitizer	No data	No data	No data	No data	No data	No	No data
Eye irritant	Possible	No	Yes	Possible	Yes	Yes	No
Phototoxicant	No data	No data	No data	No data	No data	No	No data
General health comments	N/A	Effects consistent with endocrine disruption noted in rodents/dogs	N/A	Moderately toxic	N/A	Possible liver and thyroid toxicant	Increased incidence of liver cell adenoma and adenocarcinoma in mice
		May cause low blood pressure, hypothermia, and impaired pupillary function		Potential liver, kidney, thyroid, heart and spleen toxicant		Probable human carcinogen	

[a] Yes = known to cause a problem; No = known to not cause a problem; Possible = status has not been identified. Table adapted from Kathleen A. Lewis, *et al.*, *Human and Ecological Risk Assessment*, 2016 **22**(4): pp. 1050–1064.[17]

and birds compared to neonicotinoids.[17] Organophosphates are typically rated as having a high potential to bioconcentrate in tissue, like fat, whereas neonicotinoids are believed to have a low potential to concentrate in tissue.[264–266] The following section briefly describes proprietary and peer-reviewed toxicity studies in mammalian models. A brief summary of toxicity data for neonicotinoids is presented in Table 3.

6.1 Oxidative stress

Exposure to neonicotinoids frequently causes oxidative stress, as shown by increased lipid peroxidation, decreased glutathione levels and altered activity of key antioxidant enzymes (*e.g.*, catalase, superoxide dismutase, and glutathione peroxidase).[267–272] Imidacloprid exposure increases both nitric oxide production and transcript levels of nitric oxide synthases in the brain and liver of female rats.[270] Similarly, thiacloprid increases nitric oxide levels in polymorphonuclear leukocytes and the plasma of thiacloprid-exposed rats.[271,273] Antioxidants, such as curcumin and vitamin C, can protect tissues from neonicotinoid-induced oxidative damage.[269,272] Based on the available evidence, oxidative stress caused by exposure to neonicotinoid pesticides has been proposed to play an important role in their toxicity in non-target species.[274]

6.2 Reproductive toxicity

Multiple studies report adverse reproductive and developmental effects in mammals exposed to neonicotinoids, including higher rates of embryo death, premature birth, reduced pregnancy rate, reduced sperm production and function, reduced weight of offspring, and stillbirth.[35,262,275,276] Experimental evidence indicates that neonicotinoids affect the fertilization rate in rodent models.[275,277,278] A study investigating *in vitro* fertilization reported that direct exposure to acetamiprid or imidacloprid adversely affect the fertilization ability of mouse spermatozoa.[275] Moreover, exposure of male rats to imidacloprid significantly decreased serum levels of testosterone.[278] Similarly, exposure to imidacloprid at no observed adverse effect level (NOAEL) dose-levels caused suppression of testicular function in adult male rats.[279] Clothianidin exposure in male rats significantly increased levels of thiobarbituric acid-

reactive substances, cholesterol, and palmitic, linoleic and arachidonic acids in testis, but did not cause sperm DNA fragmentation.[280] Exposure to acetamiprid in 3 week-old mice for 180 days led to a decrease in body weight, mildly affected spermatogenesis, and decreased expression of testosterone-metabolism genes, nAChR subunit genes, and proliferation-associated genes in the testes.[281] In contrast, imidacloprid is not a primary embryotoxicant, is not teratogenic, and does not affect reproduction or development following exposure *via* the maternal diet.[66,273]

6.3 Hepatotoxicity

Hepatotoxicity from a neonicotinoid exposure is typically apparent only at high doses and frequently accompanied by a decrease in food consumption and a reduced bodyweight. Serum activities of alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase, markers of hepatotoxicity, are typically elevated in rats exposed to imidacloprid.[66,269,282] A study examined liver histological changes and enzyme activity in female albino rats exposed to a high ($1/10^{th}$ LD50) and a low ($1/50^{th}$ LD50) dose of imidacloprid for 4 weeks.[283] Histological alterations involving a rise in liver enzymes, degeneration of hepatocytes, and dilations of the central vein were observed in the high dose group.[283] Dietary exposure to imidacloprid also reduced cholesterol levels in serum in rats[66] and caused fatty degeneration in the liver of female BALB/c mice.[284] Antioxidants, such as curcumin, can protect animals from liver injury caused by neonicotinoids such as imidacloprid.[269] Liver pathology is fully reversible in rats allowed to recover after exposure to imidacloprid.[66] Similar to imidacloprid, chronic thiamethoxam exposure causes liver injury in mice, as indicated by elevated serum activities of AST and ALT.[285] In contrast, exposures to thiacloprid results in a significant decrease in AST and ALT levels in serum compared to control rats.[271]

6.4 Genotoxicity and carcinogenicity

Neonicotinoids are not mutagenic based on *in vitro* and *in vivo* studies performed as part of the registration process.[66] The Ames test generally reveals that neonicotinoids are not mutagenic;[66,286] however, a imidacloprid pesticide formulation induces G–C base pair mutations.[287] Neonicotinoids, tested either as pure insecticides or as the corresponding formulations, cause dose dependent DNA damage in human peripheral blood lymphocytes (PBLs),[288,289] human lymphocytes,[290] and HepG2[286] cells, as assessed using the comet assay. Neonicotinoids also have a significant effect in the micronucleus assay in bone-marrow cells,[287,291,292] human lymphocytes,[289,293] and HepG2 cells,[286] whereas no induction of micronuclei was observed in other studies using polychromatic erythrocytes for imidacloprid-exposed mice[294] and human lymphocytes.[291] Some, but not all studies[291] report that neonicotinoids significantly induced chromosome aberrations[287,290,292,293,295,296] and sister chromatide exchanges.[293,295] Commercial formulations with neonicotinoids as active ingredients cause DNA damage in human peripheral blood lymphocytes *in vitro*.[288]

Carcinogenicity studies with imidacloprid[66] and clothianidin[273] showed no carcinogenic effects of exposure in rodents. In contrast, dietary exposure to thiamethoxam increased the incidence of liver tumors in mice,[285,297] but not in rats.[297,298] The mode of action that results in tumor formation in mice is not relevant to rats and humans, indicating that thiamethoxam does not pose a cancer risk in humans.[297] Based upon available research, acetamiprid, clothianidin, dinotefuran, imidacloprid, and thiamethoxam are classified as "Not Likely to be Carcinogenic to Humans" by the EPA.[28–32]

6.5 Neurotoxicity

In acute neurotoxicity studies performed according to EPA guidelines, no persistent or delayed neurotoxic effects were observed.[66,299] Tremors occurred in mice exposed to neonicotinoids.[59] The results from subchronic studies performed following EPA guidelines indicate that sustained dietary exposure to neonicotinoids does not produce the principal toxicities observed in acute studies and causes little-to-no neurotoxicity. These findings are consistent with the rapid elimination of neonicotinoids in rats,[67–69] their poor penetration across the blood–brain barrier,[60,66] and their poor affinity for non-insect nAChRs.[58–64] Some studies, however, indicate that imidacloprid and thiamethoxam affect motor activity in rats.[48,269,300] Thiamethoxam appears to cause an anxiogenic effect in rats[48] and clothianidin elevates anxiety-like behavior in mice.[301]

A critical review of *in vitro*, *in vivo*, and epidemiology studies by Sheets *et al.* in 2016 [302] found systemic toxicity was common at high doses; however, no developmental neurotoxicity was observed consistent with the effects of nicotine exposure. A few studies indicate that exposure to neonicotinoids may cause developmental neurotoxicity in rodents. Gestational exposure to imidacloprid results in sensorimotor deficits in the offspring.[35] Developmental exposure to clothianidin adversely affected neurobehavioral parameters in mice[273] and cognitive function in rats.[303] Low-concentrations (>1 μM) of acetamiprid and imidacloprid exerted similar excitatory effects on neonatal rat cerebellar neurons.[304] Other studies in rats observed decreased sensorimotor performance following imidacloprid exposure, increased dopamine release following thiamethoxam and clothianidin administration, and altered behavioral and biochemical processes related to the rat cholinergic systems following thiamethoxam exposure.[35,48,305] Because these studies did not follow the recommendations for rigor and reproducibility outlined by Landis *et al.*,[306] their findings need to be interpreted with caution.

6.6 Endocrine effects

In vitro testing has characterized the effects of neonicotinoids on endocrine disruption.[307] Thiacloprid, thiamethoxam, and imidacloprid affect aromatase (CYP19) activity in a model of fetoplacental steroidogenesis. Increases in estrone and estradiol production were observed, while estriol production was inhibited. Estrogens are important during pregnancy, and

disruption of the biosynthesis of estrogens may affect the fetus along with potential effects to the mother's health. Evidence indicates that neonicotinoids may be metabolized by CYP3A7, which affects the conversion of dehydroepiandrosterone sulfate into estriol and this may be the reason for the observed decrease in estriol production. Other studies have also demonstrated that thiacloprid and imidacloprid may affect aromatase activity through promoters for aromatase, which may lead to excess estrogen production in breast tissue.[308–310] This finding is of concern because an increased estrogen production in tumors has been shown to promote cancer cell growth, with aromatase being a key regulator of this process.[311,312]

6.7 Metabolite toxicity

Neonicotinoid metabolites can be more toxic than the parent compounds.[253,313,314] In particular, metabolites formed by the removal of the nitro- or cyano-functional groups are potentially more selective[260] and bind more strongly with mammalian nAChRs.[246] For example, desnitro-imidacloprid has an affinity for mammalian nAChRs that is comparable to nicotine.[19,22,59,261] The selectivity ratio for insects compared to vertebrates changes from 565 to 0.005 when the neonicotinoid is metabolized to the desnitro metabolite.[3,18] Studies with mice found that imidacloprid and desnitro-imidacloprid activate the extracellular signal-regulated kinase[135] cascade *via* the nicotinic receptor and induce the mobilization of intracellular calcium in rat PC12h cells similar to nicotine.[261,315] Desnitro-imidacloprid, a metabolite of imidacloprid, and a descyano and an olefin derivative of thiacloprid up-regulated α4β2 nAChR sites at EC_{50}s of 870 nM; 500 nM and 22 nM, respectively. In comparison, nicotine up-regulated α4β2 nAChR at EC50 s of 760 nM and imidacloprid and thiacloprid at EC50 s of 70 000 and 19 000 nM.[22]

6.8. Knowledge gaps and research needs

A considerable body of evidence, most as proprietary studies performed for regulatory purposes, indicate that neonicotinoid insecticides are safer than other insecticides currently on the market. In contrast, the peer-reviewed literature provides conflicting results with regards to toxic endpoints affected by neonicotinoid exposure. Several factors likely contribute to the conflicting findings, including different model systems, use of pure neonicotinoids *vs.* technical formulations, high concentrations or doses that do not reflect current environmental exposure levels, and lack of scientific rigor. The mammalian toxicity of neonicotinoids, especially at environmentally relevant concentrations, is most likely not mediated by nAChRs. While adverse outcome pathways (AOPs) have been proposed for honeybees,[316] AOPs for the mammalian toxicity of neonicotinoids and their bio-transformation products have not been established to date. Based on the evidence summarized above, there remains a need to further characterize the biological plausibility of adverse outcomes associated with environmental neonicotinoid exposures using robust, reproducible, and transparent studies.

7. Human exposure and health

In humans, documented adverse health effects from neonicotinoid exposure have generally been considered limited.[18] However, rigorous scientific studies examining the risk are lacking, demonstrating the need for additional human health-based research.[26] In 2013, the EFSA Panel on Plant Protection Products and their Residues stated that there is good evidence that two neonicotinoids, acetamiprid and imidacloprid, can damage the developing human nervous system, including the brain, and exhibit harmful effects similar to those caused by nicotine.[27,72] The insecticides may alter development of neurons and brain structures associated with functions such as learning and memory.[27,72] An important aspect of the PMRA and the EPA re-evaluation is the potential for effects in non-target organisms.[13,33]

7.1 Usage risks

Neonicotinoids are generally considered safe for public and occupational uses, with minimal human health risks due to dermal, inhalation, and oral exposure routes. Draft human health risk assessment published by the EPA in 2017 for acetamiprid, clothianidin, dinotefuran, imidacloprid, and thiamethoxam indicated that exposure occurs in both residential and occupational settings.[28–32,144] Overall, the assessment findings indicate that the human exposure risks associated with neonicotinoid use were limited.

One way to evaluate the human risk of exposure to neonicotinoids is to evaluate the margin of exposure, which is a ratio of its NOAEL to its theoretical, predicted, or estimated dose or concentration of human intake. The margins-of-exposure (MOE) for residential scenarios were generally greater than the EPA's level of concern. MOEs for the assessed scenarios ranged between 42 and 80 000 000 000.[28–32,144] A MOE greater or equal to 100, the level of concern, is considered protective of human health. The only residential scenario that found an estimate of concern below the MOE of 100 focused on children aged 1–2 years old exposed through a combined dermal and incidental oral routes to pets treated with pet collars containing imidacloprid.[31] The MOE for this scenario ranged from 42–110 and assumed that pet collars contained a 1% liquid/99% dust formulation for small and large dogs and cats. Children 1–2 years old exposed to indoor bed bug treatments and pet spot-on treatments with acetamiprid had margins of exposure of 120 and 110, respectively.[28] Post application non-occupational exposure to imidacloprid used for controlling burrowing shrimp in Washington State had the highest margins of exposure. Estimates ranged from 3300 (dermal) to 80 000 000 000 (inhalation) for children and adult exposed through these routes.[31] None of the examined scenarios for thiamethoxam, dinotefuran, or clothianidin resulted in risk estimates of concern.[29,30,32] Residential exposure scenarios were generally assumed to be short-term, with the exception of pet products, which due to their preventative nature are used longer term and presented longer-term potential exposure risks.[31]

The EPA evaluated several occupational scenarios, as part of their risk assessment, including direct mixers, loaders,

applicators, and other handlers for neonicotinoid exposure.[28–32,144] Most of these risk assessment scenarios assumed handlers used a baseline layer of protection including long-sleeved shirt, long pants, shoes plus socks, no protective gloves, and no respirator. Most of the combined dermal and inhalation risk estimates were not of concern, using baseline clothing, with total margins of exposure between 2 to 1 500 000. The highest MOEs were typically found for clothianidin, imidacloprid, and thiamethoxam in on-farm seed treatment and planting exposures (2–350).[29,31,32] Mixing, loading, applying dry flowable or wettable powders and liquid formulations using manual and pressurized handguns also had elevated MOEs for acetamiprid, imidacloprid, and thiamethoxam (3.8–3000).[28,31,32] The highest reported MOE of 1 500 000 was for mixing or loading acetamiprid tree injections.[28] With additional personal protective equipment, such as double layer of clothing and/or a respirator (PF5 or PF10), most of these exposure scenarios reach acceptable MOEs greater than 100. However, this extra protection was still insufficient in some scenarios where thiamethoxam and acetamiprid exposures occurred through seed treatments, planting, and dry flowable and wettable powders with handguns and backpacks.[28,32] Dinotefuran was not assessed for occupational handler exposures because no dermal or inhalation hazards were identified.[30]

7.2 Biomonitoring

Although it is unknown what the dominant routes of exposure to neonicotinoids are for humans, exposure *via* consumption (residue or in plant tissue), drinking water, or aerosols/dust associated with application are the most cited modes of exposure.[38,184,187,317–319] Over the past decade, several studies have been conducted to assess human exposure to neonicotinoids and their metabolites.[187,198,250,254,258,318–333] These studies indicate that neonicotinoid exposure is commonplace.

Studies indicate that neonicotinoids exposure may vary by geographic region and age. For example, in Japan, several studies assessed exposure to neonicotinoids. In 2013, low concentrations of 6-chloronicotinic acid, 2-chloro-1,3-thiazole-5-carboxylic acid, and 3-furoic acid were detected in urine from 10 farmers in Japan.[258] In 2016, neonicotinoid exposure was reported to be common amongst a cohort of 223 children aged 3 years old.[318] Urine samples collected during two seasons, summer and winter, showed detection rates of 58% for dinotefuran, 25% for thiamethoxam, 21% for nitenpyram, and <16% for other neonicotinoids (acetamiprid, clothianidin, imidacloprid, thiacloprid, and thiamethoxam). Higher concentrations in urine were detected in the summer compared to winter. The study also evaluated exposure to pyrethroid and organophosphate pesticides, finding exposure to all three classes. The detection rates for neonicotinoids and organophosphate and pyrethroid metabolites all were above 80%. Interestingly, this study found the detection of neonicotinoids to be higher in children exposed to organophosphate pesticides. The study was unable to confirm whether exposure was due to diet, household/agricultural application, or a combination of the two.[318] Similarly, a study investigating exposure to sprayed thiacloprid found that young children, aged 3–6 years old, were exposed to multiple neonicotinoids on a daily basis, with inhaled thiacloprid accounting for <1% of the daily intake. Diet was believed to be the primary source of exposure.[330]

A study of 373 Japanese adults, found exposure to clothianidin (96.5%), dinotefuran (93.3%), imidacloprid (76.7%) thiamethoxam (92.0%) and desmethyl-acetamiprid (100%) were common.[198] Positive correlations were reported between clothianidin, desmethyl-acetamiprid, dinotefuran, and imidacloprid concentrations and fruit intake; dinotefuran and imidacloprid with vegetable intake; and the dinotefuran concentration with cereal intake.[198] Clothianidin, dinotefuran, and imidacloprid concentrations were also associated with drinking or smoking.[198] Two other studies conducted in 2014 and 2015 also found neonicotinoid exposure in Japanese adults.[187,319] The first study evaluated exposure in 52 adults that reported no occupational exposure to neonicotinoids.[187,319] The detection rate was >96% for four neonicotinoids–dinotefuran (100%), thiamethoxam (100%), clothianidin (96%), imidacloprid (96%), acetamiprid (>50%), thiacloprid (>50%), and nitenpyram (29%). The researchers suggested that this exposure came mainly from diet and drinking water.[319] The second study examined exposure among 95 women in Kyoto, Japan and surrounding areas between the ages of 45–75 from 1994–2011.[187] Like the other studies, this research found that exposure was commonplace and increased with time. Detection rates increased from the mid-1990s to 2011, with few detections in the 1990s and detection rates between >5% and >70% for seven different neonicotinoids in 2010 and 2011. Thiamethoxam and dinotefuran were the most common neonicotinoids found with detection rates above 70%. The geometric mean for total urinary neonicotinoids also increased over this time period with values increasing from 0.05 nmol g^{-1} creatinine in 1994 to 12.83 nmol g^{-1} creatinine in 2011.[187]

In the United States, more recent studies using National Health and Nutrition Examination Survey (NHANES) data reported exposure to neonicotinoids. In 2015–2016 NHANES found that nearly half of the United States' population 3 years of age and older may be exposed to neonicotinoids. Out of 3038 total samples, researchers commonly detected two metabolites *N*-desmethyl-acetamiprid (35%) and 5-hydroxy imidacloprid (20%), followed by clothianidin (8%), and imidacloprid (4%). Acetamiprid and thiacloprid were detected in <0.5% samples. When compared to other age ranges and ethnicities young children (3–5 years old) and Asians had higher exposures to the two metabolites. The cause of the reported differences between age and ethnic groups was not known.[331] A 2017 NHANES study conducted in Atlanta, Georgia, USA, detected two metabolites — *N*-desmethyl-acetamiprid (90%) and 5-hydroxy-imidacloprid (42%) — and three neonicotinoids: clothianidin (37%), imidacloprid (30%), and acetamiprid (2%).[328] The study was conducted among anonymous male and female donors with no documented exposure to neonicotinoids.[328] In a 2019 analytical methods development paper, researchers detected imidaclothiz, a neonicotinoid manufactured and registered for use in China, in 100% of 20 spot urine samples collected from healthy adults in Albany, New York.[329] *N*-desmethyl acetamiprid (90%),

6-chloronicotinic acid (90%), clothianidin (85%), imidacloprid (70%), and thiamethoxam (55%) were also commonly detected.

Studies have also indicated that people working on or living near farms that use neonicotinoids have high exposure to these compounds. A 2007 cross-sectional study comparing 25 non-spraying control farmers and 89 pesticide sprayers who used neonicotinoids in southeastern Spain reported a suggestive relation between neonicotinoid application and lung dysfunction.[334] The lung dysfunctions included lower total lung capacity, residual volume, and functional residual capacity.[334] Exposed farmers were also more likely to report lung irritation. Regression analyses were used to compare relations between sociodemographic factors, occupational exposure, and clinical symptoms. These analyses showed a relation between short-term exposure (>25% drop in serum cholinesterase from baseline levels) with reduced forced expired volume, and long-term exposure with reduced forced expiratory flow rate. Long-term exposure was estimated based upon a lifelong cumulative index for each worker that multiplied the average number of hours working with pesticides on a weekly basis by the average number of weeks per year, and by the lifetime number of years working with pesticides.[334] In another Spanish study from 2017, urine samples from a cohort of 36 pregnant women living in agricultural areas contained imidacloprid, acetamiprid and acetamiprid-*n*-desmethyl at concentrations between 0.2 and 1.6 μg L^{-1}.[326] Dinotefuran was detected at trace levels and acetamiprid-*n*-desmethyl was the compound most widely detected. In total, at least one of these compounds was found in 16% of subjects.[326]

Additionally, evidence that proximity to farms increases exposure to neonicotinoids has also been highlighted by studies that compared humans living in urban *versus* rural landscapes. In China and Greece, neonicotinoid levels in urine have been compared between urban and rural subjects.[323,324,332] In general, neonicotinoid concentrations increased following insecticide application. In 2015, a Chinese study found frequently detected imidacloprid and 6-chloronicotinic acid in 295 spot urine samples from rural (100% and 32%, respectively) and urban (95% and 23%, respectively) subjects.[324] The geometric mean concentration of imidacloprid increased significantly among rural adults following pesticide spraying (pre-application: 0.18 ng mL^{-1} *vs.* post-application: 0.62 ng mL^{-1}).[324] In Henan Province, pre- and post-application spot urine sampling amongst 43 randomly selected neonicotinoid applicators showed that the urinary concentration of imidacloprid increased significantly following pesticide application.[332] Imidacloprid and 6-chloronicotinic acid were detected in 100% of 43 spot urine samples collected from pesticide applicators.[332] A three-fold increase in urine concentration was observed for both analytes following field application of imidacloprid.[332] In Greece, a study of imidacloprid exposure in urine and hair also compared concentrations between urban and rural populations.[323] The study found that rural residents engaged in agriculture were more likely (66%) than their urban counterparts (0%) to have a positive detection for imidacloprid in their hair. The median and maximum concentrations of imidacloprid in hair were 0.03 ng mg^{-1} and 27 ng mg^{-1}, respectively.[323]

7.3 Health effects

Recently, several epidemiological studies have highlighted concerns for human health, including the effects of acute poisonings and possible chronic effects. In humans, acute poisonings with neonicotinoids resulted in respiratory, cardiovascular, and neurological symptoms, including death.[335–350] Subacute intoxication from food consumption, specifically fruit, vegetables, and tea, has been documented in Japan.[255] Six patients that consumed greater than 500 g per day of either domestic fruits/vegetables and/or tea reported symptoms including finger tremor, impaired short-term memory, fever, general fatigue, headache, palpitation/chest pain, abdominal pain, muscle pain/muscle weakness/muscle spasm, and cough.[254,255] Similar results were found by Marfo *et al.*[250] who found an association between *N*-desmethyl-acetamiprid concentrations in urine and increased prevalence of neurologic symptoms among symptomatic patients *versus* 50-non-symptomatic volunteers {Odds ratio: 14, 95% Confidence Interval (CI): 3.5–57}. Symptoms included memory loss, finger tremor, headache, general fatigue, palpitation/chest pain, abdominal pain, muscle pain/weakness/spasm, and cough.[250] Other studies have reported a variety of respiratory, cardiovascular and neurological symptoms such as shortness of breath, coma, and irregular heart beat (slow and rapid), low blood pressure, and dilated pupils following an acute exposure.[337,339,341,346,347,351–356] Several case reports have also detailed fatalities due to acute exposure, although mortality is generally considered uncommon.[339,341,346,347,351–354] Studies reviewing incidents of acute neonicotinoid poisoning indicate that death occurred in less than 5% of cases.[337,355,356] Abnormal electrocardiograms post neonicotinoid exposure have also been observed.[254]

Researchers have also examined the potential health effects on workers who apply pesticides. Koureas *et al.* found that neonicotinoid application was related to the induction of oxidative damage to DNA in the whole blood of 80 pesticide sprayers.[357] Seasonal exposure to neonicotinoids {Risk ratio: 2.22 (95% CI: 1.07–4.63)} had greater effects on 8-OHdG levels.[357] In Sri Lanka, neonicotinoid use among rice paddy farmers was not associated with higher risk of chronic kidney disease.[325] As discussed above, neonicotinoid spraying has also been associated with impaired lung function.[334]

Links have also been found between maternal exposure to neonicotinoids during pregnancy and adverse birth outcomes. Associations between imidacloprid exposure and an increased risk for tetralogy of Fallot {Adjusted Odds Ratio (AOR) = 2.4, (95% CI: 1.1–5.1)}, a type of congenital heart defect, autism {AOR = 2.0, (95% CI 1.0–3.9)} and anencephaly in newborns {AOR = 2.9, (95% CI: 1.0–8.2)}.[335,358–360] Exposure was estimated in each of these studies through self-report on pesticide use or residential proximity to fields where neonicotinoids had been applied. A 2019 study assessing exposure of very low birth weight infants with gestational age between 23–34 weeks, 48 hours following delivery, found a significant association between birth weight and exposure to one metabolite, *N*-

desmethyl-acetamiprid.[361] Low birth-weight infants weighing below the 10th percentile for gestational age had a higher detection rate {43% *vs.* 15%, $p < 0.05$} and higher mean concentration {0.04 *vs.* 0.02 ng g^{-1}, $p < 0.05$}, compared to infants with an appropriate gestational age (10th–90th percentile for gestational age).[361]

7.4 Knowledge gaps and research needs

Although human exposure to neonicotinoids has been observed to occur through diet and occupational exposure, there are insufficient data to definitively link neonicotinoids with potential health risks. While generally considered to be safe for humans at low concentrations, the limited research that has been conducted appears to indicate long-term potential for genotoxicity, cytotoxicity, impaired immune function and reproduction, and birth defects; and acute health effects ranging from respiratory, cardiovascular and neurological symptoms. Published epidemiological studies primarily used ecological or cross-sectional designs. These designs are limited because they are unable to assess temporal relations between exposure and outcome. In addition, exposure was primarily estimated by either using proximity to application sites as a proxy. Biomonitoring would be necessary to confirm exposure has taken place. Scientifically rigorous epidemiological studies have not been conducted to examine potential adverse health risks; case-control and cohort studies are needed to examine potential adverse health outcome related to chronic neonicotinoid exposure.

8. Conclusions

Despite widespread use of neonicotinoid insecticides, there have been relatively few published studies addressing the risks of human exposure, and some critical gaps remain in our understanding of their environmental fate and occurrence necessary to best characterize exposure. Future research studies could include the following priorities:

8.1 Use

Research has found effects when neonicotinoids are applied in combination with pyrethroids and fungicides. A combination of neonicotinoids and pyrethroids was found to lead to impaired foraging, increased worker mortality, and increased colony failure, as well as a significant enhancement in the toxicity of thiacloprid to honeybees.[191,362] Only one study was found to have assessed co-exposure to neonicotinoids and other pesticides,[318] and no studies described the potential health risks of these combinations. It is important that mixture studies consider the risks of exposure to neonicotinoids combined with other contaminants including pesticides, co-formulants, inert ingredients, fertilizers, metals, and pharmaceuticals that are used in parallel or may co-occur in the environment.[4]

Research is needed on approaches for minimizing exposure to neonicotinoids, their known metabolites, and other breakdown products. For the control of neonicotinoids in water, this includes identification of best practices that can be deployed at the edge of field, as well as engineered treatment systems for use in centralized community water systems and in home (*e.g.*, point of use).

Risks associated with occupational exposure to neonicotinoids also warrant further study. Limited research has been published that assess human exposure risks from dust. Exposure scenarios evaluated by the EPA concluded that even with additional protection, such as PF5 or PF10 respirators, exposures to neonicotinoids from seed treatments, planting, and dry flowable and wettable powders still exceeded the MOEs.[28–32,144]

8.2 Transformation products

Breakdown products for neonicotinoids are potentially more toxic than the parent compounds, but their concentrations are rarely measured complicating risk assessment.[49,246,253,313,314] Assessment of these compounds is complicated by the fact that many transformation products are not available commercially as standards for analytical analyses, further limiting the ability to effectively confirm their occurrence and measure their abundance and concentration in the environment. More research is needed to identify novel transformation products of neonicotinoids and their known metabolites and the processes responsible for their formation. Improved understanding of their toxicology and health risks would complement improved understanding of their formation. In assessing formation of breakdown products, there remains a need to consider biotransformation products, as well as transformation products generated during environmental processing and engineered treatment (*e.g.*, chemical disinfection and chemical oxidation processes in drinking water treatment).

8.3 Human exposure and health effects

Human exposure to neonicotinoids has been shown to occur through different routes, including ingestion (*e.g.*, water, food), inhalation (*e.g.*, dust), and dermal exposure, and during occupational and residential use. Although these exposures appear to take place at very low concentrations, it is currently unknown which route serves as the primary exposure pathway to humans or whether major exposure routes vary across different neonicotinoid species. While neonicotinoid analyses of food and water indicate that exposure may take place simultaneously through multiple exposure routes (*e.g.*, ingestion, inhalation, dermal contact), further studies are needed to provide a detailed evaluation of the relative contribution of neonicotinoid exposure from multiple exposure pathways.[28–32,38,118,134,144–148,167–197]

In order to evaluate the relative contribution of neonicotinoid exposure from multiple exposure pathways, the magnitude and variability of neonicotinoids in dietary sources needs to be assessed. For example, research is needed to understand the actual potential for neonicotinoid exposure and the various factors (*e.g.*, land use, private *versus* public water supply) that affect the occurrence and temporal variability of neonicotinoids in tap water. While reported concentrations in food and juice appear to be below currently acceptable daily intake levels, numerous surveys have documented that chronic neonicotinoid exposure may occur regularly through these

routes.[38,145,167–197] Several studies have measured the concentrations of these insecticides in foods, but few attempts have been made to estimate daily intake.[38,145,172,174,176,183,184,199,317,363] No case-control or cohort studies have been conducted that include an estimate of the retrospective intake of neonicotinoids *via* food. In addition, EPA exposure estimates indicate that the greatest acute and chronic exposures may occur amongst young children. More studies are needed to assess exposure at different stages of life and to identify factors that may influence risk.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the authors or the U.S. Government. This work was supported by University of Iowa's Center for Health Effects of Environmental Contamination, Iowa Institute of Public Health Research and Policy, NIOSH funded Heartland Center for Occupational Health and Safety (Training Grant No. T42OH008491), NIEHS funded Environmental Health Sciences Research Center (Grant No. P30 ES005605), the State Hygienic Laboratory at the University of Iowa, and the U.S. Geological Survey Toxics Substances Hydrology Program. Gregory H. LeFevre and David M. Cwiertny were supported by a grant from the National Science Foundation (CBET Environmental Engineering 1803197). The authors would also like to acknowledge the contributions of members of the University of Iowa's Neonicotinoid Collaboratory.

Notes and references

1 P. Jeschke, R. Nauen, M. Schindler and A. Elbert, Overview of the status and global strategy for neonicotinoids, *J. Agr. Food Chem.*, 2010, **59**, 2897–2908.
2 C. Myers and E. Hill, *Benefits of Neonicotinoid Seed Treatments to Soybean Production*, U.S. Environmental Protection Agency, 2014.
3 M. Tomizawa and J. E. Casida, Neonicotinoid insecticide toxicology: mechanisms of selective action, *Annu. Rev. Pharmacol. Toxicol.*, 2005, **45**, 247–268.
4 M. L. Hladik, A. R. Main and D. Goulson, Environmental risks and challenges associated with neonicotinoid insecticides, *Environ. Sci. Technol.*, 2018, 3329–3335.
5 P. Jeschke and R. Nauen, Neonicotinoids—from zero to hero in insecticide chemistry, *Pest Manage. Sci.*, 2008, **64**, 1084–1098.
6 T. Shivanandappa and Y. Rajashekar, in *Advances in plant biopesticides*, Springer, 2014, pp. 323–345.
7 I. Yamamoto and J. E. Casida, in *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*, ed. J. C. Y. Izuru, Springer-Verlag, Tokyo, 1999, pp. 3–27.
8 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 and M. Wiemers, Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites, *Environ. Sci. Pollut. Res.*, 2015, **22**, 5–34.
9 H. Uneme, Chemistry of Clothianidin and related compounds, *J. Agric. Food Chem.*, 2011, **59**, 2932–2937.
10 D. Cappaert, D. G. McCullough, T. M. Poland and N. W. Siegert, Emerald ash borer in North America: a research and regulatory challenge, *Am. Entomol.*, 2005, **51**, 152–165.
11 J. Struger and T. Fletcher, Occurrence of lawn care and agricultural pesticides in the Don River and Humber River watersheds (1998–2002), *J. Great Lakes Res.*, 2007, **33**, 887–905.
12 J. D. McCurdy, D. W. Held, J. M. Gunn and T. C. Barickman, Dew from Warm-Season Turfgrasses as a Possible Route for Pollinator Exposure to Lawn-Applied Imidacloprid, *Crop, Forage Turfgrass Manage.*, 2017, **3**, 1.
13 J. C. Anderson, C. P. Dubetz and V. P. Palace, Neonicotinoids in the Canadian Aquatic Environment: A Literature Review on Current use of products with a focus on fate, exposure and biological effects, *Sci. Total Environ.*, 2015, **1**, 409–422.
14 F. M. Fishel, *Pesticide Toxicity Profile: Neonicotinoid Pesticides*, http://edis.ifas.ufl.edu/pdffiles/PI/PI11700.pdf, accessed October 30, 2016.
15 F. M. Fishel, *Pesticide toxicity profile: carbamate pesticides*, http://edis.ifas.ufl.edu/pdffiles/PI/PI11700.pdf, accessed October 30, 2016.
16 F. M. Fishel, *Pesticide Toxicity Profile: Organophosphate Pesticides*, http://edis.ifas.ufl.edu/pi087, accessed October 30, 2016.
17 K. A. Lewis, J. Tzilivakis, D. J. Warner and A. Green, An international database for pesticide risk assessments and management, *Hum. Ecol. Risk Assess.*, 2016, **22**, 1050–1064.
18 M. Tomizawa and J. E. Casida, Selective toxicity of neonicotinoids attributable to specificity to insect and mammalian nicotinic receptors, *Annu. Rev. Entomol.*, 2003, **48**, 339–364.
19 M. Tomizawa and J. E. Casida, Minor structural changes in nicotinoid insecticides confer differential subtype selectivity for mammalian nicotinic acetylcholine receptors, *Br. J. Pharmacol.*, 1999, **127**, 115–122.
20 S. J. Lansdell and N. S. Millar, The influence of nicotinic receptor subunit composition upon agonist, alpha-bungarotoxin and insecticide (imidacloprid) binding affinity, *Neuropharmacology*, 2000, **39**, 671–679.
21 Y. Okazawa, Y. Nakagawa, M. Akamatsu, T. Ueno and S. Nishimura, Comparison of the binding activities of chloronicotinyl insecticides toward the nicotinic acetylcholine receptors from rats and houseflies, *J. Pestic. Sci.*, 2000, **25**, 40–43.
22 M. Tomizawa and J. E. Casida, Imidacloprid, thiacloprid, and their imine derivatives up-regulate the alpha 4 beta 2

nicotinic acetylcholine receptor in M10 cells, *Toxicol. Appl. Pharmacol.*, 2000, **169**, 114–120.

23 T. Hurley and P. Mitchell, Value of neonicotinoid seed treatments to US soybean farmers, *Pest Manage. Sci.*, 2017, **73**, 102–112.

24 U.S. Department of the Interior and U.S. Geological Survey, *Pesticide National Synthesis Project, Estimated Annual Agricultural Pesticide Use*, https://water.usgs.gov/nawqa/pnsp/usage/maps/index.php.

25 N. Seltenrich, Catching Up with Popular Pesticides: More Human Health Studies Are Needed on Neonicotinoids, *Environ. Health Perspect.*, 2017, **125**, A41.

26 A. M. Cimino, A. L. Boyles, K. A. Thayer and M. J. Perry, Effects of neonicotinoid pesticide exposure on human health: a systematic review, *Environ. Health Perspect.*, 2016, **125**, 155–162.

27 EFSA Panel on Plant Protection Products and their Residues (PPR), Scientific Opinion on the developmental neurotoxicity potential of acetamiprid and imidacloprid, *EFSA J.*, 2013, **11**(12), 3471.

28 U.S. Environmental Protection Agency, Acetamiprid, Draft Human Health Risk Assessment for Registration Review Journal, 2017.

29 U.S. Environmental Protection Agency, *Clothianidin: Draft Human Health Risk Assessment in Support of Registration*, https://www.regulations.gov/docket?D=EPA-HQ-OPP-2011-0865.

30 U.S. Environmental Protection Agency, *Dinotefuran: Human Health Draft Risk Assessment for Registration Review.*, https://www.regulations.gov/docket?D=EPA-HQ-OPP-2011-0920.

31 U.S. Environmental Protection Agency, *Imidacloprid: Human Health Draft Risk Assessment for Registration Review*, https://www.regulations.gov/docket?D=EPA-HQ-OPP-2008-0844.

32 U.S. Environmental Protection Agency, *Thiamethoxam: Draft Human Health Risk Assessment for Registration Review*, https://www.regulations.gov/docket?D=EPA-HQ-OPP-2011-0581.

33 U.S. Environmental Protection Agency, *Pollinator Protection: Schedule for Review of Neonicotinoid Pesticides*, https://www.epa.gov/pollinator-protection/schedule-review-neonicotinoid-pesticides.

34 C. Bass, I. Denholm, M. S. Williamson and R. Nauen, The global status of insect resistance to neonicotinoid insecticides, *Pestic. Biochem. Physiol.*, 2015, **121**, 78–87.

35 M. B. Abou-Donia, L. B. Goldstein, S. Bullman, T. Tu, W. A. Khan, A. M. Dechkovskaia and A. A. Abdel-Rahman, 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*, 2008, **71**, 119–130.

36 J. P. Van der Sluijs, N. Simon-Delso, D. Goulson, L. Maxim, J.-M. Bonmatin and L. P. Belzunces, Neonicotinoids, bee disorders and the sustainability of pollinator services, *Curr. Opin. Env. Sust.*, 2013, **5**, 293–305.

37 K. Matsuda, S. D. Buckingham, D. Kleier, J. J. Rauh, M. Grauso and D. B. Sattelle, Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors, *Trends Pharmacol. Sci.*, 2001, **22**, 573–580.

38 M. Chen, L. Tao, J. McLean and C. Lu, Quantitative analysis of neonicotinoid insecticide residues in foods: implication for dietary exposures, *J. Agric. Food Chem.*, 2014, **62**, 6082–6090.

39 R. Nauen, P. Jeschke, R. Velten, M. E. Beck, U. Ebbinghaus-Kintscher, W. Thielert, K. Wölfel, M. Haas, K. Kunz and G. Raupach, Flupyradifurone: a brief profile of a new butenolide insecticide, *Pest Manage. Sci.*, 2015, **71**, 850–862.

40 C. Giorio, A. Safer, F. Sánchez-Bayo, A. Tapparo, A. Lentola, V. Girolami, M. B. Van Lexmond and J.-M. Bonmatin, An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 1: new molecules, metabolism, fate, and transport, *Environ. Sci. Pollut. Res.*, 2017, 1–33.

41 M. Morita, T. Yoneda and N. Akiyoshi, Research and development of a novel insecticide, flonicamid, *J. Pestic. Sci.*, 2014, **39**, 179–180.

42 G. B. Watson, M. R. Loso, J. M. Babcock, J. M. Hasler, T. J. Letherer, C. D. Young, Y. Zhu, J. E. Casida and T. C. Sparks, Novel nicotinic action of the sulfoximine insecticide sulfoxaflor, *Insect Biochem. Mol. Biol.*, 2011, **41**, 432–439.

43 X. Shao, Z. Liu, X. Xu, Z. Li and X. Qian, Overall status of neonicotinoid insecticides in China: production, application and innovation, *J. Pestic. Sci.*, 2012, D12–D037.

44 U.S. Environmental Protection Agency, *Pesticide Chemical Search*, https://iaspub.epa.gov/apex/pesticides/f?p=chemicalsearch:1, accessed September 12, 2019.

45 European Commission, *EU – Pesticides database*, https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN, accessed September 9, 2019.

46 J. M. Bonmatin, C. Giorio, V. Girolami, D. Goulson, D. P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E. A. Mitchell, D. A. Noome, N. Simon-Delso and A. Tapparo, Environmental fate and exposure; neonicotinoids and fipronil, *Environ. Sci. Pollut. Res. Int.*, 2015, **22**, 35–67.

47 F. Sánchez-Bayo, The trouble with neonicotinoids, *Science*, 2014, **346**, 806–807.

48 K. J. A. Rodrigues, M. B. Santana, J. L. M. Do Nascimento, D. L. W. Picanco-Diniz, L. A. L. Maues, S. N. Santos, V. M. M. Ferreira, M. Alfonso, R. Duran and L. R. F. Faro, Behavioral and biochemical effects on neonicotinoid thiamethoxam on the cholinergic system in rats, *Ecotoxicol. Environ. Saf.*, 2010, **73**, 101–107.

49 K. A. Ford and J. E. Casida, Unique and common metabolites of thiamethoxam, clothianidin, and dinotefuran in mice, *Chem. Res. Toxicol.*, 2006, **19**, 1549–1556.

50 J. A. Gervais, B. Luukinen, K. Buhl and D. Stone, *Imidacloprid General Fact Sheet*, http://npic.orst.edu/factsheets/imidagen.html, accessed October 30, 2016.

51 E. D. Gundelfinger and R. Schulz, in *Handbook of Experimental Pharmacology. vol. 144: Neuronal Nicotinic Receptors*, Springer, Berlin, 2000, pp. 497–521.
52 K. Matsuda, S. Kanaoka, M. Akamatsu and D. B. Sattelle, Diverse Actions and Target-Ste Selectivity of Neonicotinoids: Structural Insights, *Mol. Pharmacol.*, 2009, **76**, 1–10.
53 S. P. Arneric, M. Holladay and M. Williams, Neuronal nicotinic receptors: A perspective on two decades of drug discovery research, *Biochem. Pharmacol.*, 2007, **74**, 1092–1101.
54 J. A. Dani and D. Bertrand, Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system, *Annu. Rev. Pharmacol. Toxicol.*, 2007, **47**, 699–729.
55 E. D. Levin and A. H. Rezvani, Nicotinic interactions with antipsychotic drugs, models of schizophrenia and impacts on cognitive function, *Biochem. Pharmacol.*, 2007, **74**, 1182–1191.
56 J.-P. Changeux and A. Tally, Nicotinic receptors, allosteric proteins and medicine, *Trends Mol. Med.*, 2008, **14**, 93–102.
57 U.S. Environmental Protection Agency, *Thiacloprid - Notice of Registration Review Case Closure*, https://www.regulations.gov/document?D=EPA-HQ-OPP-2012-0218-0018.
58 M.-Y. Liu, J. Lanford and J. E. Casida, Relevance of [3H] Imidacloprid Binding Site in House Fly Head Acetylcholine Receptor to Insecticidal Activity of 2-Nitromethylene- and 2-Nitroimino-imidazolidines, *Pestic. Biochem. Physiol.*, 1993, **46**, 200–206.
59 S. L. Chao and J. E. Casida, Interaction of imidacloprid metabolites and analogs with the nicotinic acetylcholine receptor of mouse brain in relation to toxicity, *Pestic. Biochem. Physiol.*, 1997, **58**, 77–88.
60 I. Yamamoto, G. Yabuta, M. Tomizawa, T. Saito, T. Miyamoto and S. Kagabu, Molecular Mechanism for Selective Toxicity of Nicotinoids and Neonicotinoids, *J. Pestic. Sci.*, 1995, **20**, 33–40.
61 R. Zwart, M. Oortgiesen and H. P. M. Vijverberg, The nitromethylene heterocycle 1-(pyridin-3-yl-methyl)-2-nitromethylene-imidazolidine distinguishes mammalian from insect nicotinic receptor subtypes, *Eur. J. Pharmacol.*, 1992, **228**, 165–169.
62 R. Zwart, M. Oortgiesen and H. P. M. Vijverberg, Nitromethylene Heterocycles: Selective Agonists of Nicotinic Receptors in Locust Neurons Compared to Mouse N1E-115 and BC3H1 Cells, *Pestic. Biochem. Physiol.*, 1994, **48**, 202–213.
63 I. Yamamoto, M. Tomizawa, T. Saito, T. Miyamoto, E. C. Walcott and K. Sumikawa, Structural factors contributing to insecticidal and selective actions of neonicotinoids, *Arch. Insect Biochem. Physiol.*, 1998, **37**, 24–32.
64 K. Matsuda, S. D. Buckingham, J. C. Freeman, M. D. Squire, H. A. Baylis and D. B. Sattelle, Effects of the alpha subunit on imidacloprid sensitivity of recombinant nicotinic acetylcholine receptors, *Br. J. Pharmacol.*, 1998, **123**, 518–524.
65 R. Bal, S. Erdogan, G. Theophilidis, G. Baydas and M. Naziroglu, Assessing the effects of the neonicotinoid insecticide imidacloprid in the cholinergic synapses of the stellate cells of the mouse cochlear nucleus using whole-cell patch-clamp recording, *Neurotoxicology*, 2010, **31**, 113–120.
66 L. P. Sheets, *Imidacloprid: A Neonicotinoid Insecticide in Hayes' Handbook of Pesticide Toxicology*, Journal, 3rd edn, 2010, pp. 2055–2064.
67 J. Thyssen and L. Machemer, in *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor*, Springer, 1999, pp. 213–222.
68 A. Akayama and I. Minamida, in *Nicotinoid insecticides and the nicotinic acetylcholine receptor*, Springer, Tokyo, 1999, pp. 127–148.
69 I. Yamamoto and J. E. Casida, *Nicotinoid insecticides and the nicotinic acetylcholine receptor*, 1999.
70 B. C. Gerwick and T. C. Sparks, Natural products for pest control: an analysis of their role, value and future, *Pest Manage. Sci.*, 2014, **70**, 1169–1185.
71 C. M. Benbrook, Trends in glyphosate herbicide use in the United States and globally, *Environ. Sci. Eur.*, 2016, **28**, 3.
72 European Food Safety Authority, Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid, *EFSA J.*, 2013, **11**, 3068–3055.
73 M. R. Douglas and J. F. Tooker, Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops, *Environ. Sci. Technol.*, 2015, **49**, 5088–5097.
74 J. F. Tooker, M. R. Douglas and C. H. Krupke, Neonicotinoid seed treatments: limitations and compatibility with integrated pest management, *Agric. Environ. Lett.*, 2017, **2**(1), DOI: 10.2134/ael2017.08.0026.
75 National Pesticide Information Center and Oregon State University, *NPIC Product Research Online*, http://npic.orst.edu/NPRO/, accessed September 4, 2019.
76 D. Herms, D. McCullough, C. Clifford, D. Smitley, F. Miller and W. Cranshaw, *Insecticide options for protecting ash trees from emerald ash borer*, 3rd edn, http://www.emeraldashborer.info/documents/Multistate_EAB_Insecticide_Fact_Sheet.pdf.
77 A. Alford and C. H. Krupke, Translocation of the neonicotinoid seed treatment clothianidin in maize, *PLoS One*, 2017, **12**, e0173836.
78 CISION PR Newswire, *Global Seed Treatment Industry*, https://www.prnewswire.com/news-releases/global-seed-treatment-industry-300896380.html, accessed February 13, 2020.
79 B. Haire, *Are seed treatments worth the investment?*, http://southeastfarmpress.com/soybeans/are-seed-treatments-worth-investment.
80 M. R. Douglas and J. F. Tooker, Large scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest

management in US field crops, *Environ. Sci. Technol.*, 2015, **49**, 5088–5097.
81 C. D. Osteen and J. Fernandez-Cornejo, Economic and policy issues of US agricultural pesticide use trends, *Pest Manage. Sci.*, 2013, **69**, 1001–1025.
82 M. Henry, M. Beguin, F. Requier, O. Rollin, J.-F. Odoux, P. Aupinel, J. Aptel, S. Tchamitchian and A. Decourtye, A common pesticide decreases foraging success and survival in honey bees, *Science*, 2012, **336**, 348–350.
83 European Food Safety Authority, *Bee Health: EU takes additional measures on pesticides to better protect Europe's bees*, http://europa.eu/rapid/press-release_IP-13-708_en.htm.
84 European Commission, *Neonicotinoids: Current status of the neonicotinoids in the EU*, https://ec.europa.eu/food/plant/pesticides/approval_active_substances/approval_renewal/neonicotinoids_en.
85 Pest Management Regulatory Agency, *Re-evaluation Decision RVD2019-04, Thiamethoxam and Its Associated End-use Products: Pollinator Re-evaluation*, https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/decisions-updates/reevaluation-decision/2019/thiamethoxam.html.
86 Pest Management Regulatory Agency, *Re-evaluation Decision RVD2019-05, Clothianidin and Its Associated End-use Products: Pollinator Re-evaluation*, https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/decisions-updates/reevaluation-decision/2019/clothianidin.html.
87 Pest Management Regulatory Agency, *Re-evaluation Decision RVD2019-06, Imidacloprid and Its Associated End-use Products: Pollinator Re-evaluation*, https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/decisions-updates/reevaluation-decision/2019/imidacloprid.html.
88 Health Canada, *Health Canada to consult on plan to phase out most uses of the neonicotinoids clothianidin and thiamethoxam*, https://www.canada.ca/en/health-canada/news/2018/08/health-canada-to-consult-on-plan-to-phase-out-most-uses-of-the-neonicotinoids-clothianidin-and-thiamethoxam.html.
89 U.S. Environmental Protection Agency, *Product Cancellation Order for Certain Pesticide Registrations*, Fed. Regist., May 20, 2019.
90 National Caucus of Environmental Legislators, *2019 Neonicotinoid Legislation*, https://www.quorum.us/spreadsheet/external/IcBmFYETcsZMXEBUXfaH/.
91 T. J. Wood and D. Goulson, The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013, *Environ. Sci. Pollut. Res.*, 2017, **24**, 17285–17325.
92 D. Goulson, Review: An overview of the environmental risks posed by neonicotinoid insecticides, *J. Appl. Ecol.*, 2013, **50**, 977–987.
93 C. A. Morrissey, P. Mineau, J. H. Devries, F. Sanchez-Bayo, M. Liess, M. C. Cavallaro and K. Liber, Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review, *Environ. Int.*, 2015, **74**, 291–303.
94 M. B. van Lexmond, J.-M. Bonmatin, D. Goulson and D. A. Noome, Worldwide integrated assessment on systemic pesticides, *Environ. Sci. Pollut. Res.*, 2015, **22**, 1–4.
95 European Food Safety Authority, Conclusion regarding the peer review of the pesticide risk assessment of the active substance imidacloprid, *EFSA J.*, 2008, **6**, 148r.
96 Canadian Council of Ministers of the Environment, *Canadian Water Quality Guidelines: Imidacloprid. Scientific Supporting Document*, http://www.ccme.ca/files/Resources/supporting_scientific_documents/imidacloprid_ssd_1388.pdf.
97 S. T. Kurwadkar, D. Dewinne, R. Wheat, D. G. McGahan and F. L. Mitchell, Time dependent sorption behavior of dinotefuran, imidacloprid and thiamethoxam, *J. Environ. Sci. Health B*, 2013, **48**, 237–242.
98 A. Tapparo, D. Marton, C. Giorio, A. Zanella, L. Solda, M. Marzaro, L. Vivan and V. Girolami, 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.
99 T. C. Van Dijk, M. A. Van Staalduinen and J. P. Van der Sluijs, Macro-invertebrate decline in surface water polluted with imidacloprid, *PLoS One*, 2013, **8**, e62374.
100 D. Nuyttens and P. Verboven, Dust Emission from Pesticide Treated Seeds During Seed Drilling, *Outlooks Pest Manage.*, 2015, **26**, 215–219.
101 V. Girolami, M. Marzaro, L. Vivan, L. Mazzon, M. Greatti, C. Giorio, D. Marton and A. Tapparo, Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication, *J. Appl. Entomol.*, 2012, **136**, 17–26.
102 D. Pochi, M. Biocca, R. Fanigliulo, P. Gallo and P. Pulcini, Sowing of seed dressed with thiacloprid using a pneumatic drill modified for reducing abrasion dust emissions, *Bull. Insectology*, 2015, **68**, 273–279.
103 M. Biocca, E. Conte, P. Pulcini, E. Marinelli and D. Pochi, 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*, 2011, **46**, 438–448.
104 V. Girolami, M. Marzaro, L. Vivan, L. Mazzon, C. Giorio, D. Marton and A. Tapparo, Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers, *J. Appl. Entomol.*, 2013, **137**, 35–44.
105 L. G. Forero, V. Limay-Rios, Y. Xue and A. Schaafsma, Concentration and movement of neonicotinoids as particulate matter downwind during agricultural practices using air samplers in southwestern Ontario, Canada, *Chemosphere*, 2017, **188**, 130–138.

106 J. M. Bonmatin, I. Moineau, R. Charvet, M. E. Colin, C. Fleche and E. R. Bengsch, in *Environmental Chemistry*, Springer, 2005, pp. 483–494.
107 M. L. Hladik, S. Bradbury, L. A. Schulte, M. Helmers, C. Witte, D. W. Kolpin, J. D. Garrett and M. Harris, Neonicotinoid insecticide removal by prairie strips in row-cropped watersheds with historical seed coating use, *Agric., Ecosyst. Environ.*, 2017, **241**, 160–167.
108 C. de Perre, T. M. Murphy and M. J. Lydy, Fate and effects of clothianidin in fields using conservation practices, *Environ. Toxicol. Chem.*, 2015, **34**, 258–265.
109 A. Schaafsma, V. Limay-Rios, T. Baute, J. Smith and Y. Xue, Neonicotinoid Insecticide Residues in Surface Water and Soil Associated with Commercial Maize (Corn) Fields in Southwestern Ontario, *PLoS One*, 2015, **10**, e0118139.
110 T. Xu, D. G. Dyer, L. L. McConnell, S. Bondarenko, R. Allen and O. Heinemann, Clothianidin in agricultural soils and uptake into corn pollen and canola nectar after multiyear seed treatment applications, *Environ. Toxicol. Chem.*, 2016, **35**, 311–321.
111 M. L. Hladik and D. W. Kolpin, First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA, *Environ. Chem.*, 2015, **13**, 12–20.
112 A. Schaafsma, V. Limay-Rios, Y. Xue, J. Smith and T. Baute, Field-scale examination of neonicotinoid insecticide persistence in soil as a result of seed treatment use in commercial maize (corn) fields in southwestern Ontario, *Environ. Toxicol. Chem.*, 2016, **35**, 295–302.
113 European Commission, Draft assessment Report (DAR) – Public Version – Initial risk assessment provided by the rapporteur Member State Germany for the existing active substance imidacloprid of the Third Stage (Part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC, 2006.
114 M. Lamers, M. Anyusheva, N. La, V. V. Nguyen and T. Streck, Pesticide Pollution in Surface-and Groundwater by Paddy Rice Cultivation: A Case Study from Northern Vietnam, *Clean: Soil, Air, Water*, 2011, **39**, 356–361.
115 M. L. Hladik and D. L. Calhoun, *Analysis of the herbicide diuron, three diuron degradates, and six neonicotinoid insecticides in water-Method details and application to two Georgia streams*, report 2012-5206, Reston, VA, 2012.
116 M. L. Hladik, S. R. Corsi, D. W. Kolpin, A. K. Baldwin, B. R. Blackwell and J. E. Cavallin, Year-round presence of neonicotinoid insecticides in tributaries to the Great Lakes, USA, *Environ. Pollut.*, 2018, **235**, 1022–1029.
117 M. L. Hladik, D. W. Kolpin and K. M. Kuivila, Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA, *Environ. Pollut.*, 2014, **193**, 189–196.
118 K. L. Klarich, N. C. Pflug, E. M. DeWald, M. L. Hladik, D. W. Kolpin, D. M. Cwiertny and G. H. LeFevre, Occurrence of Neonicotinoid Insecticides in Finished Drinking Water and Fate during Drinking Water Treatment, *Environ. Sci. Technol. Lett.*, 2017, **4**, 168–173.
119 C. Zhang, D. Tian, X. Yi, T. Zhang, J. Ruan, R. Wu, C. Chen, M. Huang and G. Ying, Occurrence, distribution and seasonal variation of five neonicotinoid insecticides in surface water and sediment of the Pearl Rivers, South China, *Chemosphere*, 2019, **217**, 437–446.
120 B. Z. Bradford, A. S. Huseth and R. L. Groves, Widespread detections of neonicotinoid contaminants in central Wisconsin groundwater, *PLoS One*, 2018, **13**, e0201753.
121 F. Sanchez-Bayo and R. V. Hyne, Detection and analysis of neonicotinoids in river waters–development of a passive sampler for three commonly used insecticides, *Chemosphere*, 2014, **99**, 143–151.
122 K. Starner and K. S. Goh, 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.
123 J. Kreuger, S. Graaf, J. Patring and S. Adielsson, *Pesticides in surface water in areas with open ground and greenhouse horticultural crops in Sweden 2008*, 2010.
124 A. S. Huseth and R. L. Groves, Environmental Fate of Soil Applied Neonicotinoid Insecticides in an Irrigated Potato Agroecosystem, *PLoS One*, 2014, **9**, e97081.
125 H. Johnson, M. Juenemann, M. MacDonald, S. Matteson, J. Paddock, K. Rassumussen, M. Ribikawskis, B. Schaefer, D. Timm and D. Tollefson, *2016 Water Quality Monitoring Report: January–December 2016*, http://www.mda.state.mn.us/sites/default/files/inline-files/2016wqmrpt.pdf.
126 V. Guzsvány, J. Csanádi and F. Gaál, NMR study of the influence of pH on the persistence of some neonicotinoids in water, *Acta Chim. Slov.*, 2006, **53**, 52.
127 R. A. Mulligan, P. L. Tomco, M. W. Howard, T. T. Schempp, D. J. Stewart, P. M. Stacey, D. B. Ball and R. S. Tjeerdema, Aerobic versus anaerobic microbial degradation of clothianidin under simulated california rice field conditions, *J. Agric. Food Chem.*, 2016, **64**, 7059–7067.
128 Z. Liu, Y. Dai, G. Huang, Y. Gu, J. Ni, H. Wei and S. Yuan, 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 Manage. Sci.*, 2011, **67**, 1245–1252.
129 M. A. Sarkar, S. Roy, R. K. Kole and A. Chowdhury, Persistence and metabolism of imidacloprid in different soils of West Bengal, *Pest Manage. Sci.*, 2001, **57**, 598–602.
130 A. R. Main, J. V. Headley, K. M. Peru, N. L. Michel, A. J. Cessna and C. A. Morrissey, Widespread use and frequent detection of neonicotinoid insecticides in wetlands of Canada's Prairie Pothole Region, *PLoS One*, 2014, **9**, e92821.
131 C. H. Krupke, G. J. Hunt, B. D. Eitzer, G. Andino and K. Given, Multiple routes of pesticide exposure for honey bees living near agricultural fields, *PLoS One*, 2012, **7**, e29268.
132 D. Nuyttens, W. Devarrewaere, P. Verboven and D. Foqué, Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review, *Pest Manage. Sci.*, 2013, **69**, 564–575.

133 A. R. Main, N. L. Michel, M. C. Cavallaro, J. V. Headley, K. M. Peru and C. A. Morrissey, *Snowmelt transport of neonicotinoid insecticides to Canadian Prairie wetlands*, 2016.

134 Y. Wan, Y. Wang, W. Xia, Z. He and S. Xu, Neonicotinoids in raw, finished, and tap water from Wuhan, Central China: Assessment of human exposure potential, *Sci. Total Environ.*, 2019, **675**, 513–519.

135 J. Struger, J. Grabuski, S. Cagampan, E. Sverko, D. McGoldrick and C. H. Marvin, Factors influencing the occurrence and distribution of neonicotinoid insecticides in surface waters of southern Ontario, Canada, *Chemosphere*, 2017, **169**, 516–523.

136 Health Canada, *Guidelines for Canadian Drinking Water Quality – Summary Table*, https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/ewh-semt/alt_formats/pdf/pubs/water-eau/sum_guide-res_recom/sum_guide-res_recom-eng.pdf.

137 U.S. Environmental Protection Agency, *National Primary Drinking Water Regulations*, https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations.

138 European Parliament and Council, Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, *Off. J. Eur. Communities: Legis.*, 1998, **L330**, 32–54.

139 European Parliament and Council, Directive 2000/60/EC of the of 23 October 2000 establishing a framework for community action in the field of water policy, *Off. J. Eur. Communities: Legis.*, 2000, L327.

140 European Parliament and Council, Directive 2006/118/EC of 12 December 2006 on the protection of groundwater against pollution and deterioration, *Off. J. Eur. Communities: Legis.*, 2006, **372**, 19–31.

141 European Parliament and Council, Directive 2013/39/EU of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, *Off. J. Eur. Communities: Legis.*, 2013, L226.

142 European Parliament and Council, European Union Directive 2008/105/EC of the European Parliament and of the Council on environmental quality standards in the field of water policy, *Off. J. Eur. Communities: Legis.*, 2008, **348**, 84–97.

143 L. H. Nowell, P. W. Moran, T. S. Schmidt, J. E. Norman, N. Nakagaki, M. E. Shoda, B. J. Mahler, P. C. Van Metre, W. W. Stone and M. W. Sandstrom, Complex mixtures of dissolved pesticides show potential aquatic toxicity in a synoptic study of Midwestern US streams, *Sci. Total Environ.*, 2018, **613**, 1469–1488.

144 U.S. Food and Drug Administration, *Memorandum of Understanding Between The Environmental Protection Agency and The United States Department of Health*, Education and Welfare Food and Drug Administration, https://www.fda.gov/about-fda/domestic-mous/mou-225-73-8010.

145 H. A. Craddock, D. Huang, P. C. Turner, L. Quirós-Alcalá and D. C. Payne-Sturges, Trends in neonicotinoid pesticide residues in food and water in the United States, 1999–2015, *Environ. Health*, 2019, **18**, 7.

146 K. L. Klarich Wong, D. T. Webb, M. R. Nagorzanski, D. W. Kolpin, M. L. Hladik, D. M. Cwiertny and G. H. LeFevre, Chlorinated byproducts of neonicotinoids and their metabolites: An unrecognized human exposure potential?, *Environ. Sci. Technol. Lett.*, 2019, **6**, 98–105.

147 T. Sultana, C. Murray, S. Kleywegt and C. D. Metcalfe, Neonicotinoid pesticides in drinking water in agricultural regions of southern Ontario, Canada, *Chemosphere*, 2018, **202**, 506–513.

148 J. M. Montiel-León, S. V. Duy, G. Munoz, M. Amyot and S. Sauvé, Evaluation of on-line concentration coupled to liquid chromatography tandem mass spectrometry for the quantification of neonicotinoids and fipronil in surface water and tap water, *Anal. Bioanal. Chem.*, 2018, **410**, 2765–2779.

149 World Health Organization, *Drinking-water*, https://www.who.int/news-room/fact-sheets/detail/drinking-water.

150 C. A. Dieter, M. A. Maupin, R. R. Caldwell, M. A. Harris, T. Ivahnenko, J. K. Lovelace, N. L. Barber and K. S. Linsey, *Water Availability and Use Science Program: Estimated Use of Water in the United States In 2015*, U.S. Geological Survey Circular 1441, 2018.

151 U.S. Environmental Protection Agency, *Potential Well Water Contaminants and Their Impacts*, https://www.epa.gov/privatewells/potential-well-water-contaminants-and-their-impacts, accessed August 30, 2019.

152 O. Schmoll, G. Howard, J. Chilton and I. Chorus, *Protecting groundwater for health: managing the quality of drinking-water sources*, World Health Organization, 2006.

153 L. A. DeSimone and P. A. Hamilton, *Quality of water from domestic wells in principal aquifers of the United States, 1991-2004*, U.S. Geological Survey Circular 1332, 2009.

154 N. M. Gatto, M. Cockburn, J. Bronstein, A. D. Manthripragada and B. Ritz, Well-water consumption and Parkinson's disease in rural California, *Environ. Health Perspect.*, 2009, **117**, 1912–1918.

155 T. Yoshida, H. Yamauchi and G. F. Sun, Chronic health effects in people exposed to arsenic via the drinking water: dose–response relationships in review, *Toxicol. Appl. Pharmacol.*, 2004, **198**, 243–252.

156 J. D. Ayotte, D. Baris, K. P. Cantor, J. Colt, G. R. Robinson, J. H. Lubin, M. Karagas, R. N. Hoover, J. F. Fraumeni and D. T. Silverman, Bladder cancer mortality and private well use in New England: an ecological study, *Br. J. Prev. Soc. Med.*, 2006, **60**, 168–172.

157 M. Inoue-Choi, R. R. Jones, K. E. Anderson, K. P. Cantor, J. R. Cerhan, S. Krasner, K. Robien, P. J. Weyer and M. H. Ward, Nitrate and nitrite ingestion and risk of ovarian cancer among postmenopausal women in Iowa, *Int. J. Cancer*, 2015, **137**, 173–182.

158 A. P. Sanders, T. A. Desrosiers, J. L. Warren, A. H. Herring, D. Enright, A. F. Olshan, R. E. Meyer and R. C. Fry, Association between arsenic, cadmium, manganese, and lead levels in private wells and birth defects prevalence in North Carolina: a semi-ecologic study, *BMC Public Health*, 2014, **14**, 955.

159 L. C. Magalhaes, T. E. Hunt and B. D. Siegfried, Efficacy of neonicotinoid seed treatments to reduce soybean aphid populations under field and controlled conditions in Nebraska, *J. Econ. Entomol.*, 2009, **102**, 187–195.

160 K. A. Stoner and B. D. Eitzer, Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*), *PLoS One*, 2012, **7**, e39114.

161 J. Bonmatin, I. Moineau, R. Charvet, C. Fleche, M. Colin and E. Bengsch, A LC/APCI-MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens, *Anal. Chem.*, 2003, **75**, 2027–2033.

162 M.-P. Chauzat, J.-P. Faucon, A.-C. Martel, J. Lachaize, N. Cougoule and M. Aubert, A survey of pesticide residues in pollen loads collected by honey bees in France, *J. Econ. Entomol.*, 2006, **99**, 253–262.

163 V. Girolami, L. Mazzon, A. Squartini, N. Mori, M. Marzaro, A. Di Bernardo, M. Greatti, C. Giorio and A. Tapparo, 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.

164 J. C. Wise, R. Vanderpoppen and C. Vandervoort, Curative activity of insecticides on Rhagoletis pomonella (Diptera: Tephritidae) in apples, *J. Econ. Entomol.*, 2009, **102**, 1884–1890.

165 D. Mota-Sanchez, B. Cregg, E. Hoffmann, J. Flore and J. C. Wise, Penetrative and dislodgeable residue characteristics of 14C-insecticides in apple fruit, *J. Agric. Food Chem.*, 2012, **60**, 2958–2966.

166 E. J. Hoffmann, C. Vandervoort and J. C. Wise, Plum curculio (Coleoptera: Curculionidae) adult mortality and associated fruit injury after exposure to field-aged insecticides on tart cherry branches, *J. Econ. Entomol.*, 2010, **103**, 1196–1205.

167 S. K. Pramanik, J. Bhattacharyya, S. Dutta, P. K. Dey and A. Bhattacharyya, Persistence of Acetamiprid in/on Mustard (*Brassica juncea* L.), *Bull. Environ. Contam. Toxicol.*, 2006, **76**, 356–360.

168 D. Sanyal, D. Chakma and S. Alam, Persistence of a neonicotinoid insecticide, acetamiprid on chili (*Capsicum annum* L.), *Bull. Environ. Contam. Toxicol.*, 2008, **81**, 365–368.

169 P. Venkateswarlu, K. R. Mohan, C. R. Kumar and K. R. Seshaiah, Monitoring of multi-class pesticide residues in fresh grape samples using liquid chromatography with electrospray tandem mass spectrometry, *Food Chem.*, 2007, **105**, 1760–1766.

170 Y.-l. Yu, J.-l. Wu, M. Stahler and W. Pestemer, Residual dynamics of thiacloprid in medical herbs marjoram, thyme, and camomile in soil, *J. Environ. Sci.*, 2007, **19**, 205–209.

171 A. A. Romeh, T. M. Mekky, R. A. Ramadan and M. Y. Hendawi, Dissipation of profenofos, imidacloprid and penconazole in tomato fruits and products, *Bull. Environ. Contam. Toxicol.*, 2009, **83**, 812.

172 P. Wang, X. Yang, J. Wang, J. Cui, A. Dong, H. Zhao, L. Zhang, Z. Wang, R. Xu and W. Li, Multi-residue method for determination of seven neonicotinoid insecticides in grains using dispersive solid-phase extraction and dispersive liquid–liquid micro-extraction by high performance liquid chromatography, *Food Chem.*, 2012, **134**, 1691–1698.

173 S. Liu, Z. Zheng, F. Wei, Y. Ren, W. Gui, H. Wu and G. Zhu, Simultaneous determination of seven neonicotinoid pesticide residues in food by ultraperformance liquid chromatography tandem mass spectrometry, *J. Agric. Food Chem.*, 2010, **58**, 3271–3278.

174 U. Kapoor, M. Srivastava, A. K. Srivastava, D. Patel, V. Garg and L. Srivastava, 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.*, 2013, **32**, 723–727.

175 S. H. Abd-Alrahman, Residue and dissipation kinetics of thiamethoxam in a vegetable-field ecosystem using QuEChERS methodology combined with HPLC–DAD, *Food Chem.*, 2014, **159**, 1–4.

176 G. T. Bakırcı, D. B. Y. Acay, F. Bakırcı and S. Ötleş, Pesticide residues in fruits and vegetables from the Aegean region, Turkey, *Food Chem.*, 2014, **160**, 379–392.

177 G. C. Andrade, S. H. Monteiro, J. G. Francisco, L. A. Figueiredo, A. A. Rocha and V. L. Tornisielo, Effects of types of washing and peeling in relation to pesticide residues in tomatoes, *J. Braz. Chem. Soc.*, 2015, **26**, 1994–2002.

178 J. X. Huang, C. Y. Liu, D. H. Lu, J. J. Chen, Y. C. Deng and F. H. Wang, Residue behavior and risk assessment of mixed formulation of imidacloprid and chlorfenapyr in chieh-qua under field conditions, *Environ. Monit. Assess.*, 2015, **187**, 650.

179 G. M. Teló, E. Marchesan, R. Zanella, M. Limberger de Oliveira, L. L. Coelho and M. L. Martins, Residues of fungicides and insecticides in rice field, *Agron. J.*, 2015, **107**, 851–863.

180 A. K. Bhattacherjee and A. Dikshit, Dissipation kinetics and risk assessment of thiamethoxam and dimethoate in mango, *Environ. Monit. Assess.*, 2016, **188**, 165.

181 N. J. Balfour, N. L. Carreck, H. E. Blanchard and F. L. W. Ratnieks, Size matters: Significant negative relationship between mature plant mass and residual neonicotinoid levels in seed-treated oilseed rape and maize crops, *Agric., Ecosyst. Environ.*, 2016, **215**, 85–88.

182 Q. Fang, Y. Shi, H. Cao, Z. Tong, J. Xiao, M. Liao, X. Wu and R. Hua, Degradation dynamics and dietary risk assessments of two neonicotinoid insecticides during lonicera japonica planting, drying, and tea brewing processes, *J. Agric. Food Chem.*, 2017, **65**, 1483–1488.

183 O. Golge, F. Hepsag and B. Kabak, Health risk assessment of selected pesticide residues in green pepper and cucumber, *Food Chem. Toxicol.*, 2018, **121**, 51–64.

184 C. Lu, C.-H. Chang, C. Palmer, M. Zhao and Q. Zhang, Neonicotinoid residues in fruits and vegetables: An integrated dietary exposure assessment approach, *Environ. Sci. Technol.*, 2018, **52**, 3175–3184.

185 D. Zywitz, M. Anastassiades and E. Scherbaum, *Analysis of Neonicotinoid Insecticides in Fruits and Vegetables using LC-MS (MS)*, http://cvuas.xn–untersuchungsmter-bw-nzb.de/pdf/poster_neonicotinoid_insecticides_eprw.pdf.

186 U.S. Department of Agriculture, Pesticide Data Program, *Annual Summary Calendar Year 2015*, https://www.ams.usda.gov/datasets/pdp, accessed 09/28, 2018.

187 J. Ueyama, K. H. Harada, A. Koizumi, Y. Sugiura, T. Kondo, I. Saito and M. Kamijima, Temporal Levels of Urinary Neonicotinoid and Dialkylphosphate Concentrations in Japanese Women Between 1994 and 2011, *Environ. Sci. Technol.*, 2015, **49**, 14522–14528.

188 R. Juraske, F. Castells, A. Vijay, P. Muñoz and A. Antón, Uptake and persistence of pesticides in plants: measurements and model estimates for imidacloprid after foliar and soil application, *J. Hazard. Mater.*, 2009, **165**, 683–689.

189 A. Kamel, Refined Methodology for the Determination of Neonicotinoid Pesticides and Their Metabolites in Honey Bees and Bee Products by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), *J. Agric. Food Chem.*, 2010, **58**, 5926–5931.

190 E. A. D. Mitchell, B. Mulhauser, M. Mulot, A. Mutabazi, G. Glauser and A. Aebi, A worldwide survey of neonicotinoids in honey, *Science*, 2017, **358**, 109–111.

191 F. Sanchez-Bayo and K. Goka, Pesticide residues and bees-a risk assessment, *PLoS One*, 2014, **9**, e94482.

192 T. Blacquiere, G. Smagghe, C. A. M. van Gestel and V. Mommaerts, Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment, *Ecotoxicology*, 2012, **21**, 973–992.

193 N. Campillo, P. Vinas, G. Ferez-Melgarejo and M. Hernandez-Cordoba, Liquid chromatography with diode array detection and tandem mass spectrometry for the determination of neonicotinoid insecticides in honey samples using dispersive liquid-liquid microextraction, *J. Agric. Food Chem.*, 2013, **61**, 4799–4805.

194 M. Gbylik-Sikorska, T. Sniegocki and A. Posyniak, Determination of neonicotinoid insecticides and their metabolites in honey bee and honey by liquid chromatography tandem mass spectrometry, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2015, **990**, 132–140.

195 G. Tanner and C. Czerwenka, LC-MS/MS Analysis of Neonicotinoid Insecticides in Honey: Methodology and Residue Findings in Austrian Honeys, *J. Agric. Food Chem.*, 2011, **59**, 12271–12277.

196 A. Jones and G. Turnbull, Neonicotinoid concentrations in UK honey from 2013, *Pest Manage. Sci.*, 2016, **72**, 1897–1900.

197 G. Codling, Y. Al Naggar, J. P. Giesy and A. J. Robertson, Concentrations of neonicotinoid insecticides in honey, pollen and honey bees (Apis mellifera L.) in central Saskatchewan, Canada, *Chemosphere*, 2016, **144**, 2321–2328.

198 K. H. Harada, K. Tanaka, H. Sakamoto, M. Imanaka, T. Niisoe, T. Hitomi, H. Kobayashi, H. Okuda, S. Inoue, K. Kusakawa, M. Oshima, K. Watanabe, M. Yasojima, T. Takasuga and A. Koizumi, Biological Monitoring of Human Exposure to Neonicotinoids Using Urine Samples, and Neonicotinoid Excretion Kinetics, *PLoS One*, 2016, **11**, e0146335.

199 Q. Zhang, Z. Lu, C.-H. Chang, C. Yu, X. Wang and C. Lu, Dietary risk of neonicotinoid insecticides through fruit and vegetable consumption in school-age children, *Environ. Int.*, 2019, **126**, 672–681.

200 *New Zealand Food Safety, Pesticide maximum residue level legislation around the world*, https://www.mpi.govt.nz/growing-and-harvesting/plant-products/pesticide-maximum-residue-levels-mrls-for-plant-based-foods/pesticide-maximum-residue-level-legislation-around-the-world/, accessed August 22, 2019.

201 U.S. Environmental Protection Agency, *Pesticide Tolerances: Thiamethoxam*, https://www.regulations.gov/document?D=EPA-HQ-OPP-2015-0705-0006, accessed August 22, 2019.

202 U.S. Environmental Protection Agency, *Pesticide Tolerances: Thiacloprid*, https://www.regulations.gov/document?D=EPA-HQ-OPP-2010-0311-0004, accessed August 22, 2019.

203 U.S. Environmental Protection Agency, *Pesticide Tolerances: Dinotefuran*, https://www.regulations.gov/document?D=EPA-HQ-OPP-2012-0060-0005, accessed August 22, 2019.

204 U.S. Environmental Protection Agency, *Pesticide Tolerances: Acetamiprid*, https://www.regulations.gov/document?D=EPA-HQ-OPP-2011-0792-0005, accessed August 22, 2019.

205 U.S. Environmental Protection Agency, *Pesticide Tolerances: Clothianidin*, https://www.regulations.gov/document?D=EPA-HQ-OPP-2010-0217-0044, accessed August 22, 2019.

206 U.S. Environmental Protection Agency, *Pesticide Tolerances: Imidacloprid*, https://www.regulations.gov/document?D=EPA-HQ-OPP-2012-0204-0005, accessed August 22, 2019.

207 U.S. Environmental Protection Agency, *Acetamiprid. Human Health Assessment Scoping Document in Support of Registration Review*, https://www.regulations.gov/document?D=EPA-HQ-OPP-2012-0329-0004.

208 Health Canada, *Maximum Residue Limits for Pesticides*, http://pr-rp.hc-sc.gc.ca/mrl-lrm/index-eng.php, accessed September 9. 2019.

209 *The Japan Food Chemical Research Foundation, Maximum Residue Limits (MRLs) List of Agricultural Chemicals in Foods*, http://db.ffcr.or.jp/front/, accessed September 9, 2019.

210 World Health Organization, *Inventory of evaluations performed by the Joint Meeting on Pesticide Residues (JMPR)*, http://apps.who.int/pesticide-residues-jmpr-database, accessed August 22, 2019.

211 J. E. Casida, Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance, *J. Agric. Food Chem.*, 2011, **59**, 2923–2931.

212 M. Tomizawa, D. L. Lee and J. E. Casida, Neonicotinoid Insecticides: Molecular Features Conferring Selectivity for Insect versus Mammalian Nicotinic Receptors, *J. Agric. Food Chem.*, 2000, **48**, 6016–6024.

213 Y.-J. Dai, W.-W. Ji, T. Chen, W.-J. Zhang, Z.-H. Liu, F. Ge and S. Yuan, Metabolism of the Neonicotinoid insecticides acetamiprid and thiacloprid by the yeast Rhodotorula mucilaginosa strain IM-2, *J. Agric. Food Chem.*, 2010, **58**, 2419–2425.

214 H.-J. Zhang, Q.-W. Zhou, G.-C. Zhou, Y.-M. Cao, Y.-J. Dai, W.-W. Ji, G.-D. Shang and S. Yuan, Biotransformation of the neonicotinoid insecticide thiacloprid by the bacterium Variovorax boronicumulans strain J1 and mediation of the major metabolic pathway by nitrile hydratase, *J. Agric. Food Chem.*, 2012, **60**, 153–159.

215 Y.-J. Dai, S. Yuan, F. Ge, T. Chen, S.-c. Xu and J.-p. Ni, Microbial hydroxylation of imidacloprid for the synthesis of highly insecticidal olefin imidacloprid, *Appl. Microbiol. Biotechnol.*, 2006, **71**, 927–934.

216 J. Wang, H. Hirai and H. Kawagishi, Biotransformation of acetamiprid by the white-rot fungus Phanerochaete sordida YK-624, *Appl. Microbiol. Biotechnol.*, 2012, **93**, 831–835.

217 Z. Lu, J. K. Challis and C. S. Wong, Quantum Yields for Direct Photolysis of Neonicotinoid Insecticides in Water: Implications for Exposure to Nontarget Aquatic Organisms, *Environ. Sci. Technol. Lett.*, 2015, **2**, 188–192.

218 S. A. Todey, A. M. Fallon and W. A. Arnold, Neonicotinoid insecticide hydrolysis and photolysis: Rates and residual toxicity, *Environ. Toxicol. Chem.*, 2018, **37**, 2797–2809.

219 K. Z. Aregahegn, D. Shemesh, R. B. Gerber and B. J. Finlayson-Pitts, Photochemistry of Thin Solid Films of the Neonicotinoid Imidacloprid on Surfaces, *Environ. Sci. Technol.*, 2017, **51**, 2660–2668.

220 P. Moza, K. Hustert, E. A. Feicht and A. Kettrup, Photolysis of imidacloprid in aqueous solution, *Chemosphere*, 1998, **36**, 497–502.

221 R. Zabar, D. Dolenc, T. Jerman, M. Franko and P. Trebse, Photolytic and photocatalytic degradation of 6-chloronicotinic acid, *Chemosphere*, 2011, **85**, 861–868.

222 Y. Gong, J. Chen, H. Wang and J. Li, Separation and Identification of Photolysis Products of Clothianidin by Ultra-Performance Liquid Tandem Mass Spectrometry, *Anal. Lett.*, 2012, **45**, 2483–2492.

223 M. L. Dell'arciprete, L. Santos-Juanes, A. A. Sanz, R. Vicente, A. M. Amat, J. P. Furlong, D. O. Martire and M. C. Gonzalez, Reactivity of hydroxyl radicals with neonicotinoid insecticides: mechanism and changes in toxicity, *Photochem. Photobiol. Sci.*, 2009, **8**, 1016–1023.

224 R. Karmakar, S. B. Singh and G. Kulshrestha, Persistence and transformation of thiamethoxam, a neonicotinoid insecticide, in soil of different agroclimatic zones of India, *Bull. Environ. Contam. Toxicol.*, 2006, **76**, 400–406.

225 R. Karmakar, S. B. Singh and G. Kulshrestha, Kinetics and mechanism of the hydrolysis of thiamethoxam, *J. Environ. Sci. Health B*, 2009, **44**, 435–441.

226 Z. Liqing, L. Guoguang, S. Dezhi and Y. Kun, Hydrolysis of Thiamethoxam, *Bull. Environ. Contam. Toxicol.*, 2006, **76**, 942–949.

227 J. A. Leiva, P. Nkedi-Kizza, K. T. Morgan and J. A. Qureshi, Imidacloprid Sorption Kinetics, Equilibria, and Degradation in Sandy Soils of Florida, *J. Agric. Food Chem.*, 2015, **63**, 4915–4921.

228 G. Pandey, S. J. Dorrian, R. J. Russell and J. G. Oakeshott, Biotransformation of the neonicotinoid insecticides imidacloprid and thiamethoxam by Pseudomonas sp. 1G, *Biochem. Biophys. Res. Commun.*, 2009, **380**, 710–714.

229 G.-C. Zhou, Y. Wang, S. Zhai, F. Ge, Z.-H. Liu, Y.-J. Dai, S. Yuan and J.-y. Hou, Biodegradation of the neonicotinoid insecticide thiamethoxam by the nitrogen-fixing and plant-growth-promoting rhizobacterium Ensifer adhaerens strain TMX-23, *Appl. Microbiol. Biotechnol.*, 2013, **97**, 4065–4074.

230 H. Yang, X. Wang, J. Zheng, G. Wang, Q. Hong, S. Li, R. Li and J. Jiang, Biodegradation of acetamiprid by Pigmentiphaga sp. D-2 and the degradation pathway, *Int. Biodeterior. Biodegrad.*, 2013, **85**, 95–102.

231 G. Wang, W. Yue, Y. Liu, F. Li, M. Xiong and H. Zhang, Biodegradation of the neonicotinoid insecticide Acetamiprid by bacterium Pigmentiphaga sp. strain AAP-1 isolated from soil, *Bioresour. Technol.*, 2013, **138**, 359–368.

232 H. Tang, J. Li, H. Hu and P. Xu, A newly isolated strain of Stenotrophomonas sp. hydrolyzes acetamiprid, a synthetic insecticide, *Process Biochem.*, 2012, **47**, 1820–1825.

233 T. Chen, Y.-J. Dai, J.-F. Ding, S. Yuan and J.-P. Ni, N-demethylation of neonicotinoid insecticide acetamiprid by bacterium Stenotrophomonas maltophilia CGMCC 1.1788, *Biodegradation*, 2008, **19**, 651–658.

234 F. Ge, L.-Y. Zhou, Y. Wang, Y. Ma, S. Zhai, Z.-H. Liu, Y.-J. Dai and S. Yuan, Hydrolysis of the neonicotinoid insecticide thiacloprid by the N2-fixing bacterium Ensifer meliloti CGMCC 7333, *Int. Biodeterior. Biodegrad.*, 2014, **93**, 10–17.

235 L. Y. Zhou, L. J. Zhang, S. L. Sun, F. Ge, S. Y. Mao, Y. Ma, Z. H. Liu, Y. J. Dai and S. Yuan, Degradation of the neonicotinoid insecticide acetamiprid via the N-carbamoylimine derivate (IM-1-2) mediated by the nitrile hydratase of the nitrogen-fixing bacterium Ensifer meliloti CGMCC 7333, *J. Agric. Food Chem.*, 2014, **62**, 9957–9964.

236 M. Cycon, A. Markowicz, S. Borymski, M. Wojcik and Z. Piotrowska-Seget, Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities, *J. Environ. Manage.*, 2013, **131**, 55–65.

237 M. Cycon and Z. Piotrowska-Seget, Biochemical and microbial soil functioning after application of the insecticide imidacloprid, *J. Environ. Sci.*, 2015, **27**, 147–158.

238 B. Mahapatra, T. Adak, N. K. B. Patil, G. P. Pandi G, G. B. Gowda, N. N. Jambhulkar, M. K. Yadav, P. Panneerselvam, U. Kumar, S. Munda and M. Jena, Imidacloprid application changes microbial dynamics

and enzymes in rice soil, *Ecotoxicol. Environ. Saf.*, 2017, **144**, 123–130.

239 M. Chagnon, D. Kreutzweiser, E. A. Mitchell, C. A. Morrissey, D. A. Noome and J. P. Van der Sluijs, Risks of large-scale use of systemic insecticides to ecosystem functioning and services, *Environ. Sci. Pollut. Res. Int.*, 2015, **22**, 119–134.

240 T. Mori, J. Wang, Y. Tanaka, K. Nagai, H. Kawagishi and H. Hirai, Bioremediation of the neonicotinoid insecticide clothianidin by the white-rot fungus Phanerochaete sordida, *J. Hazard. Mater.*, 2017, **321**, 586–590.

241 J. L. Brunet, A. Badiou and L. P. Belzunces, In vivo metabolic fate of [14C]-acetamiprid in six biological compartments of the honeybee, Apis mellifera L, *Pest Manage. Sci.*, 2005, **61**, 742–748.

242 K. A. Ford and J. E. Casida, Chloropyridinyl neonicotinoid insecticides: diverse molecular substituents contribute to facile metabolism in mice, *Chem. Res. Toxicol.*, 2006, **19**, 944–951.

243 K. A. Ford and J. E. Casida, Comparative Metabolism and Pharmacokinetics of Seven Neonicotinoid Insecticides in Spinach, *J. Agric. Food Chem.*, 2008, **56**, 10168–10175.

244 M. Tokieda, M. Ozawa, S. Kobayashi and T. Gomyo, Methods of determination of acetamiprid and its degradation products in soil by gas chromatography, *J. Pestic. Sci.*, 1999, **24**, 181–185.

245 A. Elbert, M. Haas, B. Springer, W. Thielert and R. Nauen, Applied aspects of neonicotinoid uses in crop protection, *Pest Manage. Sci.*, 2008, **64**, 1099–1105.

246 K. A. Ford, *Metabolism, pharmacokinetics and toxicology of the neonicotinoid insecticides in mice*, University of California, Berkeley, 2008.

247 K. A. Ford, J. E. Casida, D. Chandran, A. G. Gulevich, R. A. Okrent, K. A. Durkin, R. Sarpong, E. M. Bunnelle and M. C. Wildermuth, Neonicotinoid insecticides induce salicylate-associated plant defense responses, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 17527–17532.

248 C. P. Muerdter and G. H. LeFevre, Synergistic Lemna Duckweed and Microbial Transformation of Imidacloprid and Thiacloprid Neonicotinoids, *Environ. Sci. Technol. Lett.*, 2019.

249 X. Shi, R. A. Dick, K. A. Ford and J. E. Casida, Enzymes and inhibitors in neonicotinoid insecticide metabolism, *J. Agric. Food Chem.*, 2009, **57**, 4861–4866.

250 J. T. Marfo, K. Fujioka, Y. Ikenaka, S. M. M. Nakayama, H. Mizukawa, Y. Aoyama, M. Ishizuka and K. Taira, Relationship between Urinary N-Desmethyl-Acetamiprid and Typical Symptoms including Neurological Findings: A Prevalence Case-Control Study, *PLoS One*, 2015, **10**, e0142172.

251 H. J. Renaud, J. Y. Cui, M. Khan and C. D. Klaassen, Tissue distribution and gender-divergent expression of 78 cytochrome P450 mRNAs in mice, *Toxicol. Sci.*, 2011, **124**, 261–277.

252 R. Vila, M. Kurosaki, M. M. Barzago, M. Kolek, A. Bastone, L. Colombo, M. Salmona, M. Terao and E. Garattini, Regulation and Biochemistry of Mouse Molybdoflavoenzymes The DBA/2 Mouse is selectively deficient in the expression of aldehyde oxidase homologues 1 and 2 and represents a unique source for purification and characterization of aldehyde oxidase, *J. Biol. Chem.*, 2004, **279**, 8668–8683.

253 K. M. Kasiotis and K. Marchera, Neonicotinoids and their Metabolites in Human Biomonitoring: A Review, *Hell. Plant Prot. J.*, 2015, **8**, 33–45.

254 K. Taira, Human neonicotinoids exposure in Japan, *Jpn. J. Clin. Ecol.*, 2014, **23**, 14–24.

255 K. Taira, Y. Aoyama, T. Kawakami, M. Kamata and T. Aoi, Detection of chrolopyridinyl neonicotinoid insecticide metabolite 6-chloronicotinic acid in the urine: Six cases with subacute nicotinic symptoms, *Jpn. J. Clin. Toxicol.*, 2011, **24**, 222–230.

256 K. Taira, K. Fujioka and Y. Aoyama, Qualitative profiling and quantification of neonicotinoid metabolites in human urine by liquid chromatography coupled with mass spectrometry, *PLoS One*, 2013, **8**, e80332.

257 F. J. Uroz, F. J. Arrebola, F. J. Egea-Gonzalez and J. L. Martinez-Vidal, Monitoring of 6-chloronicotinic acid in human urine by gas chromatography-tandem mass spectrometry as indicator of exposure to the pesticide imidacloprid, *Analyst*, 2001, **126**, 1355–1358.

258 H. Nomura, J. Ueyama, T. Kondo, I. Saito, K. Murata, T. Iwata, S. Wakusawa and M. Kamijima, Quantitation of neonicotinoid metabolites in human urine using GC-MS, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2013, **941**, 109–115.

259 D. A. Schulz-Jander and J. E. Casida, Imidacloprid insecticide metabolism: human cytochrome P450 isozymes differ in selectivity for imidazolidine oxidation versus nitroimine reduction, *Toxicol. Lett.*, 2002, **132**, 65–70.

260 D. A. Schulz-Jander, W. M. Leimkuehler and J. E. Casida, Neonicotinoid insecticides: reduction and cleavage of imidacloprid nitroimine substituent by liver microsomal and cytosolic enzymes, *Chem. Res. Toxicol.*, 2002, **15**, 1158–1165.

261 J.-L. Brunet, M. Maresca, J. Fantini and L. P. Belzunces, Human intestinal absorption of imidacloprid with Caco-2 cells as enterocyte model, *Toxicol. Appl. Pharmacol.*, 2004, **194**, 1–9.

262 D. Gibbons, C. Morrissey and P. Mineau, A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife, *Environ. Sci. Pollut. Res.*, 2015, **22**, 103–118.

263 W. Han, Y. Tian and X. Shen, Human exposure to neonicotinoid insecticides and the evaluation of their potential toxicity: An overview, *Chemosphere*, 2018, **192**, 59–65.

264 J. R. Roberts and J. R. Reigart, *Recognition and management of pesticide poisonings*, U.S. Environmental Protection Agency, 1999.

265 V. Freed, R. Haque, D. Schmedding and R. Kohnert, Physicochemical properties of some organophosphates in

relation to their chronic toxicity, *Environ. Health Perspect.*, 1976, **13**, 77–81.

266 U.S. Environmental Protection Agency, *Chapter 5: Organophosphate insecticides*, https://www.epa.gov/pesticide-worker-safety/recognition-and-management-pesticide-poisonings.

267 A. Annabi, I. B. Dhouib, A. J. Lamine, N. E. Golli, N. Gharbi, S. E. Fazâa and M. M. Lasram, Recovery by N-acetylcysteine from subchronic exposure to Imidacloprid-induced hypothalamic–pituitary–adrenal (HPA) axis tissues injury in male rats, *Toxicol. Mech. Methods*, 2015, **25**, 524–531.

268 U. Kapoor, M. K. Srivastava, S. Bhardwaj and L. P. Srivastava, Effect of imidacloprid on antioxidant enzymes and lipid peroxidation in female rats to derive its No Observed Effect Level (NOEL), *J. Toxicol. Sci.*, 2010, **35**, 577–581.

269 M. Lonare, M. Kumar, S. Raut, P. Badgujar, S. Doltade and A. Telang, Evaluation of imidacloprid-induced neurotoxicity in male rats: a protective effect of curcumin, *Neurochem. Int.*, 2014, **78**, 122–129.

270 V. Duzguner and S. Erdogan, Chronic exposure to imidacloprid induces inflammation and oxidative stress in the liver & central nervous system of rats, *Pestic. Biochem. Physiol.*, 2012, **104**, 58–64.

271 B. Aydin, Effects of thiacloprid, deltamethrin and their combination on oxidative stress in lymphoid organs, polymorphonuclear leukocytes and plasma of rats, *Pestic. Biochem. Physiol.*, 2011, **100**, 165–171.

272 K. S. El-Gendy, N. M. Aly, F. H. Mahmoud, A. Kenawy and A. K. El-Sebae, The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid, *Food Chem. Toxicol.*, 2010, **48**, 215–221.

273 T. Tanaka, Reproductive and neurobehavioral effects of clothianidin administered to mice in the diet, *Birth Defects Res., Part B*, 2012, **95**, 151–159.

274 X. Wang, A. Anadón, Q. Wu, F. Qiao, I. Ares, M.-R. Martínez-Larrañaga, Z. Yuan and M.-A. Martínez, Mechanism of neonicotinoid toxicity: Impact on oxidative stress and metabolism, *Annu. Rev. Pharmacol. Toxicol.*, 2018, **58**, 471–507.

275 Y.-h. Gu, Y. Li, X.-f. Huang, J.-f. Zheng, J. Yang, H. Diao, Y. Yuan, Y. Xu, M. Liu, H.-J. Shi and W.-p. Xu, Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development in vitro, *PLoS One*, 2013, **8**, e70112.

276 E. H. Berheim, J. A. Jenks, J. G. Lundgren, E. S. Michel, D. Grove and W. F. Jensen, Effects of Neonicotinoid Insecticides on Physiology and Reproductive Characteristics of Captive Female and Fawn White-tailed Deer, *Sci. Rep.*, 2019, **9**, 4534.

277 R. Bal, G. Turk, O. Yilmaz, E. Etem, T. Kuloglu, G. Baydas and M. Naziroglu, Effects of clothianidin exposure on sperm quality, testicular apoptosis and fatty acid composition in developing male rats, *Cell Biol. Toxicol.*, 2012, **28**, 187–200.

278 G. Najafi, M. Razi, A. Hoshyar, S. Shahmohamadloo and S. Feyzi, The effect of chronic exposure with imidacloprid insecticide on fertility in mature male rats, *Int. J. Fertil. Steril.*, 2010, **4**, 9–16.

279 R. Bal, G. Türk, M. Tuzcu, O. Yilmaz, T. Kuloglu, R. Gundogdu, S. Gür, A. Agca, M. Ulas and Z. Çambay, Assessment of imidacloprid toxicity on reproductive organ system of adult male rats, *J. Environ. Sci. Health B*, 2012, **47**, 434–444.

280 R. Bal, G. Türk, M. Tuzcu, Ö. Yılmaz, T. Kuloğlu, G. Baydaş, M. Naziroğlu, Z. Yener, E. Etem and Z. Tuzcu, Effects of the neonicotinoid insecticide, clothianidin, on the reproductive organ system in adult male rats, *Drug Chem. Toxicol.*, 2013, **36**, 421–429.

281 H. Terayama, N. Qu, H. Endo, M. Ito, H. Tsukamoto, K. Umemoto, S. Kawakami, Y. Fujino, M. Tatemichi and K. Sakabe, Effect of acetamiprid on the immature murine testes, *Int. J. Environ. Health Res.*, 2018, **28**, 683–696.

282 M. Mohany, G. Badr, I. Refaat and M. El-Feki, Immunological and histological effects of exposure to imidacloprid insecticide in male albino rats, *Afr. J. Pharm. Pharmacol.*, 2011, **5**, 2106–2114.

283 H. K. Toor, G. K. Sangha and K. S. Khera, Imidacloprid induced histological and biochemical alterations in liver of female albino rats, *Pestic. Biochem. Physiol.*, 2013, **105**, 1–4.

284 P. C. Badgujar, S. K. Jain, A. Singh, J. S. Punia, R. P. Gupta and G. A. Chandratre, Immunotoxic effects of imidacloprid following 28 days of oral exposure in BALB/c mice, *Environ. Toxicol. Pharmacol.*, 2013, **35**, 408–418.

285 T. Green, A. Toghill, R. Lee, F. Waechter, E. Weber and J. Noakes, Thiamethoxam induced mouse liver tumors and their relevance to humans. Part 1: mode of action studies in the mouse, *Toxicol. Sci.*, 2005, **86**, 36–47.

286 J. Bianchi, D. C. Cabral-de-Mello and M. A. Marin-Morales, Toxicogenetic effects of low concentrations of the pesticides imidacloprid and sulfentrazone individually and in combination in in vitro tests with HepG2 cells and Salmonella typhimurium, *Ecotoxicol. Environ. Saf.*, 2015, **120**, 174–183.

287 N. U. Karabay and M. G. Oguz, Cytogenetic and genotoxic effects of the insecticides, imidacloprid and methamidophos, *Genet. Mol. Res.*, 2005, **4**, 653–662.

288 M. E. Calderón-Segura, S. Gómez-Arroyo, R. Villalobos-Pietrini, C. Martínez-Valenzuela, Y. Carbajal-López, M. del Calderón-Ezquerro, J. Cortés-Eslava, R. García-Martínez, D. Flores-Ramírez, M. Rodríguez-Romero, P. Méndez-Pérez and E. Bañuelos-Ruíz, Evaluation of genotoxic and cytotoxic effects in human peripheral blood lymphocytes exposed in vitro to neonicotinoid insecticides news, *J. Toxicol.*, 2012, **2012**, 612647.

289 C. Costa, V. Silvari, A. A. Melchini, S. Catania, J. J. A. Heffron, A. Trovato and R. D. De Pasquale, Genotoxicity of imidacloprid in relation to metabolic activation and composition of the commercial product, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.*, 2009, **672**, 40–44.

290 S. Feng, Z. Kong, X. Wang, P. Peng and E. Y. Zeng, Assessing the genotoxicity of imidacloprid and RH-5849 in human

peripheral blood lymphocytes in vitro with comet assay and cytogenetic tests, *Ecotoxicol. Environ. Saf.*, 2005, **61**, 239–246.

291 G. Demsia, D. Vlastos, M. Goumenou and D. P. Matthopoulos, Assessment of the genotoxicity of imidacloprid and metalaxyl in cultured human lymphocytes and rat bone-marrow, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.*, 2007, **634**, 32–39.

292 P. Bagri, V. Kumar and A. K. Sikka, Assessment of imidacloprid-induced mutagenic effects in somatic cells of Swiss albino male mice, *Drug Chem. Toxicol.*, 2016, **39**, 412–417.

293 A. Y. Kocaman and M. Topaktas, In vitro evaluation of the genotoxicity of acetamiprid in human peripheral blood lymphocytes, *Environ. Mol. Mutagen.*, 2007, **48**, 483–490.

294 Y. Zang, Y. Zhong, Y. Luo and Z. Kong, Genotoxicity of two novel pesticides for the earthworm, Eisenia fetida, *Environ. Pollut.*, 2000, **108**, 271–278.

295 A. Y. Kocaman, E. Rencuzogullari and M. Topaktas, In vitro investigation of the genotoxic and cytotoxic effects of thiacloprid in cultured human peripheral blood lymphocytes, *Environ. Toxicol.*, 2014, **29**, 631–641.

296 V. Sekeroglu, Z. A. Sekeroglu and H. Kefelioglu, Cytogenetic effects of commercial formulations of deltamethrin and/or thiacloprid on Wistar rat bone marrow cells, *Environ. Toxicol.*, 2013, **28**, 524–531.

297 T. Pastoor, P. Rose, S. Lloyd, R. Peffer and T. Green, Case study: weight of evidence evaluation of the human health relevance of thiamethoxam-related mouse liver tumors, *Toxicol. Sci.*, 2005, **86**, 56–60.

298 T. Green, A. Toghill, R. Lee, F. Waechter, E. Weber, R. Peffer, J. Noakes and M. Robinson, Thiamethoxam induced mouse liver tumors and their relevance to humans. Part 2: species differences in response, *Toxicol. Sci.*, 2005, **86**, 48–55.

299 L. P. Sheets, in *Handbook of Neurotoxicology: Volume I*, ed. E. J. Massaro, Humana Press, Totowa, NJ, 2002, pp. 79–87, DOI: 10.1007/978-1-59259-132-9_6.

300 S. Bhardwaj, M. K. Srivastava, U. Kapoor and L. P. Srivastava, A 90 days oral toxicity of imidacloprid in female rats: morphological, biochemical and histopathological evaluations, *Food Chem. Toxicol.*, 2010, **48**, 1185–1190.

301 T. Hirano, S. Yanai, T. Omotehara, R. Hashimoto, Y. Umemura, N. Kubota, K. Minami, D. Nagahara, E. Matsuo, Y. Aihara, R. Shinohara, T. Furuyashiki, Y. Mantani, T. Yokoyama, H. Kitagawa and N. Hoshi, The combined effect of clothianidin and environmental stress on the behavioral and reproductive function in male mice, *J. Vet. Med. Sci.*, 2015, 77, 1207–1215.

302 L. P. Sheets, A. A. Li, D. J. Minnema, R. H. Collier, M. R. Creek and R. C. Peffer, A critical review of neonicotinoid insecticides for developmental neurotoxicity, *Crit. Rev. Toxicol.*, 2016, **46**, 153–190.

303 H. H. Özdemir, M. Kara, O. Yumrutas, F. Uckardes, E. Eraslan, C. F. Demir and R. Bal, Determination of the effects on learning and memory performance and related gene expressions of clothianidin in rat models, *Cogn. Neurodynamics*, 2014, **8**, 411–416.

304 J. Kimura-Kuroda, Y. Komuta, Y. Kuroda, M. Hayashi and H. Kawano, Nicotine-like effects of the neonicotinoid insecticides acetamiprid and imidacloprid on cerebellar neurons from neonatal rats, *PLoS One*, 2012, **7**, e32432.

305 I. M. de Oliveria, B. V. Nunes, D. R. Barbosa, A. M. Pallares and L. R. F. Faro, Effects of the neonicotinoids thiametoxam and clothianidin on in vivo dopamine release in rat striatum, *Toxicol. Lett.*, 2010, **192**, 294–297.

306 S. C. Landis, S. G. Amara, K. Asadullah, C. P. Austin, R. Blumenstein, E. W. Bradley, R. G. Crystal, R. B. Darnell, R. J. Ferrante, H. Fillit, R. Finkelstein, M. Fisher, H. E. Gendelman, R. M. Golub, J. L. Goudreau, R. A. Gross, A. K. Gubitz, S. E. Hesterlee, D. W. Howells, J. Huguenard, K. Kelner, W. Koroshetz, D. Krainc, S. E. Lazic, M. S. Levine, M. R. Macleod, J. M. McCall, R. T. Moxley III, K. Narasimhan, L. J. Noble, S. Perrin, J. D. Porter, O. Steward, E. Unger, U. Utz and S. D. Silberberg, A call for transparent reporting to optimize the predictive value of preclinical research, *Nature*, 2012, **490**, 187–191.

307 E. Caron-Beaudoin, R. Viau, A.-A. Hudon-Thibeault, C. Vaillancourt and J. T. Sanderson, The use of a unique co-culture model of fetoplacental steroidogenesis as a screening tool for endocrine disruptors: The effects of neonicotinoids on aromatase activity and hormone production, *Toxicol. Appl. Pharmacol.*, 2017, **332**, 15–24.

308 E. Caron-Beaudoin, R. Viau and J. T. Sanderson, Effects of Neonicotinoid Pesticides on Promoter-Specific Aromatase (CYP19) Expression in Hs578t Breast Cancer Cells and the Role of the VEGF Pathway, *Environ. Health Perspect.*, 2018, **126**, 047014.

309 É. Caron-Beaudoin, M. S. Denison and J. T. Sanderson, Effects of neonicotinoids on promoter-specific expression and activity of aromatase (CYP19) in human adrenocortical carcinoma (H295R) and primary umbilical vein endothelial (HUVEC) cells, *Toxicol. Sci.*, 2015, **149**, 134–144.

310 S. Schmidt, Promotional Consideration: A Potential Mechanistic Link between Neonicotinoid Insecticides and Hormone-Dependent Breast Cancer, *Environ. Health Perspect.*, 2018, **126**, 114001.

311 V. R. Agarwal, S. E. Bulun, M. Leitch, R. Rohrich and E. R. Simpson, Use of alternative promoters to express the aromatase cytochrome P450 (CYP19) gene in breast adipose tissues of cancer-free and breast cancer patients, *J. Clin. Endocrinol. Metab.*, 1996, **81**, 3843–3849.

312 N. Irahara, Y. Miyoshi, T. Taguchi, Y. Tamaki and S. Noguchi, Quantitative analysis of aromatase mRNA expression derived from various promoters (I. 4, I. 3, PII and I. 7) and its association with expression of TNF-α, IL-6 and COX-2 mRNAs in human breast cancer, *Int. J. Cancer*, 2006, **118**, 1915–1921.

313 R. Nauen, U. Ebbinghaus-Kintscher and R. Schmuck, Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in Apis mellifera

(Hymenoptera: Apidae), *Pest Manage. Sci.*, 2001, **57**, 577–586.

314 M. la Farre, S. Perez, L. Kantiani and D. Barcelo, Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment, *TrAC, Trends Anal. Chem.*, 2008, **27**, 991–1007.

315 H. Nakayama, T. Numakawa, T. Ikeuchi and H. Hatanaka, Nicotine-induced phosphorylation of extracellular signal-regulated protein kinase and CREB in PC12h cells, *J. Neurochem.*, 2001, **79**, 489–498.

316 C. A. LaLone, D. L. Villeneuve, J. Wu-Smart, R. Y. Milsk, K. Sappington, K. V. Garber, J. Housenger and G. T. Ankley, Weight of evidence evaluation of a network of adverse outcome pathways linking activation of the nicotinic acetylcholine receptor in honey bees to colony death, *Sci. Total Environ.*, 2017, **584**, 751–775.

317 Q. Zhang, Z. Li, C. Chang, J. Lou, M. Zhao and C. Lu, Potential human exposures to neonicotinoid insecticides: A review, *Environ. Pollut.*, 2018, **236**, 71–81.

318 A. Osaka, J. Ueyama, T. Kondo, H. Nomura, Y. Sugiura, I. Saito, K. Nakane, A. Takaishi, H. Ogi and S. Wakusawa, Exposure characterization of three major insecticide lines in urine of young children in Japan—neonicotinoids, organophosphates, and pyrethroids, *Environ. Res.*, 2016, **147**, 89–96.

319 J. Ueyama, H. Nomura, T. Kondo, I. Saito, Y. Ito, A. Osaka and M. Kamijima, Biological monitoring method for urinary neonicotinoid insecticides using LC-MS/MS and its application to Japanese adults, *J. Occup. Health*, 2014, **56**, 461–468.

320 F. J. Uroz, F. J. Arrebola, F. J. Egea-González and J. L. Martinez-Vidal, Monitoring of 6-chloronicotinic acid in human urine by gas chromatography-tandem mass spectrometry as indicator of exposure to the pesticide imidacloprid, *Analyst*, 2001, **126**, 1355–1358.

321 K. Taira, Y. Aoyama, T. Kawakami, M. Kamata and T. Aoi, [Detection of chloropyridinyl neonicotinoid insecticide metabolite 6-chloronicotinic acid in the urine: six cases with subacute nicotinic symptoms], *Jpn. J. Toxicol.*, 2011, **24**, 222–230.

322 K. Taira, Health effects of neonicotinoid insecticides-Part1: Physicochemical characteristics and case reports, *Jpn. J. Ecol.*, 2012, **21**, 24–34.

323 M. P. Kavvalakis, M. N. Tzatzarakis, E. P. Theodoropoulou, E. G. Barbounis, A. K. Tsakalof and A. M. Tsatsakis, Development and application of LC-APCI-MS method for biomonitoring of animal and human exposure to imidacloprid, *Chemosphere*, 2013, **93**, 2612–2620.

324 L. Wang, T. Liu, F. Liu, J. Zhang, Y. Wu and H. Sun, Occurrence and profile characteristics of the pesticide imidacloprid, preservative parabens, and their metabolites in human urine from rural and urban China, *Environ. Sci. Technol.*, 2015, **49**, 14633–14640.

325 R. Kabata, S. Nanayakkara, S. Stmld, K. H. Harada, R. Chandrajith, T. Hitomi, T. Abeysekera, T. Takasuga and A. Koizumi, Neonicotinoid concentrations in urine from chronic kidney disease patients in the North Central Region of Sri Lanka, *J. Occup. Health*, 2015, 15-0140-BR.

326 M. López-García, R. Romero-González, M. Lacasaña and A. G. Frenich, Semiautomated determination of neonicotinoids and characteristic metabolite in urine samples using TurboFlow™ coupled to ultra high performance liquid chromatography coupled to Orbitrap analyzer, *J. Pharm. Biomed. Anal.*, 2017, **146**, 378–386.

327 Q. Zhang, X. Wang, Z. Li, H. Jin, Z. Lu, C. Yu, Y.-f. Huang and M. Zhao, Simultaneous determination of nine neonicotinoids in human urine using isotope-dilution ultra-performance liquid chromatography–tandem mass spectrometry, *Environ. Pollut.*, 2018, **240**, 647–652.

328 S. E. Baker, A. B. Serafim, P. Morales-Agudelo, M. Vidal, A. M. Calafat and M. Ospina, Quantification of DEET and neonicotinoid pesticide biomarkers in human urine by online solid-phase extraction high-performance liquid chromatography-tandem mass spectrometry, *Anal. Bioanal. Chem.*, 2019, **411**, 669–678.

329 M. Honda, M. Robinson and K. Kannan, A simple method for the analysis of neonicotinoids and their metabolites in human urine, *Environ. Chem.*, 2019, **16**, 171–178.

330 Y. Ikenaka, Y. Miyabara, T. Ichise, S. Nakayama, C. Nimako, M. Ishizuka and C. Tohyama, Exposures of children to neonicotinoids in pine wilt disease control areas, *Environ. Toxicol. Chem.*, 2019, **38**, 71–79.

331 M. Ospina, L.-Y. Wong, S. Baker, A. B. Serafim, P. Morales-Agudelo and A. M. Calafat, Exposure to neonicotinoid insecticides in the US general population: Data from the 2015–2016 national health and nutrition examination survey, *Environ. Res.*, 2019, 108555.

332 Y. Tao, D. Phung, F. Dong, J. Xu, X. Liu, X. Wu, Q. Liu, M. He, X. Pan and R. Li, Urinary monitoring of neonicotinoid imidacloprid exposure to pesticide applicators, *Sci. Total Environ.*, 2019, **669**, 721–728.

333 T. Zhang, S. Song, X. Bai, Y. He, B. Zhang, M. Gui, K. Kannan, S. Lu, Y. Huang and H. Sun, A nationwide survey of urinary concentrations of neonicotinoid insecticides in China, *Environ. Int.*, 2019, **132**, 105114.

334 A. F. Hernández, I. Casado, G. Pena, F. Gil, E. Villanueva and A. Pla, Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects, *Inhalation Toxicol.*, 2008, **20**, 839–849.

335 P.-C. Lin, H.-J. Lin, Y.-Y. Liao, H.-R. Guo and K.-T. Chen, Acute Poisoning with Neonicotinoid Insecticides: A Case Report and literature Review, *Basic Clin. Pharmacol. Toxicol.*, 2013, **112**, 282–286.

336 I.-W. Wu, J.-L. Lin and E.-T. Cheng, Acute poisoning with the neonicotinoid insecticide imidacloprid in N-methyl pyrrolidone, *J. Toxicol., Clin. Toxicol.*, 2001, **39**, 617–621.

337 D. H. Phua, C. C. Lin, M.-L. Wu, J.-F. Deng and C.-C. Yang, Neonicotinoid insecticides: an emerging cause of acute pesticide poisoning, *Clin. Toxicol.*, 2009, **47**, 336–341.

338 F. Mohamed, I. Gawarammana, T. A. Robertson, M. S. Roberts, C. Palangasinghe, S. Zawahir, S. Jayamanne, J. Kandasamy, M. Eddleston and N. A. Buckley, Acute human self-poisoning with

imidacloprid compound: a neonicotinoid insecticide, *PLoS One*, 2009, **4**, e5127.

339 N.-C. Huang, S.-L. Lin, C.-H. Chou, Y.-M. Hung, H.-M. Chung and S.-T. Huang, Fatal ventricular fibrillation in a patient with acute imidacloprid poisoning, *Am. J. Emerg. Med.*, 2006, **24**, 883–885.

340 R. Agarwal and R. Srinivas, Severe neuropsychiatric manifestations and rhabdomyolysis in a patient with imidacloprid poisoning, *Am. J. Emerg. Med.*, 2007, **25**, 844–845.

341 S. Shadnia and H. H. Moghaddam, Fatal intoxication with imidacloprid insecticide, *Am. J. Emerg. Med.*, 2008, **26**, e631–e634.

342 M. Todani, T. Kaneko, H. Hayashida, K. Kaneda, R. Tsuruta, S. Kasaoka and T. Maekawa, Acute poisoning with neonicotinoid insecticide acetamiprid, *Japanese Journal of Toxicology*, 2008, **21**, 387–390.

343 A. K. Panigrahi, D. Subrahmanyam and K. K. Mukku, Imidacloprid poisoning: a case report, *Am. J. Emerg. Med.*, 2009, **27**, 256.e5–256.6.

344 A. D. Karatas, Severe central nervous system depression in a patient with acute imidacloprid poisoning, *Am. J. Emerg. Med.*, 2009, **27**, 1171.e5–1171.e7.

345 T. Imamura, Y. Yanagawa, K. Nishikawa, N. Matsumoto and T. Sakamoto, Two cases of acute poisoning with acetamiprid in humans, *Clin. Toxicol.*, 2010, **48**, 851–853.

346 R. Iyyadurai, I. A. George and J. V. Peter, Imidacloprid poisoning—newer insecticide and fatal toxicity, *J. Med. Toxicol.*, 2010, **6**, 77–78.

347 I.-J. Yeh, T.-J. Lin and D.-Y. Hwang, Acute multiple organ failure with imidacloprid and alcohol ingestion, *Am. J. Emerg. Med.*, 2010, **28**, 255.e1–255.e3.

348 P. Chwaluk, Acute inhalation imidacloprid poisoning–a case report, *Przegl. Lek.*, 2010, **67**, 619–620.

349 P. C. Lin, H. J. Lin, Y. Y. Liao, H. R. Guo and K. T. Chen, Acute poisoning with neonicotinoid insecticides: a case report and literature review, *Basic Clin. Pharmacol. Toxicol.*, 2013, **112**, 282–286.

350 D. David, I. A. George and J. V. Peter, Toxicology of the newer neonicotinoid insecticides: imidacloprid poisoning in a human, *Clin. Toxicol.*, 2007, **45**, 485–486.

351 P. Proença, H. Teixeira, F. Castanheira, J. Pinheiro, P. V. Monsanto, E. P. Marques and D. N. Vieira, Two fatal intoxication cases with imidacloprid: LC/MS analysis, *Forensic Sci. Int.*, 2005, **153**, 75–80.

352 C. Fuke, T. Nagai, K. Ninomiya, M. Fukasawa, Y. Ihama and T. Miyazaki, Detection of imidacloprid in biological fluids in a case of fatal insecticide intoxication, *Leg. Med.*, 2014, **16**, 40–43.

353 K. V. Vinod, S. Srikant, G. Thiruvikramaprakash and T. K. Dutta, A fatal case of thiacloprid poisoning, *Am. J. Emerg. Med.*, 2015, **33**, 310.e5–310.e6.

354 N. Nistor, O. E. Frăsinariu and V. Ştreangă, *Acute Poisoning with Neonicotinoid Insecticide, Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis*, 2017, vol. 107.

355 J. C. Kim, B. H. So, H. J. Kim, H. M. Kim, J. H. Park, S. M. Choi, K. N. Park and K. H. Choi, Clinical characteristics of patients with neonicotinoid insecticide poisoning, *Journal of the Korean Society of Clinical Toxicology*, 2010, **8**, 24.

356 M. Forrester, Neonicotinoid insecticide exposures reported to six poison centers in Texas, *Hum. Exp. Toxicol.*, 2014, **33**, 568–573.

357 M. Koureas, A. Tsezou, A. Tsakalof, T. Orfanidou and C. Hadjichristodoulou, Increased levels of oxidative DNA damage in pesticide sprayers in Thessaly Region (Greece). Implications of pesticide exposure, *Sci. Total Environ.*, 2014, **496**, 358–364.

358 A. P. Keil, J. L. Daniels and I. Hertz-Piccioto, Autism Spectrum disorder, flea and tick medication, and adjustments for exposure misclassification: the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study, *Environ. Health*, 2014, **13**, 3.

359 S. L. Carmichael, W. Yang, E. Roberts, S. E. Kegley, A. M. Padula, P. B. English, E. J. Lammer and G. M. Shaw, Residential agricultural pesticide exposures and risk of selected congenital heart defects among offspring in the San Joaquin Valley of California, *Environ. Res.*, 2014, **135**, 133–138.

360 W. Yang, S. L. Carmichael, E. M. Roberts, S. E. Kegley, A. M. Padula, P. B. English and G. M. Shaw, Residential Agricultural Pesticide Exposures and Risk of Neural Tube Defects and Orofacial Clefts among Offspring in the San Joaquin Valley of California, *Am. J. Epidemiol.*, 2014, **179**, 740–748.

361 G. Ichikawa, R. Kuribayashi, Y. Ikenaka, T. Ichise, S. M. Nakayama, M. Ishizuka, K. Taira, K. Fujioka, T. Sairenchi and G. Kobashi, LC-ESI/MS/MS analysis of neonicotinoids in urine of very low birth weight infants at birth, *PLoS One*, 2019, **14**, e0219208.

362 R. Schmuck, T. Stadler and H. W. Schmidt, 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 Manage. Sci.*, 2003, **59**, 279–286.

363 C.-H. Chang, D. MacIntosh, B. Lemos, Q. Zhang and C. Lu, Characterization of daily dietary intake and the health risk of neonicotinoid insecticides for the US population, *J. Agric. Food Chem.*, 2018, **66**, 10097–10105.

