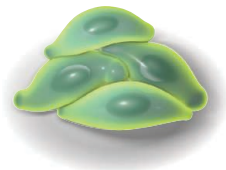
Pinpointing extrasolar planets *p. 809*

Diverse progenitor pools in the lung *p. 810*



PERSPECTIVES

ENVIRONMENTAL SCIENCE

The trouble with neonicotinoids

Chronic exposure to widely used insecticides kills bees and many other invertebrates

By **Francisco Sánchez-Bayo**

Four decades ago, DDT and other pesticides that cause environmental harm were banned. Since then, newly developed pesticides have had to conform to stricter environmental standards. Yet, recent studies highlight the subtle but deadly impacts of neonicotinoids—the most widely used insecticides in the world—on ecosystems (*1*–*3*). In contrast to other insecticides, neonicotinoids are systemic, meaning that they are highly soluble and thus absorbed by the plant. They produce delayed mortality in arthropods after chronic exposure to sublethal doses but are not very toxic to vertebrates. It has taken more than a decade to unravel some of the mechanisms through which neonicotinoids affect the integrity of ecosystems. Although gaps in knowledge remain, there is a strong case for stricter regulation of these pesticides.

Neonicotinoids are mainly applied as granules into the soil or as seed-dressings during crop planting. Seeds are coated with 1 to 17 mg per kg, depending on crops and compounds. As plants grow, they take up 2 to 20% of the insecticide and distribute it to all parts of the plant, including leaves, flowers, pollen, and nectar. The resulting concentrations of 5 to 10 μg per liter [parts per billion (ppb)] in the sap are sufficient to control sucking and chewing insect pests (see the figure). However, pollinators such as bees, butterflies, moths, and hoverflies are equally exposed; where neonicotinoids are used, 11 to 24% of pollen and 17 to 65% of nectar is contaminated with these insecticides (*3*).

Soon after the neonicotinoid imidacloprid was introduced in France in 1994, beekeepers noticed that their honey bee colonies were weakening or disappearing. The ensuing investigation found that this and another systemic insecticide (fipronil) were

Hidden killers. Neonicotinoids applied to seeds or soils spread into the environment, killing many nontarget anthropods.

PHOTO: MARA RADEVA/THINKSTOCK

particularly toxic to bees, with acute dietary LD_{50}'s (dose to kill 50% of bees) of 2.5 to 5 ng per bee (*4*). Forager bees do not die immediately after visiting flowers in treated crops because residue levels are below their acute LD_{50} and bees only ingest part of what they collect; the rest is taken to the hive. It is the daily sublethal doses the pollinators ingest that are the problem. Effects include olfactory learning, memory, and locomotory impairment and inhibited feeding (*5*). In a laboratory study, chronic ingestion of 4 to 8 ppb imidacloprid resulted in 50% survival of honey bee workers after 30 days (*6*).

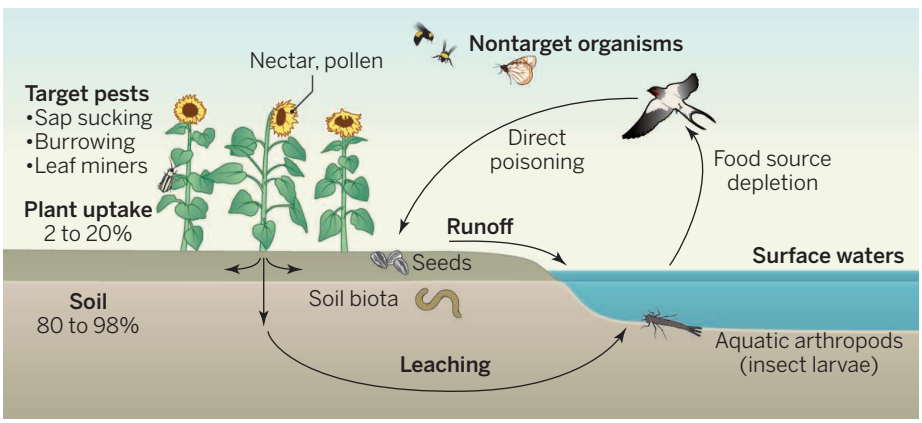
Whether these observations apply to bees in the natural environment has been a contentious question, because the performance of the hives does not change significantly. There are several reasons for this apparent lack of effect. The amount of honey produced is usually higher in contaminated hives because feeding inhibition and death of workers result in excess honey stores. Also, some undetectable sublethal effects cause mortality after a time lag (*1*). Finally, honey bee colonies compensate forager losses by producing hundreds of new workers daily; colonies thus usually overcome the initial effects during spring and summer and may survive the winter apparently unscathed. However, colony growth is usually hampered by queen failure in the next season (*7*), indicating that the queen suffers the effects of long-term intoxication. Bumble bees produce 85% fewer queens per colony when exposed to field-realistic concentrations of imidacloprid (*8*).

Concurrent with the widespread use of neonicotinoids, honey bees have experienced an increase in viral diseases, some of which are propagated by a mite parasite (*Varroa destructor*) that undermines bee health. Pollen from monoculture crops also weakens the bees' immune system, making them more susceptible to pathogens such as Nosema. These confounding factors can be blamed for the declines in honey bees but cannot account for the parallel decline in wild and bumble bees.

Di Prisco *et al.* (*1*) have established that sublethal doses of two neonicotinoids (clothianidin and imidacloprid) cause bee immune deficiency that triggers viral infections. This causal link helps to explain the time lag between initial exposure and mortality: The cascade of effects prompted by the insecticides involves irreversible biological pathways that are not observable until death takes place (*9*).

Although bees have captured most of the attention, neonicotinoids are equally toxic to ants, termites, parasitoids, and aquatic insect larvae, particularly mayflies, caddisflies, stoneflies, and midges. They are also toxic to decomposer amphipods, woodlice, and most crustaceans, but water fleas are very tolerant (*4*).

Because most neonicotinoids persist in soils for a year or more and are water soluble, 80 to 98% of residues remaining in the soil of treated crops eventually move into surface waters or leach into groundwater. Recent surveys from nine countries show 80% of surface waters contaminated with neonicotinoids at levels of 0.14 to 18 ppb, which are sublethal to aquatic arthropods (*10*, *11*). However, as in bees, chronic toxicity in all these organisms involves delayed and cumulative lethal effects over time (*12*). Experiments in aquatic model ecosystems treated with single or repeated dosages of imidacloprid confirm this: midges, ostracods, and mayflies disappear; their populations do not recover while residues in water are above 1 ppb (*13*). After 8 years of field monitoring, Van Dijk *et al.* (*11*) reported that imidacloprid concentrations as low as 0.01 ppb led to significant reduction of macroinvertebrates in surface waters.



Fate of neonicotinoids and pathways of environmental contamination.

Feeding inhibition has been observed in several decomposer organisms exposed to chronic, sublethal concentrations of imidacloprid, but starvation alone is insufficient to explain the lack of recovery and increased mortality with time (*14*). Continuous contamination of the aquatic environment with neonicotinoids may undermine the invertebrate resource base of aquatic ecosystems (*11*), thereby indirectly reducing populations of fish, birds, bats, frogs, and other animals that feed on them. Indeed, the steady decline of five species of birds in the Netherlands over the past two decades correlates with imidacloprid contamination of surface waters during the same period (*2*).

The effects of neonicotinoid residues on soil biota remain largely unknown, but the extreme efficiency with which these insecticides eliminate grub populations in turf is worrisome (*4*). This issue requires more study, because the ecosystem services provided by soil organisms are essential for sustainable agricultural production (*15*). Scattered seeds coated with high concentrations of neonicotinoids may also pose a risk to birds and rodents, despite the higher tolerance of vertebrates due to their distinct nicotinic receptor subunits (*4*).

Mechanisms that underpin chronic neonicotinoid effects on terrestrial and aquatic arthropods include immune suppression and feeding inhibition. While these and other issues are investigated further, current knowledge calls for a reconsideration of current prophylactic seed treatments with neonicotinoids. Such treatments are the main source of soil and water contamination; are often unnecessary, as they either do not increase yields or are not profitable; and go against the principles of integrated pest management (*15*). ■

Faculty of Agriculture and Environment, The University of Sydney, Eveleigh, NSW 2015, Australia. E-mail: sanchezbayo@mac.com

ILLUSTRATION: P. HUEY/*SCIENCE*

REFERENCES

1. G. Di Prisco *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 18466 (2013).
2. C. A. Hallmann *et al.*, *Nature* **511**, 341 (2014).
3. F. Sánchez-Bayo, K. Goka, *PLOS ONE* **9**, e94482 (2014).
4. L. W. Pisa *et al.*, *Environ. Sci. Pollut. Res.*, 10.1007/s11356 (2014).
5. A. Decourtye, J. Devillers, in *Advances in Experimental Medicine and Biology: Insect Nicotinic Acetylcholine Receptors*, S. H. Thany, Ed. (Landes Bioscience, Austin, TX, 2009), pp. 85–95.
6. F.-X. Dechaume Moncharmont, A. Decourtye, C. Hennequet-Hantier, O. Pons, M. H. Pham-Delègue, *Environ. Toxicol. Chem.* **22**, 3088 (2003).
7. C. Sandrock *et al.*, *PLOS ONE* **9**, e103592 (2014).
8. P. R. Whitehorn, S. O'Connor, F. L. Wackers, D. Goulson, *Science* **336**, 351 (2012).
9. G. Rondeau *et al.*, *Sci. Rep.* **4**, 5566 (2014).
10. A. R. Main *et al.*, *PLOS ONE* **9**, e92821 (2014).
11. T. C. Van Dijk *et al.*, *PLOS ONE* **8**, e62374 (2013).
12. M. A. Beketov, M. Liess, *Environ. Toxicol. Chem.* **27**, 461 (2008).
13. D. Hayasaka *et al.*, *Ecotoxicol. Environ. Saf.* **80**, 355 (2012).
14. A.-M. Nyman *et al.*, *PLOS ONE* **8**, e62472 (2013).
15. M. Chagnon *et al.*, *Environ. Sci. Pollut. Res.*, 10.1007/s11356 (2014).

10.1126/science.1259159

Environ Sci Pollut Res (2015) 22:35–67
DOI 10.1007/s11356-014-3332-7

Environmental fate and exposure; neonicotinoids and fipronil

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

Received: 27 May 2014 / Accepted: 11 July 2014 / Published online: 7 August 2014

Abstract Systemic insecticides are applied to plants using a wide variety of methods, ranging from foliar sprays to seed treatments and soil drenches. Neonicotinoids and fipronil are among the most widely used pesticides in the world. Their popularity is largely due to their high toxicity to invertebrates, the ease and flexibility with which they can be applied, their long persistence, and their systemic nature, which ensures that they spread to all parts of the target crop. However, these properties also increase the probability of environmental contamination and exposure of nontarget organisms. Environmental contamination occurs via a number of routes including dust generated during drilling of dressed seeds, contamination and accumulation in arable soils and soil water, runoff into waterways, and uptake of pesticides by nontarget plants via their roots or dust deposition on leaves. Persistence in soils, waterways, and nontarget plants is variable but can be prolonged; for example, the half-lives of neonicotinoids in soils can exceed 1,000 days, so they can accumulate when used repeatedly. Similarly, they can persist in woody plants for periods exceeding 1 year. Breakdown results in toxic metabolites, though concentrations of these in the environment are rarely measured. Overall, there is strong evidence that soils, waterways, and plants in agricultural environments and neighboring areas are contaminated with variable levels of

Responsible editor: Philippe Garrigues

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Introduction

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

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

Chemical properties

Volatility (air)

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

Sorption to soil particles (soil)

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

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

Solubility (water)

In general terms, the systemic activity of compounds increases with increasing solubility due to improved uniformity in the distribution of the active ingredient in the formulation (Koltzenburg et al. 2010) and increased bioavailability of the pesticide (Pierobon et al. 2008). Transport and translocation are positively correlated with solubility (Chamberlain 1992). The solubility of neonicotinoids in water depends on multiple factors such as water temperature and pH as well as the physical state of the pesticide applied. The molecular weight of the neonicotinoids is between 250 and 300 g/mol, and solubility ranges between 184 (moderate) and 590.000 mg/L (high) for thiacloprid and nitenpyram, respectively, at 20 °C and at pH 7 (Carbo et al. 2008; Jeschke et al. 2011; PPDB 2012) (Table 1). When compared to the neonicotinoids, fipronil has a low solubility at 3.78 mg/L under the same conditions and has a larger molecular weight (437.15 g/mol) (Tingle et al. 2003). However, even lower solubilities ranging between 1.90 and 2.40 mg/L at pH 5 and pH 9, respectively were also reported.

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

Environmental fate—abiotic

Air—environmental exposure by neonicotinoid and fipronil, contaminated dust

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

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

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

[a] USEPA (2010)

History and background

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

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

Developments

It is now known that the dissemination of neonicotinoid-contaminated dust is exacerbated by the addition of seed lubricants during planting. In North America, for instance, talc, graphite, or a combination of these minerals in a finely powdered form is typically mixed with seeds to minimize friction and ensure smooth seed flow during planting (Krupke et al. 2012). Lubricants are added directly into the planter with pesticide-treated seeds; inevitably some amount of lubricant powder fails to adhere to seeds during the sowing process. This residual lubricant remains behind in the planter to be exhausted, either immediately (i.e., during seed sowing) or later during routine cleaning of planting equipment. Because this powder comes into direct contact with treated seeds, it can act as a carrier of abraded seed coating. In fact, residual talc lubricant has been shown to contain high concentrations of seed treatment compounds, including the protectant fungicides metalaxyl and trifloxystrobin, and up to 15,000 μg/g of neonicotinoid active ingredients (Krupke et al. 2012), a concentration several orders of magnitude above the contact lethal dose for honeybees.

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

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

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

Conclusions

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

Soil—environmental fate and exposure of neonicotinoid insecticides in soils

Introduction

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

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

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

Temporal dynamics

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

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

Half-life—ranges (soil)

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

Calculated half-lives of imidacloprid in soil range over 1 order of magnitude from 100 to 1,230 days following application (Baskaran et al. 1999). The shortest recorded half-life of imidacloprid in the field is 107 days in turf-covered soils in the humid subtropical climate of Georgia, USA (Cox 2001), while according to Belzunces and Tasei (1997), the half-life of imidacloprid ranges between 188 and 249 days. However, ranges of 27 to 229 days, 997–1,136 days (in laboratory studies) (Scorza et al. 2004; Fossen 2006), 455–518 days (Fernandez-Bayo et al. 2009), 28–46 days (in India) (Sarkar et al. 2001), and even 1,000 days in soil and bedding material (Baskaran et al. 1999) have been reported. The half-life for imidacloprid in soils of seed-treated fields was about 270 days in France (Bonmatin et al. 2005a). However, no decrease in concentration was observed over a 1-year period following treatment in a field test in Minnesota (Cox 2001). Half-life of imidacloprid ranged from 3 to 4 months to over 1 year in soils in the USA (US EPA 1993a) and was longer under higher pH conditions (Sarkar et al. 2001). Based on data in Anon (2006), Goulson (2013) calculated the half-life of 1,250 days for loam in the UK.

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

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

Effect of water content (soil)

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

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

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

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

Dose dependency of decay

Decay of pesticides has been shown to depend on the dose applied. We did not find any studies on this topic for neonicotinoids, but, in the case of fipronil, dissipation was shown to be rapid (24 days) at relatively low dose (56–112 g active ingredient/ha) (Chopra et al. 2011). Fipronil was also found to exhibit a dose-dependent rate of decay within a similar range (0.15, 0.75 and 3.0 g active ingredient/m^2) in Australian Red Earth loam soils (Ying and Kookana 2006). The time for 50 % loss of active ingredients to occur increased approximately fourfold from low to high application rates (145–166 days at lowest rate to 514–613 days at highest rate). Although we did not find published reports of dose-dependent decay among neonicotinoid insecticides, we raise this as a possible further factor affecting concentrations in soils.

Effect of temperature on decay

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

Leaching and other causes of concentration changes

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

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

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

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

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

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

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

Mobility of fipronil and of its metabolites (desulfinyl, sulfide, and sulfone derivatives) was observed down to 15 cm, but only traces were found at higher depths (15–30 cm) in three Australian Red Earth loam soils (sandy, loamy, and clay) overlain by 5 cm of quartzite sand. However, experimental plots were covered by plastic liners and fiber cement during the course of the experiment, thus limiting the leaching due to rain (Ying and Kookana 2006). The same authors reported an experiment on two repacked soils (sandy loamy and clay, respectively) with alternative wet-dry weekly cycles (7 days dry followed by 20 mm of rain). Fipronil was added at a high concentration (3 g/m^2 active ingredient, which in a parallel experiment was shown to result in longest half-life), and bromide was used as a tracer. Mobility was minimal in both soils and not related to the behavior of bromide (highly leached in the sandy loamy soil but not in the clay soil) (Ying and Kookana 2006). Limited fipronil mobility was also demonstrated in Australian soils despite rather dry conditions: although measured annual rainfall was only 432.1 mm, mostly falling during the second half of the experiment, significant downward movement of fipronil was measured (Ying and Kookana 2006). Fipronil was found to bind to soil organic matter, increasing in the range 0.1–6.5 % (Bobé et al. 1997; Gunasekara et al. 2007) and this may explain the low bioaccumulation measured in fungi grown on compost with different concentration of fipronil (Carvalho et al. 2014).

Conclusions

Neonicotinoid and fipronil concentrations in soils typically decline rapidly after application, by hydrolytic, photolytic, and microbial degradation, by plant uptake, by sorption to soil particles, and by leaching to receiving waters. However, in some soil conditions, neonicotinoid and fipronil concentrations can persist, and possibly accumulate, for months or years. Persistence is highest under cool, dry conditions and, at least for neonicotinoids but possibly also for fipronil, in soils with high organic matter content. Given that neonicotinoids and fipronil are widely used in agricultural settings and can persist in drier, organic-enriched soils, which are common in agricultural fields, their residues in agricultural soils may pose a risk to soil organisms (Pisa et al. 2014, this issue). The uptake of soil-borne residues by plants expands this risk of exposure to other nontarget organisms such as those feeding on living or decomposing plant material, and those collecting nectar and pollen, although little is known about biologically-relevant concentrations found in nontarget plants and the effects of these concentrations upon other organisms.

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

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

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

Introduction

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

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

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

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

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

pH The degradation rates of neonicotinoids and fipronil in water also vary with pH. PPDB (2012) and US EPA (2005) reports that imidacloprid is stable at a pH between 5 and 7, while the half-life time at pH 9 is about 1 year at 25 °C, thereby indicating a decreasing DT50 with increasing pH. Thuyet et al. (2013) studied degradation of imidacloprid and fipronil at pH levels relevant for rice paddies. Kept at 18.2± 0.4 °C and in the dark, the initial concentrations of 60 and 3 μg/L, respectively, for analytical-grade imidacloprid and fipronil, were based on field-realistic concentrations found in paddy fields after application of these pesticides. After an initial decrease in concentration on the first 7 days, the concentration of imidacloprid remained stable at pH 7, but continued to decrease at pH 10. The authors estimated a DT50 of 182 and 44.7 days for imidacloprid at pH 7 and 10. However, Sarkar et al. (1999) found an average half-life of 36.2 days at pH 4, which increased to 41.6 days at pH 9. It should be noted that these results were obtained with commercial formulations (Confidor and Gaucho) at an ambient temperature of 30± 5 °C, which is a very wide range. The relatively high temperature will increase the degradation rate, making these results difficult to translate to the majority of field conditions.

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

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

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

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

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

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

Sources of contamination in water

Systemic pesticides used on agricultural fields, grass, turf, or hard surfaces such as lawns, golf courses, or concrete may contaminate surface and/or groundwater through (foliar) runoff, as well as through leaching, (subsurface) drains, spillage, greenhouse wastewater, and spray or dust drift (Gerecke et al. 2002). In addition, water on the soil surface of treated fields, temporary pondage, may contain high concentrations of systemic pesticides (Main et al. 2014). In sporadic events, flooding of greenhouses and the subsequent emptying thereof into surface water may result in severe contamination locally. In addition, when applied as stem injection to trees, the falling leaves in autumn may provide a source of contamination to water bodies (Kreutzweiser et al. 2007). Figure 1 provides an overview.

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

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

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

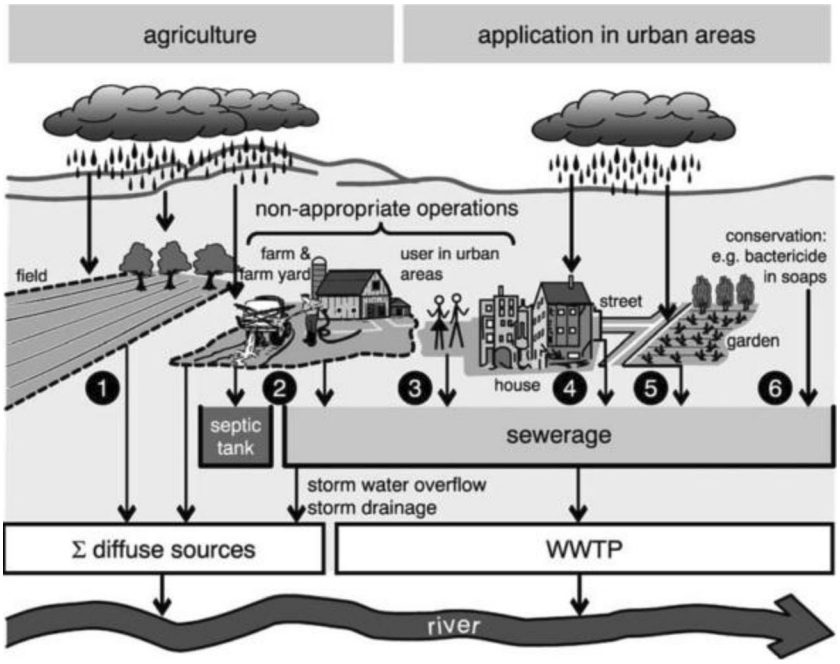


Fig. 1 Important applications and major pathways for pesticide transport into surface waters. *1* Field—spray and dust drift during application, surface runoff, and leaching with subsequent transport through drainage channels during rain events. *2* Farm and farmyard—improper operations (e.g., filling of sprayers, washing of measuring utilities, disposing of packing material, driving with seeping sprayers, and cleaning of spraying equipment). These operations are done either at locations, which are drained to the sewerage, to the septic tank or into surface waters. *3* Like 2 for pesticide users in urban areas. *4* Pesticides in building material—leaching during rain events. *5* Applications on lawns, streets, and road embankments—runoff during rain events. *6* Protection of materials—e.g., products containing antifouling ingredients that get into the sewerage (e.g., detergents and cosmetics) (source, Gerecke et al. 2002)

and these residues can be washed off during rain events leading to contamination of surface waters. Climate change is expected to play a role in altering pesticide environmental fate in the future. The likelihood of runoff increases with precipitation levels, with increased frequency and intensity of storm events and with increasing pest pressure under climate change effects. As a consequence, the risk of pesticide runoff is likely to be elevated (Kattwinkel et al. 2011). Bloomfield et al. (2006) examined the impacts of this for pesticide behavior in groundwater and surface water in the UK. Pesticide mobility is expected to increase through more frequent heavy rainfall events, increased soil erosion, and cracking of soils leading to faster by-pass flows in winter. In the drier periods, lower flow in rivers also has the potential to increase pesticide concentration and accumulation in sediments (Masiá et al. 2013). On the other hand, higher soil and surface water temperatures due to climate change will decrease some pesticide half-life times. While the overall impact is difficult to predict, increased transport to surface and groundwater of soluble substances such as several neonicotinoids seems likely. For clothianidin, for example, increased mobility is expected, but not the predicted decrease in half-life time as clothianidin is not sensitive to temperature changes. The future increased potential of such pesticides to reach and accumulate in surface and groundwater is an aspect that requires attention and warrants further research. Similarly, increases in the risk of flooding, especially in greenhouses, could result in washing out of systemic pesticides to the environment (Blom et al. 2008).

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

Exposure

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

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

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

Fipronil was detected in the Mermentau and Calcasieu River Basins in the USA, in more than 78 % of water samples from the study area. The metabolites fipronil sulfone and fipronil sulfide were detected more often than the parent compound in 81.7 and 90.0 % of the samples, respectively (Mize et al. 2008). In an earlier report by Demcheck et al. (2004), the accumulation of fipronil degradates in sediment in the same area was reported (100 % of samples). Both authors report that higher concentrations of fipronil and its metabolites were connected to changes in aquatic invertebrate communities, notably a decrease in abundance and diversity. Contamination with fipronil has also an impact on fish as exemplified by Baird et al. (2013).

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

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

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

Conclusion

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

Environmental fate and exposure in plants

Introduction

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

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

Uptake by the roots and leaves

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

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

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

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

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

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

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

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

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

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

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

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

The draft assessment report (DAR) of thiamethoxam in 2001 (EFSA 2013b) includes studies of distribution and metabolism of ^{14}C-oxadiazin- and ^{14}C-thiazol-thiamethoxam investigated in corn (seed treatment); pear and cucumber (foliar application); lettuce, potato, tobacco, and rice (soil and foliar treatment). All applications show high and fast uptake (e.g., 23 % recovered activity in the plant within day 1, 27 % of the applied amount being found after 28 h in leaves), where the product is continuously taken up from the soil reservoir for at least 100 days. The metabolism of thiamethoxam is very rapid, both inside the plant and following foliar application (photodegradation, 30 % degradation in 12 h of sun). Clothianidin is the main metabolite of this active ingredient.

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

Transport of products within the plant

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

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

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

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

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

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

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

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

Exposure

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

Foliage

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

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

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

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

Tree treatment

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

Cowles et al. (2006) studied the concentrations of imidacloprid in Hemlock (*Tsuga* spp.) needles, twigs, and sap using soil and trunk injection methods and found residues after 1 month and up to 3 years after application. The detected concentration of imidacloprid in needles and twigs ranged from stable to increasing at times during the 3 years after application. This was more often the case when a soil injection was used, possibly due to continued uptake through the roots. These findings indicate the relative stability of imidacloprid once it is absorbed by the tree. Tattar et al. (1998) studied imidacloprid translocation in Eastern Hemlock (*Tsuga canadensis*), White Pine (*Pinus strobus*), and Pin Oak (*Quercus palustris*) using soil and trunk applications. Although a continuous increase in imidacloprid concentration was observed in *Q. palustris* and *T. canadensis* after soil application, the restricted sample size ($n=6$) and sampling period render these results inconclusive with regard to the persistence of imidacloprid in these tree species. In addition, the concentration of imidacloprid in *P. strobus* needles began to decrease 12 weeks after treatment, indicating that the degradation of imidacloprid in tree foliage may be species-dependent. Multiple factors can be hypothesized to play a role in this mechanism including exposure to light, temperature differences, and the efficiency of translocation within the tree.

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

Guttation and related risk for honeybees

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

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

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

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

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

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

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

In addition, several climatic variables can affect neonicotinoid concentration in guttation drops of corn seedlings. Preliminary experiments in Italy demonstrate that under high humidity conditions (close to saturation, a situation that often occurs during the morning in spring) insecticide concentrations can be 10 times lower than those observed in guttations formed during the following sunny hours. This difference could be relevant especially in the warmer area of Europe. Moreover, guttation production by corn seedlings may be dramatically reduced or ended under low humidity conditions (RH 50–60 %). Rain can reduce the concentration of insecticide in guttations by about 10 times with respect to the values observed the day before a rainfall event. Sunny conditions and a moderate wind can promote water evaporation and affect the concentration of insecticide in guttation drops. On the contrary, strong winds can dislodge droplets off leaves, eliminating any concentration effects that would otherwise occur if droplets remained on the leaves. Finally, soil moisture and composition only moderately affect the insecticide concentration of guttation droplets (APENET 2011), suggesting that air humidity is a significant environmental factor to consider in the case of guttations.

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

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

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

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

Resin (propolis)

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

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

Presence in plant reproductive organs and fruits

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

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

Concerns regarding the contamination of fruits and vegetables with regard to human health are beyond the scope of the present study. However, the translocation of residues of systemic products into fruits can be achieved either by their transport through the xylem or phloem (Alsayeda et al. 2008), although the mechanisms of accumulation in fruits are not yet fully understood. Juraske et al. (2009) studied the human intake fraction of imidacloprid for unwashed tomatoes and found that it varies between 10^{-2} and 10^{-3} ($kg_{ingested}/kg_{applied}$) depending on the time of consumption. This was the case for tomato plants treated with the recommended doses in spray application as well as chemigation. Sur and Stork (2003) found that tomato and apple exhibit 21 and 28 % recovery of applied compounds following a foliar application. More than two thirds of this recovery was located on the surface of the fruits. A study by Zywitz et al. (2004), examined a range of fruit and vegetable groups for which neonicotinoid residues could be detected (LOD=3 ng/g) and quantified (limits of quantification (LOQ)=5 ng/g) (Table 3). Fruiting vegetables (tomatoes, pepper, cucumbers, courgettes, and melon) exhibited the highest number of positive samples (46.7 %), followed by leafy vegetables and fresh herbs (lettuce, cress, spinach, dill, chives, and parsley; 10 %), stone fruits (peach, nectarine, apricot, and cherry; 4.5 %), pome fruits (apple and pear; 2.9 %), and berries (strawberry, raspberry, currant, blueberry, and grape; 2.2 %). No information was provided on the method of application of neonicotinoids or the doses used. More recently, 22 % of fruits sampled in India showed the presence of imidacloprid and 2 % were above the maximum residue level (MRL) (Kapoor et al. 2013). A similar situation has been described in Turkey, with levels of acetamiprid in vegetables occurring above the allowable MRL (Sungur and Tunur 2012).

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

Pollen and nectar

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

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

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

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

Source, Zywitz et al. (2004)

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

Assessment The ecological risks of active ingredients are assessed using the hazard quotient (HQ) calculation. This approach estimates whether harmful effects of the contaminate in question may occur in the environment by comparing the Predicted Environmental Concentrations (PEC) to the Predicted No Effect Concentration (PNEC). HQ calculations do not consider the mode of insecticide application, the systemic properties, routes of exposure, or the persistence or metabolism of pesticides. Historically, these calculations have been inaccurate due to a lack of adequate analytical techniques for the quantification of residues in matrices like pollen and/or nectar. This was the case for imidacloprid and fipronil in the 1990s—the initial risk assessment assumed that flowers were not significantly contaminated with respect to the LD_{50} values for bees and so the PEC was underestimated at the time of registration (Maxim and van der Sluijs 2007). However, with the improvement of analytical techniques, the detection of residues in pollen/beebread and nectar/honey have become more accurate (Bonmatin et al. 2005a; Dively and Kamel 2012; Paradis et al. 2014), and show that the PEC values are actually significantly higher. Meanwhile, new understanding of the sublethal and chronic exposure effects on bees has improved the PNEC value, and demonstrates that this value was clearly overestimated during the registration of these products (Suchail et al. 2001; Whitehorn et al. 2012). It was only in the early 2000s that assessments were conducted for imidacloprid using accurate data (Rortais et al. 2005; Halm et al. 2006). This work considered both (1) different exposure pathways and (2) relative needs in food among various castes of honeybees (foragers, nurses, larvae, winter bees, etc.).

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

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

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

The contamination of fresh and stored honey originates from the presence of residues in nectar. Honey in beehives can be less contaminated than nectar. This situation was reported from sunflowers treated by seed dressing (Schmuck et al. 2001), but could have been due to a dilution effect, whereby mixture of treated and untreated nectar yields lower levels of contamination, as in the case of mixing pollen (*vide supra*). The opposite situation has also been described for citrus trees treated with soil applications (Byrne et al. 2014). Although the sum of processes remains poorly understood, it is known that there is an initial metabolism during transport and diverse chemical reactions and processing are conducted by workers—where the concentration factor is affected by the amount of water in the nectar (Winterlin et al. 1973) and by degradation over time leading to metabolites (Simon-Delso et al. 2014, this issue). Because foragers and in-hive bees participate in these metabolic processes, it can be assumed that in cases of high contamination of nectar, honey would not be stored in the hive so efficiently, due to deleterious effects on the global functioning of the beehive (Bogdanov 2006; EFSA 2012a).

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

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

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

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

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

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

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

Interestingly, the maximum residue levels in Table 4 are thiacloprid (1,002 ng/g), imidacloprid (912 ng/g), dinotefuran (168 ng/g), acetamiprid (134 ng/g), thiamethoxam (127 ng/g), clothianidin (41 ng/g), and fipronil (29 ng/g). For each of these compounds, these values must be interpreted with respect to the corresponding data for toxicity. However, these values represent the worst case scenarios. Further examination of exposure data shows that average levels in pollen/beebread are lower than these maximums, due to some data issuing from various types of application techniques (soil treatment, injection, spray, seed dressing, etc.). For example, it has been reported that aerial treatments represent a significantly higher source of contamination than seed-dressing treatments (Thompson 2012; EFSA 2012a). This explains the high variability of results when concentrations are ranked by decades. However, when imidacloprid was used as a seed dressing, mean residue levels were mostly found to be in the range of 1–10 ng/g and variability among crops was not so high (sunflower, maize, and canola), whereas spray or soil application led to higher values, by 1 order of magnitude. To a lesser extent, this was also observed for clothianidin and thiamethoxam. Therefore, averaged data must also be considered to gain a better idea of the average contamination of pollen/beebread: thiacloprid (75 ng/g), dinotefuran (45 ng/g), thiamethoxam (29 ng/g), imidacloprid (20 ng/g), clothianidin (9 ng/g), acetamiprid (3 ng/g), and fipronil (1.6 ng/g) (Sanchez-Bayo and Goka 2014). As a consequence, the latter values are the most relevant for toxicity studies for nontarget species.

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

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

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

Insecticide[a]	Detection rate[b] (%)	Range[c] (ng/g)	Mean[d] or magnitude[e,f] (ng/g)	Maximum[f] (ng/g)	Reference[g]
Acetamiprid	24.1	1–1,000	3	134	Sanchez-Bayo and Goka (2014)
	45	0.1–100	4.1	26.1	Pohorecka et al. (2012)
	3.1	10–1,000	59.3	134	Mullin et al. (2010)
Clothianidin	11	1–100	9.4	41.2	Sanchez-Bayo and Goka (2014)
		0.1–100	0.1[h] to 17.1[h]	21.1[h]	Dively and Kamel (2012)
		1–10	1[i] to 4[i]	7	Pilling et al. (2014)
	11	1–10	1.8	3.7	Pohorecka et al. (2012)
		1–100	3.9	10.7	Krupke et al. (2012)
		1–100			In EFSA (2013a):
			7.38-	36.88	See estimate for maize
			5.95-	19.04	See estimate for rape
				3.29	See estimate for sunflower
				15	See Schöning 2005 (DAR)
		1–10		2.59	Cutler and Scott-Dupree (2007)
		1–10		2.8	Scott-Dupree and Spivak (2001)
		1–10			In EFSA (2012a):
				10.4	See Nikolakis et al. (2009) (DAR)
			2.6-	2.9	See Maus and Schöening (2001) (DAR)
				4.1	See Schmuck and Schöening (2001a) (DAR)
				3.3	See Schmuck and Schöening (2000b) (DAR)
				2.5	See Maus and Schöening (2001c) (DAR)
				3.1	See Schmuck and Schöening (2001d) (DAR)
				5.4	See Maus and Schöening (2001e) (DAR)
			3.3-	6.2	See Maus and Schöening (2001f, g) (DAR)
Dinotefuran	1	10–1,000	45.3	168.1	Sanchez-Bayo and Goka (2014)
	100	10–1,000	11.2 to 88.3+17.1[j]	147+21.1[j]	Dively and Kamel (2012)
	1	1–10	4	7.6	Stoner and Eitzer (2013)
Imidacloprid	16.2	1–1,000	19.7	912	Sanchez-Bayo and Goka (2014)
		0.1–1,000	0.1 to 80.2+19.1[k]	101+27.5[k]	Dively and Kamel (2012)
	9.1	1–1,000	30.8	216	Rennich et al. (2012)
	2.9	1–1,000	39	206+554[l]+152[l]	Mullin et al. (2010)
	40.5	0.1–10	0.9	5.7	Chauzat et al. (2011)
		1–100	14	28	Stoner and Eitzer (2012)
	12.1	1–100	5.2+5.6[l]	70+5.6[l]	Stoner and Eitzer (2013)
		10–100	13	36	Laurent and Rathahao (2003)
	87.2	0.1–100	2.1	18	Bonmatin et al. (2005)
		1–100	9.39	10.2	Byrne et al. (2014)
		1–100	2.6	12	Wiest et al. (2011)
	83	0.1–100	3	11	Bonmatin et al. (2003)
		1–100			In EFSA (2013c):
			3-	15	See Stork (1999) (Germany 2005, DAR)
			3.45-	4.6	See Germany 2005 (DAR)
		1–10			In EFSA (2012a):
			1.56-	8.19	See Schmuck et al. (2001) (DAR)
				3.3	See Stork (1999) (Germany 2005, DAR)
		1–10	4.4-	7.6	Scott-Dupree and Spivak (2001)
	49.4	1–10	1.2		Chauzat et al. (2006)
		1–10	3.3-	3.9	Schmuck et al. (2001)
	0.8	1–10	1.35	<12	Lambert et al. (2013)
		0.1–1		<0.5	Thompson et al. (2013)
Thiacloprid	17.7	100–1,000	75.1	1,002.2	Sanchez-Bayo and Goka (2014)
	62	1–1,000	89.1	1,002.2	Pohorecka et al. (2012)
	2	1–1,000	187.6	326	Rennich et al. (2012)
	5.4	1–1,000	23.8	115	Mullin et al. (2010)

Table 4 (continued)

Insecticide[a]	Detection rate[b] (%)	Range[c] (ng/g)	Mean[d] or magnitude[e,f] (ng/g)	Maximum[f] (ng/g)	Reference[g]
	1.3	1–100	22.3	68	Stoner and Eitzer (2013)
		1–1,000			In EFSA (2012a):
			150-	277	See Von der Ohe (DAR)
			9-	36	See Schatz and Wallner (2009) (DAR)
		1–100	10 to 30	90	Skerl et al. (2009)
Thiamethoxam	12.8	10–1,000	28.9	127	Sanchez-Bayo and Goka (2014)
		0.1–1,000	0.1 to 95.2+26.8[h]	127+35.1[h]	Dively and Kamel (2012)
	0.3 %	10–100	53.3	53.3	Mullin et al. (2010)
		1–100	12	35	Stoner and Eitzer (2012)
	37	1–10	3.8	9.9	Pohorecka et al. (2012)
	1	1–10	2.8	4.1	Stoner and Eitzer (2013)
		1–100	3[i] to 7[i]	12	Pilling et al. (2014)
		1–100	1.7	6.2 to 20.4	Krupke et al. (2012)
		1–100			In EFSA (2013b):
			13.41-	21.51	See estimate for maize
			2.37-	3.02	See estimate for sunflower
			4.59-	19.29	See estimate for rape
			4-	12	See Hecht-Rost (2007); Hargreaves (2007) (DAR)
		1–10	2.3 to 2.7		Thompson et al. (2013)
		0.1–10			In EFSA (2012a):
			2.5-	4.2	See Schuld (2001a) (DAR)
				4.6	See Schuld (2001b) (DAR)
				3.6	See Barth (2001) (DAR)
				1.1	See Balluf (2001) (DAR)
				3.2	See Schur (2001c) (DAR)
6-CNA	33	0.1–10	1.2	9.3	Chauzat et al. (2011)
	57.3	0.1–10	1.2		Chauzat et al. (2009)
	44.4	0.1–10	1.2		Chauzat et al. (2006)
Fipronil	2.8 and 3.7[m]	1–100	1.6	29	Sanchez-Bayo and Goka (2014)
	0.3	1–100	28.5	28.5	Mullin et al. (2010)
	6.5	0.1–10	1.2+1.0+1.7[m]	0.3+1.5+3.7[m]	Chauzat et al. (2011)
	0.6	1–10	2.8	3.5	Stoner and Eitzer (2013)
	3.7[m]	1–10	2 to 2.3[m]	4	Bernal et al. (2010)
	49[m]	0.1–10	0.8[m]	8.3[m]	Bonmatin et al. (2007)
	12.4	0.1–10	1.2	1.2+1.7+1[m]	Chauzat et al. (2009)
		1–10		1.9 and 6.4	In EFSA (2013d): see Kerl (2005) (DAR)

6-CNA (6-chloro-nicotinic acid)

[a] Active ingredient

[b] Proportion of positive analyses (see text)

[c] Classified by decade

[d] Mean value from positive analyses

[e] The lowest value of quantified data is followed by a hyphen, the highest value is in the next column

[f] The highest value of quantified data

[g] The sources are related to the original works for avoiding data duplications, and data from DARs (draft assessment report) are available in the cited EFSA reviews

[h] Clothianidin issuing from thiamethoxam

[i] Median values

[j] When data include the UF (1-methyl-3-(tetrahydro-3-furylmethyl)urea) derivative

[k] When data include the derivatives of imidacloprid (olefin, 5-OH, urea, desnitro olefin, desnitro HCl, and 6-CNA)

[l] When data include the derivatives of imidacloprid (5-OH, olefin, or 6-CNA)

[m] Data include some fipronil derivatives (sulfone-, sufide-, or desulfynyl-fipronil)

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

Insecticide[a]	Detection rate[b] (%)	Range[c] (ng/g)	Mean[d] or magnitude[e,f] (ng/g)	Maximum[f] (ng/g)	Reference[g]
Acetamiprid	51	0.1–100	2.4	13.3	Sanchez-Bayo and Goka (2014); Pohorecka et al. (2012)
		0.1–1,000		112.8	Paradis et al. (2014)
Clothianidin	17	0.1–10	1.9	10.1	Sanchez-Bayo and Goka (2014)
		0.1–100	0.1[h] to 4[h]	12.2[h]	Dively and Kamel (2012)
	17	1–10	2.3	10.1	Pohorecka et al. (2012)
		0.1–10	0.9-	2.2	Cutler and Scott-Dupree (2007); Johnson et al. (2010)
		0.1–1	1[i]	1	Pilling et al. (2014)
	100	10–1,000	89-	319	Larson et al. (2013)
		0.1–100	5	16	Thompson et al. (2013)
		0.1–10	1-	3	Wallner (2009)
		0.1–10			In EFSA (2012a):
			1.2-	8.6	See Schmuk and Shöening (2000a) (DAR)
			0.3-	1	See Maus and Schöening (2002a) (DAR)
			2.8-	3	See Maus and Schöening (2001b) (DAR)
				5.4	See Maus and Schöening (2001c) (DAR)
		0.1–10	0.9-	3.7	Scott-Dupree and Spivak (2001)
		0.1–10	0.32		EFSA (2013a) (estimate)
Dinotefuran		1–100	13.7	21.6	Sanchez-Bayo and Goka (2014)
	100	1–100	2.1+0.1[j] to 9.2+4.1[j]	10.8+10.8[j]	Dively and kamel (2012)
Imidacloprid	21.4	1–100	6	72.8	Sanchez-Bayo and Goka (2014)
		10–100	13.37 to 72.81	95.2	Byrne et al. (2014)
		0.1–100	0.1 to 11.2+6.4[k]	13.7+9.4[k]	Dively and Kamel (2012)
	21.8	0.1–10	0.7	1.8	Chauzat et al. (2011)
		100–1,000		660[j]	Paine et al. (2011)
		100–1,000		171	Larson et al. (2013)
		1–100	6.6+1.1+0.2[l]	16+2.4+0.5[l]	Krischik et al. (2007)
		0.1–100	0.1 to 11.2+6.4[k]	13.7+9.4[k]	Dively and Kamel (2012)
		1–100	10.3	14	Stoner and Eitzer (2012)
		1–10			In EFSA (2012a):
			3.45-	4.6	See Stork (1999) (DAR)
			1.59-	8.35	See Germany (2005) (DAR)
	29.7	0.1–10	0.7+1.2[l]		Chauzat et al. (2009)
		0.1–10	1.9		Schmuck et al. (2001)
	21	0.1–10	0.6	2	Pohorecka et al. (2012)
		0.1–10	0.2[l]-	3.9[l]	Wiest et al. (2011)
	2.1	0.1–10	0.14[l]	<3.9[l]	Lambert et al. (2013)
		0.1–1	0.6-	0.8	Scott-Dupree and Spivak (2001)
		0.1–1	0.45	0.5	Thompson et al. (2013)
Thiacloprid	64	1–1,000	6.5	208.8	Sanchez-Bayo and Goka (2014); Pohorecka et al. (2012)
		1–100	1.8	36	Schatz and Wallner (2009)
		1–100		33	Johnson et al. (2010)
		1–100		11.6	Paradis et al. (2014)
Thiamethoxam	65	0.1–100	6.4	17	Sanchez-Bayo and Goka (2014)
		0.1–100	0.1 to 9.5+4[h]	12.2+6.4[h]	Dively and Kamel (2012)
	65	0.1–100	4.2	12.9	Pohorecka et al. (2012)
		0.1–10	0.7 to 2.4[i]+1[i]	4,7+1	Pilling et al. (2014)
		1–100	11	20	Stoner and Eitzer (2012)
		0.1–10	0.59	4	EFSA (2013b): see Hecht-Rost (2007) (DAR)
		0.1–10		1.5 and 3.9	Thompson et al. (2013)

Table 5 (continued)

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

6-CNA (6-chloro-nicotinic acid)

[a] Active ingredient

[b] Proportion of positive analyses (see text)

[c] Classified by decade

[d] Mean value from positive analyses

[e] The lowest value of quantified data is followed by a hyphen, the highest value is in the next column

[f] The highest value of quantified data

[g] The sources are related to the original works for avoiding data duplications, and data from DARs (draft assessment report) are available in the cited EFSA reviews

[h] Clothianidin issuing from thiamethoxam

[i] Median values

[j] When data include the UF (1-methyl-3-(tetrahydro-3-furylmethyl)urea) derivative

[k] When data include the derivatives of imidacloprid (olefin, 5-OH, urea, desnitro olefin, desnitro HCl, and 6-CNA)

[l] When data include the derivatives of imidacloprid (5-OH, olefin, or 6-CNA)

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

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

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

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

Finally, nontarget species are very likely to be exposed to multiple pesticides (insecticides, herbicides, and several fungicides) simultaneously or at different points in time, and via multiple routes including pollen and nectar. This is especially relevant for treated fruit trees. In the cases of neonicotinoids and fipronil, variability of exposure data remains high between and within studies, due to variability of (1) pesticide applications, (2) the crops considered, (3) the samples analyzed, and (4) measurement methods. Variability will be difficult to improve and assess because field trials demand robust protocols that are difficult to manage, and also the required sensitive analytical techniques are costly to utilize. Therefore, despite the large methodological progress that has been made in the last decade, the question of exposure inherently leads to heterogeneous results and remains the object of discussion. Despite this variability, which does not imply inaccuracy of measurements in real situations, studies worldwide demonstrate the exposure of nontarget species to these pesticides. This exposure, specifically through nectar and pollen, has proved harmful for bees and other pollinators (Pisa et al. 2014, this issue).

Honeydew

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

Conclusion

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

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

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

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

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

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

References

Aajoud A, Raveton M, Azrou-Isghi D, Tissut M, Ravanel P (2008) How can the fipronil insecticide access phloem? J Agric Food Chem 56: 3732–3737

Ahmed N, Kanan H, Inanaga S, Ma Y, Sugimoto Y (2001) Impact of pesticide seed treatments on aphid control and yield of wheat in the Sudan. Crop Prot 20:929–934

Alsayeda H, Pascal-Lorber S, Nallanthigal C, Debrauwer L, Laurent F (2008) Transfer of the insecticide [14C] imidacloprid from soil to tomato plants. Environ Chem Lett 6:229–234

Anon (2006) Draft assessment Report (DAR)—public version—initial risk assessment provided by the Rapporteur Member State Germany for the existing active substance imidacloprid. Volume 3, Annex B, B.8, February 2006. http://dar.efsa.europa.eu/dar-web/provision. Accessed 21 June 2014

APENET (2011) Effects of coated maize seed on honey bees. Report based on results obtained from the third year (2011) activity of the APENET project. http://www.reterurale.it/apenet . Accessed 21 June 2014

Armbrust KL, Peeler HB (2002) Effects of formulation on the run-off of imidacloprid from turf. Pest Manag Sci 58:702–706

Bacey J (2003) Environmental fate of imidacloprid. California Department of Pesticide Regulation, California, p 8

Baird S, Garrison A, Jones J, Avants J, Bringolf R, Black M (2013) Enantioselective toxicity and bioaccumulation of fipronil in fathead minnows (*Pimephales promelas*) following water and sediment exposures. Environ Toxicol Chem 32:222–227

Balsari P, Manzone M, Marucco P, Tamagnone M (2013) Evaluation of seed dressing dust dispersion from maize sowing machines. Crop Prot 51:19–23

Baskaran S, Kookana RS, Naidu R (1999) Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal application rates. Pestic Sci 55:1222–1228

Baur P, Marzouk H, Schönherr J, Terence Grayson B (1997) Partition coefficients of active ingredients between plant cuticle and adjuvants as related to rates of foliar uptake. J Agric Food Chem 45:3659–3665

Belzunces L, Tasei J-N (1997) Report on the effects of seed treatments in sunflower Gaucho® (imidacloprid). Impacts on populations of bee colonies and the honey. Commission d'Etude de la Toxicité des Produits Antiparasitaires à Usage Agricole et des Produits assimilés, Ministère de l'Agriculture, Paris, 27pp

Bernal J, Garrido-Bailon E, Nozal MJD, Gonzalez-Porto AV, Martin-Hernandez R, Diego JC, Jiménez JJ, Bernal JL, Higes M (2010) Overview of pesticide residues in stored pollen and their potential effect on bee colony (*Apis mellifera*) losses in Spain. J Econ Entomol 103:1964–1971

Bijleveld van Lexmond M, Bonmatin JM, Goulson D, Noome DA (2014) Worldwide integrated assessment on systemic pesticides. Global collapse of the entomofauna: exploring the role of systemic insecticides. Environ Sci Pollut Res. doi:10.1007/s11356-014-3220-1

Bobé A, Coste CM, Coope J (1997) Factors influencing the adsorption of fipronil on soils. J Agric Food Chem 45:4861–4865

Bogdanov S (2006) Contaminants of bee products. Apidologie 37:1–18

Blom G, Paulissen M, Vos C, Agricola H (2008) Effecten van klimaatverandering op landbouw en natuur – nationale knelpuntenkaart en adaptatiestrategieën. Report 182; plant research international B.V., pp. I-3. (In Dutch) http://edepot.wur.nl/7956. Accessed 21 June 2014

Bloomfield JP, Williams RJ, Gooddy DC, Cape JN, Guha P (2006) Impacts of climate change on the fate and behaviour of pesticides in surface and groundwater—a UK perspective. Sci Total Environ 369:163–177

Bobe A, Coste CM, Cooper JF (1997) Factors influencing the adsorption of fipronil in soils. J Agric Food Chem 45:4861–4865

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

Bonmatin J-M, Moineau I, Charvet R, Colin ME, Fleche C, Bengsch ER (2005a) Behaviour of imidacloprid in fields. toxicity for honey bees. In: Lichtfouse E, Schwarzbauer J, Robert D (eds). Environmental chemistry. Springer, Berlin. pp. 483–494. http://link.springer.com/chapter/10.1007%2F3-540-26531-7_44. Accessed 21 June 2014

Bonmatin J-M, Marchand PA, Charvet R, Moineau I, Bengsch ER, Colin ME (2005b) Quantification of imidacloprid uptake in maize crops. J Agric Food Chem 53:5336–5341

Bonmatin J-M, Marchand PA, Cotte JF, Aajoud A, Casabianca H, Goutailler G, Courtiade M (2007) Bees and systemic insecticides (imidacloprid, fipronil) in pollen: subnano-quantification by HPLC/MS/MS and GC/MS. In: Del Re AAM, Capri E, Fragoulis, Trevisan M (eds) Environmental fate and ecological effects of pesticide. La Goliardica Pavese, Pavia, (It), pp. 827–824. http://www.cabdirect.org/abstracts/20083103467.html;jsessionid=8EE58D309B91521CB0CFECD7D2568525. Accessed 21 June 2014

Briggs GG, Bromilow RH, Evans AA, Williams M (1983) Relationships between lipophilicity and the distribution of non-ionised chemicals in barley shoots following uptake by the roots. Pestic Sci 14:492–500

Broznic D, Milin C (2012) Effects of temperature on sorption–desorption processes of imidacloprid in soils of Croatian coastal regions. J Env Sci Health B Pestic Food Contam Agric Wastes 47:779–794

Broznic D, Marinic JTM, Juresic GC, Petkovic O, Milin C (2012) Hysteretic behavior of imidacloprid sorption–desorption in soils of Croatian coastal regions. Soil Sediment Contam 21:850–871

Buchholz A, Nauen R (2002) Translocation and translaminar bioavailability of two neonicotinoid insecticides after foliar application to cabbage and cotton. Pest Manag Sci 58:10–16

Bugbee B, Koerner G (2002) Yield comparisons and unique characteristics of the dwarf wheat cultivar 'USU-Apogee' research: super dwarf cultivar studies: 'APOGEE' WHEAT

Burgerstein A (1887) Materialien zu einer monographie betreffend die erscheinungen der transpiration der pflanzen. Zool-Bot Ges Wien 37:691–782

Byrne FJ, Visscher PK, Leimkuehler B, Fischer D, Grafton-Cardwell EE, Morse JG (2014) Determination of exposure levels of honey bees foraging on flowers of mature citrus trees previously treated with imidacloprid. Pest Manag Sci 70:470–482

Carbo L, Dores EFGC, Ribeiro ML (2008) Determination of pesticides multiresidues in shallow groundwater in a cotton-growing region of Mato Grosso, Brazil. J Braz Chem Soc 00(00):1111–1117. http://www.scielo.br/pdf/jbchs/v19n6/a09v19n6.pdf. Accessed 21 June 2014

Carvalho MA, Marques SC, Martos ET, Rigitano RLO, Dias ES (2014) Bioaccumulation of insecticide in *Agaricus subrufescens*. Hortic Bras 32:159–162

CCME (2007) Canadian water quality guidelines; imidacloprid. Scientific supporting document. Canadian Council of Ministers of the Environment, Winnipeg. http://www.ccme.ca/assets/pdf/imidacloprid_ssd_1388.pdf. Accessed 21 June 2014

Chamberlain P (1992) Sprayable agricultural compositions

Chamberlain K, Tench AJ, Williams RH, Bromilow RH (1995) Phloem translocation of pyridinecarboxylic acids and related imidazolinone herbicides in *Ricinus communis*. Pestic Sci 45:69–75

Chagnon M, Kreutzweiser DP, Mitchell EAD, Morrissey CA, Noome DA, van der Sluijs JP (2014) Risks of large scale use of systemic insecticides to ecosystem functioning and services. Environ Sci Pollut Res. doi:10.1007/s11356-014-3277-x

Chauzat MP, Faucon JP, Martel AC, Lachaize J, Cougoule N, Aubert M (2006) A survey of pesticide residues in pollen loads collected by honey bees in France. J Econ Entomol 99:253–262

Chauzat MP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon JP (2009) Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France. Environ Entomol 38:514–523

Chauzat MP, Martel AC, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M, Carpentier P, Faucon JP (2011) An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France. Environ Toxicol Chem 30:103–111

Chopra I, Chauhan R, Kumari B, Dahiya KK (2011) Fate of fipronil in cotton and soil under tropical climatic conditions. Bull Environ Contam Toxicol 86:242–245

Cowles RS, Montgomery ME, Cheah CASJ (2006) Activity and residues of imidacloprid applied to soil and tree trunks to control hemlock woolly adelgid (Hemiptera: Adelgidae) in forests. J Econ Entomol 99:1258–1267

Cox L, Koskinen W, Yen P (1997) Sorption–desorption of imidacloprid and its metabolites in soils. J Agric Food Chem 45:1468–1472

Cox L, Koskinen W, Celis R, Yen P, Hermosin M, Cornejo J (1998a) Sorption of imidacloprid on soil clay mineral and organic components. Soil Sci Soc Am J 62:911–915

Cox L, Koskinen W, Yen P (1998b) Changes in sorption of imidacloprid with incubation time. Soil Sci Soc Am J 62:342–347

Cox L, Koskinen W, Yen P (1998c) Influence of soil properties on sorption–desorption of imidacloprid. J Environ Sci Health B 33:123–134

Cox C (2001) Insecticide factsheet—imidacloprid. J Pestic Reform 21:15–21

Cresswell JE (2011) A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. Ecotoxicology 20:149–157

Curtis LC (1944) The influence of guttation fluid on pesticides. Phytopathology 34:196–205

Cutler GC, Scott-Dupree CD (2007) Exposure to clothianidin seed-treated canola has no long-term impact on honey bees. J Econ Entomol 100:765–772

DeCant J (2010) Clothianidin registration of Prosper T400 seed treatment on mustard seed (oilseed and condiment) and Poncho/Votivo seed treatment on cotton. U.S. Environmental Protection Agency, Washington DC

Demcheck DK, Tollett RW, Mize SV, Skrobialowski SC, Fendick Jr. RB, Swarzenski CM, Porter SD (2004) Water quality in the Acadian-Pontchartrain drainages, Louisiana and Mississippi, 1999–2001: Reston, VA, U.S. Geological Survey Circular 1232, p47

Dieckmann Y, Christian F, Ishaque M, Kerl W, Köhle H et al. (2010a) Agrochemical formulations comprising co-polymers based on ethylenically unsaturated dicarboxylic mono and diesters. Patent No. US 2010/0063167 A1. 1–14

Dieckmann Y, Görth FC, Ishaque M, Kerl W, Köhle H, Kreuz K, Langewald J, Münster I, Picard L (2010b) Agrochemical formulations comprising 1-vinyl-2-pyrrolidinone co-polymers. USA. http://www.google.com/patents/US20100075849. Accessed 21 June 2014

Dieckmann Y, Ishaque M, Muenster I, Picard L, Benz A et al. (2010c) Systemicity enhancers. Patent No. US 2010/0204045 A1, 1–21

Dively GP, Kamel A (2012) Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. J Agric Food Chem 60:4449–4456

Donnarumma L, Pulcini P, Pochi D, Rosati S, Lusco L, Conte E (2011) Preliminary study on persistence in soil and residues in maize of imidacloprid. J Environ Sci Health B 46:469–472

Duby G, Boutry M (2009) The plant plasma membrane proton pump ATPase: a highly regulated P-type ATPase with multiple physiological roles. Pflugers Arch 457:645–655

EFSA (2012a) Panel on Plant Protection Products and their Residues (PPR); Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA J 10(5):2668, 275 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014

EFSA (2012b) Statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe. EFSA J 10(6):2752, 27 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014

EFSA (2013a) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance clothianidin. EFSA J 11(1):3066, 58 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014

EFSA (2013b) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance thiamethoxam. EFSA

J 11(1):3067, 68 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014

EFSA (2013c) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance imidacloprid. EFSA J 11(1):3068, 55 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014

EFSA (2013d) Conclusion on the peer review of the pesticide risk assessment for bees for the active substance fipronil. EFSA J 11(5):3158, 51 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014

EFSA (2013e) Evaluation of the FERA study on bumble bees and consideration of its potential impact on the EFSA conclusions on neonicotinoids. EFSA J 11(6):3242, 20 pp. www.efsa.europa.eu/efsajournal. Accessed 21 June 2014

EFSA (2013f) Guidance on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees). EFSA J 11(7):3295, 266 ppwww.efsa.europa.eu/efsajournal. Accessed 21 June 2014

Fantke P, Juraske R (2013) Variability of pesticide dissipation half-lives in plants. Environ Sci Technol 16:3548–3562

Felsot AS, Cone W, Yu J, Ruppert JR (1998) Distribution of imidacloprid in soil following subsurface drip chemigation. Bull Environ Contam Toxicol 60:363–370

Felsot AS, Ruppert JR (2002) Imidacloprid residues in Willapa bay (Washington state) water and sediment following application for control of burrowing shrimp. J Agric Food Chem 50:4417–4423

Fernandez-Bayo JD, Nogales R, Romero E (2009) Effect of vermicomposts from wastes of the wine and alcohol industries in the persistence and distribution of imidacloprid and diuron on agricultural soils. J Agric Food Chem 57:5435–5442

Flores-Céspedes F, González-Pradas E, Fernández-Pérez M, Villafranca-Sánchez M, Socías-Viciana M, Urea-Amate MD (2002) Effects of dissolved organic carbon on sorption and mobility of imidacloprid in soil. J Environ Qual 31:880–888

Fossen M (2006) Environmental fate of imidacloprid. Environmental Monitoring Department of Pesticide Regulation. 1001 I Street Sacramento, CA, 95812–4015. http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/Imidclprdfate2.pdf. Accessed 21 June 2014

Furlan L, Kreutzweiser DP (2014) Alternatives to neonicotinoid insecticides for pest control: case studies in agriculture and forestry. Environ Sci Pollut Res (this issue)

Gerecke AC, Schärer M, Singer HP, Muller SR, Schwarzenbach RP, Sägesser M, Ochsenbein U, Popow G (2002) Sources of pesticides in surface waters in Switzerland: pesticide load through waste water treatment plants—current situation and reduction potential. Chemosphere 48:307–315

Gibbons D, Morrissey C, Mineau P (2014) A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ Sci Pollut Res. doi:10.1007/s11356-014-3180-5

Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Di Bernardo A, Greatti M, Giorio C, Tapparo A (2009) Translocation of neonicotinoid insecticides from coated seeds to seedling guttations: a novel way of intoxication for bees. J Econ Entomol 102:1808–1815

Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A (2012) Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. J Appl Entomol 136:17–26

Girolami V, Marzaro M, Vivan L, Mazzon L, Giorio C, Marton D, Tapparo A (2013) Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers. J Appl Entomol 137:35–44

Giroux I (2003). (in French) Contamination de l'eau souterraine par les pesticides et les nitrates dans les régions en culture de pommes de terre. Campagne d'échantillonnage de 1999-2000-2001. Direction de suivi de l'état de l'environnement, Ministère de l'Environnement, Gouvernement du Québec, Québec. www.mddep.gouv.qc.ca/pesticides/pomme_terre/Pesticides_pomme_terre.pdf. Accessed 21 June 2014

Goatley JL, Lewis RW (1966) Composition of guttation fluid from rye, wheat, and barley seedlings. Plant Physiol 41:373–375

Gonçalves CM, Silva JC, Alpendurada MF (2007) Evaluation of the pesticide contamination of groundwater sampled over two years from a vulnerable zone in Portugal. J Agric Food Chem 55:6227–6235

Gonzalez-Pradas E, Urena-Amate MD, Flores-Cespedes F, Fernandez-Perez M, Garratt J, Wilkins RJ (2002) Leaching of imidacloprid and procymidone in a greenhouse of southeast of Spain. Soil Sci Soc Am J 66:1821–1828

Goulson D (2013) An overview of the environmental risks posed by neonicotinoid insecticides. J Appl Ecol 50:977–987

Greatti M, Sabatini AG, Barbattini R, Rossi S, Stravisi A (2003) Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results. Bull Insectol 56:69–72

Grosman DM, Upton WW (2006) Efficacy of systemic insecticides for protection of loblolly pine against southern pine engraver beetles (Coleoptera: Curculionidae: Scolytinae) and wood borers (Coleoptera: Cerambycidae). J Econ Entomol 99:94–101

Gunasekara AS, Truong T, Goh KS, Spurlock F, Tjeerdema RS (2007) Environmental fate and toxicology of fipronil. J Pestic Sci 32:189–199

Gupta S, Gajbhiye VT, Kalpana, Agnihotri NP (2002) Leaching behavior of imidacloprid formulations in soil. Bull Environ Contam Toxicol 68:502–508

Gupta S, Gajbhiye VT (2007) Persistence of acetamiprid in soil. Bull Environ Contam Toxicol 78:349–352

Gupta S, Gajbhiye VT, Gupta RK (2008a) Effect of light on the degradation of two neonicotinoids viz acetamiprid and thiacloprid in soil. Bull Environ Contam Toxicol 81:185–189

Gupta S, Gajbhiye VT, Gupta RK (2008b) Soil dissipation and leaching behavior of a neonicotinoid insecticide thiamethoxam. Bull Environ Contam Toxicol 80:431–437

Gustafson DI (1989) Ground water ubiquity score: a simple method for assessing pesticide leachability. Environ Toxicol Chem 8:339–357

Guzsvány V, Csanádi J, Gaál F (2006) NMR study of the influence of pH on the persistence of some neonicotinoids in water. Acta Chim Slov 53:52–57

Haith DA (2010) Ecological risk assessment of pesticide runoff from grass surfaces. Environ Sci Technol 44:6496–6502

Halm MP, Rortais A, Arnold G, Taséi JN, Rault S (2006) New risk assessment approach for systemic insecticides: the case of honey bees and imidacloprid (Gaucho). Environ Sci Technol 40:2448–2454

Hoffman EJ, Castle SJ (2012) Imidacloprid in melon guttation fluid: a potential mode of exposure for pest and beneficial organisms. J Econ Entomol 105:67–71

Horwood MA (2007) Rapid degradation of termiticides under field conditions. Aust J Entomol 46:75–78

Huseth AS, Groves RL (2013) Environmental fate of neonicotinoids: a potato case study. www.soils.wisc.edu/extension/wcmc/2013/pap/Huseth.pdf. Accessed 21 June 2014

Huseth AS, Groves RL (2014) Environmental fate of soil applied neonicotinoid insecticides in an irrigated potato agroecosystem. PLoS One 9(5):1–11

Ishaque M, Jung MR, Tuerk H, Schroeder-Grimonpont T, Reinhard K, Schnabel G, Klein CD, Holt TJ, Mascianica MP (2012) Polymers for increasing the soil mobility of low-solubility insecticides. http://www.google.com/patents/US20120053221. Accessed 21 June 2014

Jeschke P, Nauen R (2008) Neonicotinoids—from zero to hero in insecticide chemistry. Pest Manag Sci 64:1084–1098

Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. J Agric Food Chem 59: 2897–2908

Johnson RM, Ellis MD, Mullin CA, Frazier M (2010) Pesticides and honey bee toxicity—USA. Apidologie 41:312–331

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

Kapoor U, Srivastava MK, Srivastava AK, Patel DK, Garg V, Srivastava LP (2013) Analysis of imidacloprid residues in fruits, vegetables, cereals, fruit juices, and baby foods, and daily intake estimation in and around Lucknow. India Environ Toxicol Chem 32:723–727

Kasiotis KM, Anagnostopoulos C, Anastasiadou P, Machera K (2014) Pesticide residues in honeybees, honey and bee pollen by LC-MS/MS screening: reported death incidents in honeybees. Sci Total Environ 485–486:633–642

Katsuhara M, Hanba YT, Shiratake K, Maeshima M (2008) Expanding roles of plant aquaporins in plasma membranes and cell organelles. Funct Plant Biol 35:1–14

Kattwinkel M, Kühne JV, Foit K, Liess M (2011) Climate change, agricultural insecticide exposure, and risk for freshwater communities. Ecol Appl 21:2068–2081

Knipfer T, Besse M, Verdeil J-L, Fricke W (2011) Aquaporin-facilitated water uptake in barley (*Hordeum vulgare* L.) roots. J Exp Bot 62: 4115–4126

Koch R, Burkness E, Hutchison W, Rabaey T (2005) Efficacy of systemic insecticide seed treatments for protection of early-growth-stage snap beans from bean leaf beetle (Coleoptera: Chrysomelidae) foliar feeding. Crop Prot 24:734–742

Koltzenburg S, Dombo P, Oetter G, Bratz M (2010) Comb polymers and use thereof for the production of active or effective ingredient formulations. USA. http://www.google.com/patents/US20100048655. Accessed 21 June 2014

Koulman A, Lane GA, Christensen MJ, Fraser K, Tapper BA (2007) Peramine and other fungal alkaloids are exuded in the guttation fluid of endophyte-infected grasses. Phytochemistry 68:355–360

Kreuger J, Graaf S, Patring J, Adielsson S (2010) Pesticides in surface water in areas with open ground and greenhouse horticultural crops in Sweden 2008. pp. 49. http://www-mv.slu.se/webfiles/vv/CKB/Ekohydrologi_117_ENG.pdf. Accessed 21 June 2014

Kreutzweiser DP, Good K, Chartrand D, Scarr T, Thompson D (2007) Non-target effects on aquatic decomposer organisms of imidacloprid as a systemic insecticide to control emerald ash borer in riparian trees. Ecotoxicol Environ Saf 68:315–325

Kreutzweiser DP, Thompson DG, Scarr TA (2009) Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates. Ecotoxicol Environ Saf 72:1053–1057

Krischik VA, Landmark AL, Heimpel GE (2007) Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae). Environ Entomol 36:1238–1245

Krohn J, Hellpointner E (2002) Environmental fate of imidacloprid. Pflanzenschutz- Nachrichten Bayer 55:1–25

Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7:e29268

Kühnholz S, Seeley TD (1997) The control of water collection in honey bee colonies. Behav Ecol Sociobiol 41:407–422

Kurwadkar ST, Dewinne D, Wheat R, McGahan DG, Mitchell FL (2013) Time dependent sorption behavior of dinotefuran, imidacloprid and thiamethoxam. J Environ Sci Health B 48:237–242

Lambert O, Piroux M, Puyo S, Thorin C, L'Hostis M, Wiest L, Buleté A, Delbac F, Pouliquen H (2013) Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of western France. PLoS One 8:e67007

Larson JL, Redmond CT, Potter DA (2013) Assessing insecticide hazard to bumble bees foraging on flowering weeds in treated lawns. PLoS One 86:e66375

Laurent FM, Rathahao E (2003) Distribution of [(14)C]imidacloprid in sunflowers (*Helianthus annuus* L.) following seed treatment. J Agric Food Chem 51:8005–8010

Le Faouder J, Bichon E, Brunschwig P, Landelle R, Andre F, Le Bizec B (2007) Transfer assessment of fipronil residues from feed to cow milk. Talanta 73:710–717

Magalhaes LC, Hunt TE, Siegfried BD (2009) Efficacy of neonicotinoid seed treatments to reduce soybean aphid populations under field and controlled conditions in Nebraska. J Econ Entomol 102:187–195

Maienfisch P, Angst M, Brandl F, Fischer W, Hofer D, Kayser H, Kobel W et al (2001) Chemistry and biology of thiamethoxam: a second generation neonicotinoid. Pest Manag Sci 57:906–913

Main AR, Headley JV, Peru KM, Michel NL, Cessna AJ et al (2014) Widespread use and frequent detection of neonicotinoid insecticides in wetlands of Canada's prairie Pothole region. PLoS One 9(3): e92821

Marzaro M, Vivan L, Targa A, Mazzon L, Mori N, Greatti M, Petrucco Toffolo E, Di Bernardo A, Giorio C, Marton D, Tapparo A, Girolami V (2011) Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat. Bull Insectol 64:119–126

Masiá A, Campo J, Vázquez-Roig P, Blasco C, Picó Y (2013) Screening of currently used pesticides in water, sediments and biota of the Guadalquivir river basin (Spain). J Hazard Mater 263(Pt 1):95–104

Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Sattelle DB (2005) Neonicotinoids: insecticides acting on insect nicotinic acetycholine receptors. Trend Pharmacol Sci 22:573–580

Maxim L, van der Sluijs JP (2007) Uncertainty: cause or effect of stakeholders' debates? Analysis of a case study: the risk for honeybees of the insecticide Gaucho. Sci Total Environ 376:1–17

Mesnage R, Defarge N, Spiroux de Vendômois J, Séralini GE (2014) Major pesticides are more toxic to human cells than their declared active principles. Biomed Res Int 2014:179691

Misra A, Tyler G (1999) Influence of soil moisture on soil solution chemistry and concentrations of minerals in the calcicoles *Phleum phleoides* and *Veronica spicata* grown on a limestone soil. Ann Bot 84:401–410

Mize SV, Porter SD, Demcheck DK (2008) Influence of fipronil compounds and rice-cultivation land-use intensity on macroinvertebrate communities in streams of southwestern Louisiana, USA. Environ Pollut 152:491–503

Mohapatra S, Deepa M, Jagdish GK, Rashmi N, Kumar S, Prakash GS (2010) Fate of fipronil and its metabolites in/on grape leaves, berries and soil under semi arid tropical climatic conditions. Bull Environ Contam Toxicol 84:587–591

Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, Vanengelsdorp D, Pettis JS (2010) High levels of miticides and agrochemicals in north American apiaries: implications for honeybee health. PLoS One 5(3):e9754

Mullins JW (1993) Imidacloprid. A new nitroguanidine insecticide. Am Chem Soc Symp Ser 524:183–198

Nauen R, Ebbinghaus-Kintscher U, Schmuck R (2001) Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). Pest Manag Sci 57:577–586

Nauen R, Ebbinghaus-Kintscher U, Salgado VL, Kaussmann M (2003) Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. Pestic Biochem Physiol 76:55–69

Nauen R, Reckmann U, Thomzik J, Thielertand W, Baur P (2008) Biological profile of spirotetramat (Movento®)—a new two-way systemic (ambimobile) insecticide against sucking pest species. Bayer Crop Sci J 61:245–278

Nikolakis A, Chapple A, Friessleben R, Neumann P, Schad T, Schmuck R, Schnier H, Schnorbach H, Schoning R, Maus C (2009) An effective risk management approach to prevent bee damage due to the emission of abraded seed treatment particles during sowing of seeds treated with bee toxic insecticides. Julius Kühn Archiv 423:132–148

Nuyttens D, Devarrewaere W, Verboven P, Foqué D (2013) Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review. Pest Manag Sci 69:564–575

Overmyer JP, Mason BN, Armbrust KL (2005) Acute toxicity of imidacloprid and fipronil to a nontarget aquatic insect, *Simulium vittatum* Zetterstedt cytospecies IS-7. Bull Environ Contam Toxicol 74:872–879

Paine TD, Hanlon CC, Byrne FJ (2011) Potential risks of systemic imidacloprid to parasitoid natural enemies of a cerambycid attacking eucalyptus. Biol Control 56:175–178

Paradis D, Bérail G, Bonmatin JM, Belzunces LP (2014) Sensitive analytical methods for 22 relevant insecticides of 3 chemical families in honey by GC-MS/MS and LC-MS/MS. Anal Bioanal Chem 406:621–633

Pareja L, Colazzo M, Pérez-Parada A, Niell S, Carrasco-Letelier L, Besil N, Cesio MV et al (2011) Detection of pesticides in active and depopulated beehives in Uruguay. Int J Environ Res Public Health 8:3844–3858

Peña A, Rodríguez-Liébana JA, Mingorance MD (2011) Persistence of two neonicotinoid insecticides in wastewater, and in aqueous solutions of surfactants and dissolved organic matter. Chemosphere 84: 464–470

PMRA; Pest Management Regulatory Authority (2013) Action to protect bees from exposure to neonicotinoid pesticides. Published by Health Canada in Ottawa, ON. 4 pp. http://www.hc-sc.gc.ca/cps-spc/alt_formats/pdf/pest/part/consultations/_noi2013-01/noi2013-01-eng.pdf. Accessed 21 June 2014

PPDB (2012) Pesticide properties database. http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm. Accessed 21 June 2014

Pierobon M, Bouillo N, Lange RFM, Meyer K, Kolter K (2008) Use of amphiphilic copolymers as solubilising agents. http://www.google.com/patents/US20080153925. Accessed 21 June 2014

Pilling E, Campbell P, Coulson M, Ruddle N, Tornier I (2014) A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. PLoS One 8:e77193

Pisa L, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs C, Goulson D, Kreutzweiser D, Krupke C, Liess M, McField M, Morrissey C, Noome DA, Settele J, Simon-Delso N, Stark J, van der Sluijs, van Dyck H, Wiemers M (2014) Effects of neonicotinoids and fipronil on non-target invertebrates. Environ Sci Pollut Res (this issue)

Pistorius J, Bischoff G, Heimbach U, Stähler M (2009) Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. Julius Kühn Archiv 423:118–126

Pistorius J, Brobyn T, Campbell P, Forster R, Lortsch JA, Marolleau F, Maus C, Luckmann J, Suzuki H, Wallner K, Becker R (2012) Assessment of risks to honey bees posed by guttation. Julius-Kühn-Archiv 437:199–208

Pochi D, Biocca M, Fanigliulo R, Pulcini P, Conte E (2012) Potential exposure of bees, *Apis mellifera L.*, to particulate matter and pesticides derived from seed dressing during maize sowing. Bull Environ Contam Toxicol 89:354–361

Pohorecka K, Skubida P, Miszczak A, Semkiw P, Sikorski P, Zagibajło K, Teper D, Kołtowski Z, Zdańska D, Skubida M, Bober A (2012) Residues of neonicotinoid insecticides in bee collected plant materials from oilseed rape crops and their effect on bee colonies. J Apic Sci 56:115–134

Poland TM, Haack RA, Petrice TR, Miller DH, Bauer LS (2006) Laboratory evaluation of the toxicity of systemic insecticides for control of *Anoplophora glabripennis* and *Plectrodera scalator* (Coleoptera: Cerambycidae). J Econ Entomol 99:85–93

Reetz JE, Zühlke S, Spiteller M, Wallner K (2011) Neonicotinoid insecticides translocated in guttated droplets of seed-treated maize and wheat: a threat to honeybees? Apidologie 42:596–606

Rennich K, Pettis J, van Engelsdorp D, Bozarth R, Eversole H, Roccasecca K, Smith M, Stitzinger J, Andree M, Snyder R, Rice N, Evans J, Levi V, Lopez D, Rose R (2012) 2011–2012 National honey bee pests and diseases survey report. USDA. 17 p. http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/2011_National_Survey_Report.pdf. Accessed 21 June 2014

Rigitano RLO, Bromilow RH, Briggs GG, Chamberlain K (1987) Phloem translocation of weak acids in *Ricinus communis*. Pestic Sci 19:113–133

Rortais A, Arnold G, Halm MP, Touffet-Briens F (2005) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36:71–83

Rouchaud J, Gustin F, Wauters A (1994) Soil biodegradation and leaf transfer of insecticide imidacloprid applied in seed dressing in sugar beet crops. Bull Environ Contam Toxicol 53:344–350

Sanchez-Bayo F, Goka K (2006) Ecological effects of the insecticide imidacloprid and a pollutant from antidandruff shampoo in experimental rice fields. Environ Toxicol Chem 25:1677–1687

Sanchez-Bayo F, Goka K (2014) Pesticide residues and bees—a risk assessment. PLoS One 9(4):e94482

Sarkar MA, Biswas PK, Roy S, Kole RK, Chowdhury A (1999) Effect of pH and type of formulation on the persistence of imidacloprid in water. Bull Environ Contam Toxicol 63:604–609

Sarkar MA, Roy S, Kole RK, Chowdhury A (2001) Persistence and metabolism of imidacloprid in different soils of West Bengal. Pest Manag Sci 57:598–602

Schatz F, Wallner K (2009) Pflanzenschutzmittelapplikation in blühenden raps (*Brassica napus*) und deren auswirkungen auf die rückstandssituation in honig, nektar und pollen der honigbiene (*Apis mellifera L.*). Thesis (Diploma), Universität Hohenheim, Hohenheim, Germany

Schmuck R, Schöning R, Stork A, Schramel O (2001) Risk posed to honeybees (*Apis mellifera L*, Hymenoptera) by an imidacloprid seed dressing of sunflowers. Pest Manag Sci 57:225–238

Schnier HF, Wenig G, Laubert F, Simon V, Schmuck R (2003) Honey bee safety of imidacloprid corn seed treatment. Bull Insectol 56:73–76

Scorza RP, Smelt JH, Boesten JJTI, Hendriks RFA, Van der Zee SEATM (2004) Vadose zone processes and chemical transport: preferential flow of bromide, bentazon, and imidacloprid in a Dutch clay soil. J Environ Qual 33:1473–1486

Scott-Dupree CD, Spivak M (2001) The impact of Gaucho and TI-435 seed-treated canola on honey bees, *Apis mellifera* L., Université de Guelf, Ontario Canada Université

Selim HM, Jeong CY, Elbana TA (2010) Transport of Imidacloprid in soils: miscible displacement experiments. Soil Sci 175:375–381

Shawki MAA, Titera D, Kazda J, Kohoutkova J, Taborsky V (2005) Toxicity to honeybees of water guttation and dew collected from winter rape treated with Nurelle D®. Plant Protect Sci 42:9–14

Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, van Dyck H, van Praagh J, van der Sluijs JP, Whitehorn PR, Wiemers M (2014) Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites Environ Sci Pollut Res (this issue)

Singh S, Singh TN (2013) Guttation 1: chemistry, crop husbandry and molecular farming. Phytochem Rev 12:147–172

Singh S, Singh TN, Chauhan JS (2009a) Guttation in rice: occurrence, regulation, and significance in varietal improvement. J Crop Improv 23:351–365

Singh S, Singh TN, Chauhan JS (2009b) Water transport in crop plants with special reference to rice: key to crop production under global water crisis. J Crop Improv 23:194–212

Skerl MIS, Bolta SV, Cesnik HB, Gregorc A (2009) Residues of pesticides in honeybee (*Apis mellifera carnica*), bee bread and in pollen loads from treated apple orchards. Bull Environ Contam Toxicol 83: 374–377

Skrobialowski SC, Mize SV, Demcheck DK (2004) Environmental setting, water quality, and ecological indicators of surface water quality in the Mermentau river basin, southwestern Louisiana, 1998–2001. US Geological survey—resources investigations report 03-4185. http://water.usgs.gov/nawqa/bib/pubs.php?state=LA. Accessed 21 June 2014

Spiteller M (1993) Aerobic metabolism of imidacloprid, 14C-NTN 33893, in an aquatic model ecosystem. Bayer AG, Report No. PF3950

Starner K, Goh KS (2012) Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010–2011. Bull Environ Contam Toxicol 88: 316–321

Stoner KA, Eitzer BD (2012) Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (*Cucurbita pepo*). PLoS One 7(6):e39114

Stoner KA, Eitzer BD (2013) Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in Connecticut. PLoS One 8:e77550

Suchail S, Guez D, Belzunces LP (2001) Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. Environ Toxicol Chem SETAC 20:2482–2486

Sungur S, Tunur C (2012) Investigation of pesticide residues in vegetables and fruits grown in various regions of Hatay, Turkey. Food Addit Contam B Surveill 5:265–267

Sur R, Stork A (2003) Uptake, translocation and metabolism of imidacloprid in plants. Bull Insectol 56:35–40

Tanner G (2010) Development of a method for the analysis of neonicotinoid insecticide residues in honey using LC-MS/MS and investigations of neonicotinoid insecticides in matrices of importance in apiculture. Universität Wien, Vienna. http://othes.univie.ac.at/9119/ . Accessed 21 June 2014

Tapparo A, Giorio C, Marzaro M, Marton D, Soldà L, Girolami V (2011) Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds. J Environ Monit 13: 1564–1568

Tapparo A, Marton D, Giorio C, Zanella A, Soldà L, Marzaro M, Vivan L, Girolami V (2012) Assessment of the environmental exposure of honeybees to particulate matter containing neonicotinoid insecticides coming from corn coated seeds. Environ Sci Technol 46: 2592–2599

Tattar TA, Dotson JA, Ruizzo MS, Steward VB (1998) Translocation of imidacloprid in three tree species when trunk- and soil-injected. J Arboric 24:54–56

Thompson HM (2012) Interaction between pesticides and other factors in effects on bees. Supporting Publications 2012: EN-340. 204 pp. http://www.efsa.europa.eu/fr/publications.htm?text=thompson+2012+bee&p=10. Accessed 21 June 2014

Thompson H, Harrington P, Wilkins S, Pietravalle S, Sweet D, Jones A (2013) Effects of neonicotinoid seed treatments on bumble bee colonies under field conditions. The Food and Environment Research Agency, Sand Hutton, York, UK. March 2013. http://fera.co.uk/ccss/documents/defraBumbleBeeReportPS2371V4a.pdf. Accessed 21 June 2014

Thuyet DQ, Jorgenson BC, Wissel-Tyson C, Watanabe H, Young TM (2012) Wash off of imidacloprid and fipronil from turf and concrete surfaces using simulated rainfall. Sci Total Environ 414:515–524

Thuyet DQ, Watanabe H, Ok J (2013) Effect of pH on the degradation of imidacloprid and fipronil in paddy water. J Pestic Sci 38:223–227

Tingle CCD, Rother JA, Dewhurst CF, Lauer S, King WJ (2003) Fipronil: environmental fate, ecotoxicology and human health concerns. Rev Environ Contam 176:1–66

Tišler T, Jemec A, Mozetic B, Trebse P (2009) Hazard identification of imidacloprid to aquatic environment. Chemosphere 76:907–914

Tomizawa M, Casida JE (2003) Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu Rev Entomol 48:339–364

Tomizawa M, Casida JE (2005) Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu Rev Pharmacol Toxicol 45: 247–268

Trapp S (2004) Plant uptake and transport models for neutral and ionic chemicals. Environ Sci Pollut Res 11:33–39

US EPA (1993) EFGWB review of imidacloprid, environmental fate and groundwater branch. United States Environmental Protection Agency, Washington, DC

US EPA (1993b) Comparison of the leaching potential of imidacloprid (NTN) to other turf insecticides considered in the preliminary turf cluster assessment. Memo from J. Wolf, soil scientist, to H. Jacoby, chief. Washington, DC. June 15

US EPA (1996) Fipronil pesticide fact sheet. EPA-737-F-96-005. EPA, Washington DC

US EPA (2003) Office of pesticide programs, factsheet clothianidin. EPA Publ 7501C

US EPA (2005) Pesticide fate database. Environmental fate and effects division of the office of pesticide programs

US EPA (2008) Imidacloprid summary document registration review: Initial docket December 2008. Docket Number: EPA-HQ-OPP-2008-0844

US EPA (2010) Environmental fate and ecological risk assessment for the registration of clothianidin for use as a seed treatment on mustard seed (oilseed and condiment) and cotton

US EPA (2013) Pollinator risk assessment framework. http://www.epa.gov/scipoly/sap/meetings/2012/september/091112transcript.pdf. Accessed 21 June 2014

Vaknin Y, Gan-Mor S, Bechar A, Ronen B, Eisikowitch B (2000) The role of electrostatic forces in pollination. Plant Syst Evol 222:133–142

Van der Geest B (2012) Bee poisoning incidents in the Pomurje region of eastern Slovenia in 2011. Julius Kühn Archiv 437:124. http://pub.jki.bund.de/index.php/JKA/article/download/1962/2338. Accessed 21 June 2014

Van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin J-M, Belzunces LP (2013) Neonicotinoids, bee disorders and the sustainability of pollinator services. Curr Opin Environ Sustain 5:1–13

Van der Sluijs JP, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Simon-Delso N, Stark JD, Tapparo A, van Dyck H, van Praagh J, Whitehorn PR, Wiemers M (2014) Conclusions of the worldwide integrated assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. Environ Sci Pollut Res. doi:10.1007/s11356-014-3229-5

Van der Werf HMG (1996) Assessing the impact of pesticides on the environment. Agric Ecosyst Environ 60:81–96

Van Dijk TC, Van Staalduinen MA, Van der Sluijs JP (2013) Macro-invertebrate decline in surface water polluted with imidacloprid. PLoS ONE 8(5):e62374

Visscher PK, Crailsheim K, Sherman G (1996) How do honey bees (*Apis mellifera*) fuel their water foraging flights? J Insect Physiol 42: 1089–1094

Vollner L, Klotz D (1997) Leaching and degradation of pesticides in groundwater layers. In: Agency IAE (ed). Environmental behaviour of crop protection chemicals. pp. 187–203. Vienna, Austria

Wallner K (2009) Sprayed and seed dressed pesticides in pollen, nectar and honey of oilseed rape. Julius Kuhn archive 423:152–153

Westwood F, Bean KM, Dewar AM, Bromilow RH, Chamberlain K (1998) Movement and persistence of [14C]imidacloprid in sugar-beet plants following application to pelleted sugar-beet seed. Pestic Sci 52:97–103

Whitehorn PR, O'connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336:351–352

Wiest L, Buleté A, Giroud B, Fratta C, Amic S, Lambert O, Pouliquen H, Arnaudguilhem C (2011) Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection. J Chromatogr A 12185743–5756

Winterlin W, Walker G, Luce A (1973) Carbaryl residues in bees, honey, and bee bread following exposure to carbaryl via the food supply. Arch Environ Contam Toxicol 1:362–374

Ying G-G, Kookana RS (2006) Persistence and movement of fipronil termiticide with under-slab and trenching treatments. Environ Toxicol Chem SETAC 25:2045–2050

Zheng W, Liu W (1999) Kinetics and mechanism of the hydrolysis of imidacloprid. Pest Manag Sci 55:482–485

Zywitz D, Anastassiades M, Scherbaim E (2004) Analysis of neonicotinoid insecticides in fruits and vegetables using LC-MS (MS). EPRW 2004. http://www.rfb.it/bastaveleni/documenti/poster_neonicotinoid_insecticides_eprw. Accessed 21 June 2014

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

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Environmental Sustainability

Neonicotinoids, bee disorders and the sustainability of pollinator services

Jeroen P van der Sluijs[1], Noa Simon-Delso[1], Dave Goulson[2], Laura Maxim[3], Jean-Marc Bonmatin[4] and Luc P Belzunces[5]

In less than 20 years, neonicotinoids have become the most widely used class of insecticides with a global market share of more than 25%. For pollinators, this has transformed the agrochemical landscape. These chemicals mimic the acetylcholine neurotransmitter and are highly neurotoxic to insects. Their systemic mode of action inside plants means phloemic and xylemic transport that results in translocation to pollen and nectar. Their wide application, persistence in soil and water and potential for uptake by succeeding crops and wild plants make neonicotinoids bioavailable to pollinators at sublethal concentrations for most of the year. This results in the frequent presence of neonicotinoids in honeybee hives. At field realistic doses, neonicotinoids cause a wide range of adverse sublethal effects in honeybee and bumblebee colonies, affecting colony performance through impairment of foraging success, brood and larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene etc. Neonicotinoids exhibit a toxicity that can be amplified by various other agrochemicals and they synergistically reinforce infectious agents such as *Nosema ceranae* which together can produce colony collapse. The limited available data suggest that they are likely to exhibit similar toxicity to virtually all other wild insect pollinators. The worldwide production of neonicotinoids is still increasing. Therefore a transition to pollinator-friendly alternatives to neonicotinoids is urgently needed for the sake of the sustainability of pollinator ecosystem services.

Addresses
[1] Environmental Sciences, Copernicus Institute, Utrecht University, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands
[2] School of Life Sciences, University of Sussex, UK
[3] Institut des Sciences de la Communication, CNRS UPS 3088, Paris, France
[4] Centre de Biophysique Moléculaire, UPR 4301 CNRS affiliated to Orléans University and to INSERM, 45071 Orléans cedex 02, France
[5] INRA, UR 406 Abeilles & Environnement, Laboratoire de Toxicologie Environnementale, CS 40509, Avignon, France

Corresponding author: van der Sluijs, Jeroen P (j.p.vandersluijs@uu.nl)

Current Opinion in Environmental Sustainability 2013, **5**:293–305

This review comes from a themed issue on **Open issue**

Edited by **Rik Leemans** and **William Solecki**

For a complete overview see the Issue and the Editorial

Available online 6th June 2013

http://dx.doi.org/10.1016/j.cosust.2013.05.007

Introduction

The introduction to the market in the early 1990s of imidacloprid and thiacloprid opened the neonicotinoid era of insect pest control [1]. Acting systemically, this new class of neurotoxic insecticides is taken up by plants, primarily through the roots, and translocates to all parts of the plant through xylemic and phloemic transport [2]. This systemic property combined with very high toxicity to insects enabled formulating neonicotinoids for soil treatment and seed coating with typical doses from 10 to 200 g ha^{-1} high enough to provide long lasting protection of the whole plant from pest insects.

Neonicotinoids interact with the nicotinic acetylcholine receptors (nAChRs) of the insect central nervous system. They act mainly agonistically on nAChRs on the postsynaptic membrane, mimicking the natural neurotransmitter acetylcholine by binding with high affinity [3–5,6••,7•,8••]. This induces a neuronal hyper-excitation, which can lead to the insect's death within minutes [6,9]. Some of the major metabolites of neonicotinoids are equally neurotoxic, acting on the same receptors [10–12] thereby prolonging the effectiveness as systemic insecticide. The nAChR binding sites in the vertebrate nervous system are different from those in insects, and in general they have lower numbers of nicotinic receptors with high affinity to neonicotinoids, which are the reasons that neonicotinoids show selective toxicity for insects over vertebrates [9,13].

The main neonicotinoids presently on the market are imidacloprid, thiamethoxam, clothianidin, thiacloprid, dinotefuran, acetamiprid, nitenpyram and sulfoxaflor [12,14,15]. Since their introduction, neonicotinoids have grown to become the most widely used and fastest

growing class of insecticides with a 2010 global market share of 26% of the insecticide market [16] and imidacloprid the second most widely used (2008) agrochemical in the world [17]. The worldwide production of neonicotinoids is still increasing [18]. Large-scale use in Europe and US started around 2004. Neonicotinoids are nowadays authorised in more than 120 countries for more than 1000 uses [19] for the treatments of a wide range of plants including potato, rice, maize, sugar beets, cereals, oil rapeseed, sunflower, fruit, vegetables, soy, ornamental plants, tree nursery, seeds for export, and cotton.

When used as a seed coating, only 1.6–20% of the amount of active substance applied actually enters the crop to protect it [20], and the remaining 80–98.4% pollutes the environment without any intended action to plant pests. Diffusion and transformation of pesticides in the environment lead to various environmental concentrations and bioavailability, all strongly dependent on the properties of the substance [21]. Because of their high leaching potential, neonicotinoids tend to contaminate surface water and ground water [22–25]. Owing to sorption to organic matter in soil and sediments [24,26], the equilibrium partitioning over soil and water varies with soil type and is typically 1:3 ($\log P = 0.57$) [25]. In countries where monitoring data are available, high levels of neonicotinoid pollution in surface water have been reported [27–30]. In the Netherlands, 45% of 9037 water samples taken from 801 different locations in a nation-wide routine water quality monitoring scheme, over the period 1998 and 2003–2009, exceeded the 13 ng l^{-1} imidacloprid water quality standard, the median concentration being 80 ng l^{-1} and the maximum concentration found being 320 μg l^{-1}, which is acutely toxic to honeybees [27]. In the US, neonicotinoids were also found in surface water. In 108 water samples collected in 2005 from playa wetlands on the Southern High Plains, thiamethoxam was found at an average concentration of 3.6 μg l^{-1} and acetamiprid at 2.2 μg l^{-1} [30].

Neonicotinoids and their metabolites are highly persistent in soil, aquatic sediments and water. To give an example: Six years after a single soil drench application of imidacloprid, residue levels up to 19 μg kg^{-1} could be recovered in *Rhododendron* shrub blossoms [31]. Clothianidin has a half-life in soil between 148–6900 days [32], and imidacloprid 40–997 days [33]. Consequently, neonicotinoids exhibit a potential for accumulation in soil following repeated applications [23] and can be taken up by succeeding crops up to at least two years after application [34]. Imidacloprid has been detected in 97% of 33 soil samples from untreated fields on which treated corn seeds were used 1 or 2 years before the sampling [34]. Concentrations in these soil samples ranged from 1.2 to 22 μg kg^{-1} [34]. Several studies recovered neonicotinoids in wild flowers near treated fields [35,36••]. However, it remains a knowledge gap to what extent the presence in wild flowers results from systemic uptake from polluted soil and water or from direct contamination of the flowers by contaminated dust from seed drilling.

At their introduction, neonicotinoids were assumed to be more efficient than the organophosphates and carbamates that they replaced [37]. As a seed treatment, they could be used in much lower quantities and they promised to be less polluting to the environment. It is however not the quantity that is relevant but the potency to cause harm, which results from toxicity, persistence and bioavailability to non-target species. Indeed, soon after the introduction of neonicotinoids, exposure to its residues in pollen, nectar, sowing dust etc., of non-target pollinating insects became clear. This led to various harmful effects [10,37,38,39••,40,41,42••,43••].

Ecosystem services of pollinators

Amongst the wide diversity of pollinating species [44], bees are the most important. Although bee research mostly focuses on the domesticated *Apis mellifera*, over 25,000 different bee species have been identified (FAO: Pollination; URL: http://www.fao.org/agriculture/crops/core-themes/theme/biodiversity/pollination/en/). Bees provide a vital ecosystem service, playing a key role in the maintenance of biodiversity and in food and fibre production [45–47,48••,49–51]. Pollination comprises an integrated system of interactions that links earth's vegetation, wildlife and human welfare [52]. Of all flowering plants on earth, 87.5% benefits from animal pollination [53]. Globally, 87 of the leading food crops (accounting for 35% of the world food production volume) depend on animal pollination [45]. Pollinator mediated crops are of key importance in providing essential nutrients in the human food supply [54•]. The history of apiculture goes back to pre-agricultural times [55,56] and later co-developed with agriculture [57,58]. In addition, wild bees deliver a substantial and often unappreciated portion of pollination services to agriculture and wildflowers [59,60]. Bees and apiary products have a pharmacological [61,62], scientific and technological [63], poetic [64], aesthetic (springs filled with buzzing bumblebees) culinary (e.g., keeping alive traditional cuisine of patisseries with honey) and cultural value.

Global pollinator decline and emerging bee disorders

Long-term declines have been observed in wild bee populations around the world [47,65–70]. Over the past decades, a global trend of increasing honeybee disorders and colony losses has emerged [71–77]. Winter mortality of entire honeybee colonies has risen in many parts of the world [72•,73,74,75•]. When neonicotinoids were first used, beekeepers started describing different disorders and signs ranging from: bees not returning to the hive, disoriented bees, bees gathered close together in small groups on the ground, abnormal foraging behaviour, the

occurrence of massive bee losses in spring, queen losses, increased sensitivity to diseases and colony disappearance [38,40–43,77]. None of these individual signs is a unique effect of neonicotinoids, other causal factors or other agrochemicals could produce similar signs, which complicates the establishment of a causal link.

Scientific research appears to indicate no single cause explaining the increase in winter colony losses. All viruses and other pathogens that have been linked to colony collapse have been found to be present year-round also in healthy colonies [78]. That colonies remain healthy despite the presence of these infectious agents, supports the theory that colony collapse may be caused by factors working in combination. Farooqui [79•] has analysed the different hypotheses provided by science when searching for an explanation of Colony Collapse Disorder (CCD). Research points in the direction of a combination of reciprocally enhancing causes. Among those, the advance of neonicotinoid insecticides has gained more weight in light of the latest independent scientific results [80,81••,82••]. In the present article, we synthesise the state of knowledge on the role of neonicotinoids in pollinator decline and emerging bee disorders.

Multiple ways of exposure

Neonicotinoids are authorised for a wide range of agricultural and horticultural plants that flower at different times of the year. The systemic properties of neonicotinoids imply translocation to pollen, nectar, and guttation droplets [34,37,83,84]. The persistency and potential contamination of wild plants and trees surrounding the treated crops [36] and the possibility for travelling far outside the fields via surface and ground water [27] and the potential to contaminate wild plants and crops that take up polluted water, means that pollinating insects are likely to be exposed for much of the year to multiple sources of multiple neonicotinoids in their foraging area, but often at very low doses.

Honeybees' exposure to neonicotinoids can occur through ingestion, contact and inhalation (aerosols). Many possible exposure pathways can exist [85•]. Here, we aggregate exposure pathways into: first, intake of food that contain residues; second, nesting material (resin, wax etc.); third, direct contact with spray drift and dust drift during application; fourth, contact with contaminated plants, soil, water; fifth, use of cooling water in the hive; and sixth, inhalation of contaminated air. For bumble bees and other wild bees that nest in soil, contact with contaminated soil is an additional pathway of concern. Leafcutter bees use cut leaf fragments to form nest cells and can thus be exposed to residues in leaves. There are many other conceivable exposure routes, for instance, a bee hive could have been made from timber from trees treated with neonicotinoids and may thus contain residues. However, the best researched exposure pathway is via intake of food. Food with residues can be subdivided into self-collected raw food (nectar, pollen, water, honeydew, extrafloral nectar, guttation droplets, various other edible substances available in the foraging area etc.), in-hive processed food (honey, beebread, royal jelly, wax etc.), and food supplied by bee keepers (high fructose corn syrup, sugar water, sugar dough, bee candy, pollen, pollen substitutes based on soybean flower and other vegetable protein supplements etc.).

Given the large numbers of crops in which neonicotinoids are used and the large scale of use, there is a huge variability in space and time for each possible exposure pathway as well as in their relative importance for the overall exposure at a given place and time. This is further complicated by the fact that the foraging area of a honeybee colony can extend to a radius of up to 9 km around the hive which is never a homogenous landscape [86]. Additionally, suburban areas have become a stronghold for some wild bee species due to the abundance of floral resources in gardens and parks [87]. Thus, bees may be exposed to systemic insecticides which are widely used on garden flowers, vegetables, ornamental trees, and lawns. The relative importance of exposure pathways will also vary according to bee species as they have different foraging ranges, phenologies, and flight times in a day. This can be exemplified by *Osmia* bees in corn growing areas for which intake of guttation droplets may be more important than for honeybees.

Different categories of honeybees could be exposed in different ways and to varying extents [42]. For example, pollen foragers (which differ from nectar foragers) do not consume pollen, merely bringing it to the hive. The pollen is consumed by nurse bees and to a lesser extent by larvae which are thus the ones that are exposed to residues of neonicotinoids and their metabolites [88]. The exposure of nectar foragers to residues of neonicotinoids and metabolites in the nectar they gather can vary depending on the resources available in the hive environment. In addition, foragers take some honey from the hive before they leave for foraging. Depending on the distance from the hive where they forage, the honeybees are obliged to consume more or less of the nectar/honey taken from the hive and/or of the nectar collected, for energy for flying and foraging. They can therefore ingest more or less neonicotinoid residues, depending on the foraging environment [42]. Oral uptake is estimated to be highest for forager honeybees, winter honeybees and larvae [85].

Little is known about the real exposure to contaminated food for different categories of honeybees in a colony, either in terms of contact with pollen or contact with, and possible consumption of, nectar if needed. For wild bees very few data exist on exposure in the field. The amount that wild bees actually consume in the field has not been

measured. EFSA estimated that worker bees, queens and larvae of bumblebees and adult females and larvae of solitary bees are likely to have the highest oral uptake of residues [85].

In 2002, 69% of pollen samples collected by honeybees at various places in France contained residues of imidacloprid and its metabolites [89]. In a systematic sampling scheme covering 5 locations over 3 years, imidacloprid was found in 40.5% of the pollen samples and in 21.8% of the honey samples [90,91]. On the basis of data from authorisation authorities, neonicotinoid residues in nectar and pollen of treated crop plants are estimated to be in the range of below analytical detection limit (0.3 $\mu g\ kg^{-1}$) to 5.4 $\mu g\ kg^{-1}$ in nectar, the highest value corresponding to clothianidin in oilseed rape nectar, and a range of below detection limit (0.3 $\mu g\ kg^{-1}$) to 51 $\mu g\ kg^{-1}$ in pollen, the highest value corresponding to thiamethoxam in alfalfa pollen [85]. A recent review reports wider ranges for pollen: 0.2–912 $\mu g\ kg^{-1}$ for imidacloprid and 1.0–115 $\mu g\ kg^{-1}$ for thiacloprid [92]. Residues of imidacloprid, dinotefuran, and thiamethoxam plus metabolites in pumpkin treated with United States label rates reach average levels up to 122 $\mu g\ kg^{-1}$ in pollen and 17.6 $\mu g\ kg^{-1}$ in nectar [93]. Up to 346 $mg\ l^{-1}$ for imidacloprid and 146 $mg\ l^{-1}$ for thiamethoxam and 102 $mg\ l^{-1}$ clothianidin and have been found in guttation drops from leaves of plants germinated from neonicotinoid-coated seeds [84,94]. In melon, guttation levels up to 4.1 $mg\ l^{-1}$ imidacloprid were found 3 days after a top (US) label rate soil application [95]. In a US wide survey of pesticide residues in beeswax, pollen and honeybees during the 2007–2008 growing seasons, high levels of neonicotinoids were found in pollen (included in [92]) but imidacloprid was also found up to 13.6 $\mu g\ kg^{-1}$ in wax [96]. In Spain, neonicotinoids were found in beeswax samples from apiaries near fruit orchards: 11 out of 30 samples tested positive in ranges from 11 $\mu g\ kg^{-1}$ (acetamiprid) to 153 $\mu g\ kg^{-1}$ (thiacloprid) [97].

Little is known on the presence of neonicotinoids in honeydew. Given differences in life span of aphids and bees, concentrations in plant sap too low to kill aphids could translocate to honeydew and could still produce sublethal effects and chronic toxicity mortality in bees and bee colonies.

Acute and chronic effects of lethal and sublethal exposure

Pesticides can produce four types of effects on honeybees: lethal effects and sublethal effects from acute or chronic exposures.

Acute toxicity is expressed as the lethal dose (LD) at which 50% of the exposed honeybees die within 48 hours: abbreviated to 'LD50 (48 hours)'. Neonicotinoids are highly toxic (in the range of ng/bee) to honeybees [98], both when administered orally and by contact. They also have high acute toxicity to all other bee species so far tested, including various *Bombus* species, *Osmia lignaria* and *Megachile rotundata* [99–102]. *O. lignaria* is more sensitive to both clothianidin and imidacloprid than is *B. impatiens*, with *M. rotundata* more sensitive still [100]. In an acute toxicity test under semi field conditions on the Indian honeybee *Apis cerana indica*, clothianidin showed the highest toxicity, followed by imidacloprid and thiamethoxam [103].

For mass-dying of bees in spring nearby and during sowing of corn seeds coated with neonicotinoids there now is a one to one proven causal link with acute intoxication though contact with the dust cloud around the pneumatic sowing machines during foraging flights to adjacent forests (providing honeydew) or nearby flowering fields [104••,105–109]. Such mass colony losses during corn sowing have also been documented in Italy, Germany, Austria and Slovenia [110,111,104••]. In response to the incidents, the adherence of the seed coating has been improved owing to better regulations, and an improved sowing-technique has recently become compulsory throughout Europe, [112]. Despite the deployment of air deflectors in the drilling machines or improved seed coating techniques, emissions are still substantial and the dust cloud is still acutely toxic to bees [105,109,111,113–115]. Acute lethal effects of neonicotinoids dispersed as particulate matter in the air seem to be promoted by high environmental humidity which accelerates mortality [105]. Honeybees also bring the toxic dust particles they gather on their body into the hive [106]. Sunny and warm days also seem to favour the dispersal of active substances [35].

Lethal effects from chronic exposure refer to honeybee mortality that occurs after prolonged exposure. In contrast to acute lethal effects, there are no standardised protocols for measuring chronic lethal effects. Therefore, in traditional risk assessment of pesticides they are usually expressed in three ways: LD50: the dose at which 50% of the exposed honeybees die (often, but not always, within 10 days); NOEC (No Observed Effect Concentration): the highest concentration of imidacloprid producing no observed effect; and LOEC (Lowest Observed Effect Concentration): the lowest concentration of imidacloprid producing an observed effect. However, for neonicotinoids and its neurotoxic metabolites, lethal toxicity can increase up to 100,000 times compared to acute toxicity when the exposure is extended in time [10]. There has been some controversy on the findings of that study, which is discussed in detail by Maxim and Van der Sluijs [40,42]. However, the key finding that exposure time amplifies the toxicity of neonicotinoids is consistent with later findings. Micro-colonies of bumblebees fed with imidacloprid showed the same phenomenon [102]: at one tenth of the concentration of the toxin in feed, it took twice as long to produce 100% mortality in a

bumblebee microcolony. At a 100 times lower dose, it took ca. four times longer to produce 100% mortality. The measurable shortening of the life span ceases to occur only when a dose was administered, for which the (extrapolated) chronic intoxication time would be longer than the natural life span of a worker bumblebee. This implies that the standard 10 day chronic toxicity test for bees is far too short for testing neonicotinoids. Indeed, honeybees fed with one tenth of the LC50 of thiamethoxam showed a 41.2% reduction of life span [116]. Recent studies have shown that chronic toxicity of neonicotinoids can more adequately be expressed by time to 50% mortality instead of by the 10 day LD50 [117–120,121•,122]. There is a linear relation between log daily dose and log time to 50% mortality [118,120,121•]. In experiments with honeybee colonies, similar long term chronic effects have indeed been found with typical times of 14–23 weeks to collapse 25–100% of the colonies exposed to imidacloprid-contaminated food at 20 $\mu g\ kg^{-1}$ [123] and 80–120 days for 1 $mg\ kg^{-1}$ dinotefuran and 400 $\mu g\ kg^{-1}$ clothianidin [76]. Note that these studies used concentrations that are on the high end of the currently reported ranges of concentrations found in the field. However, such data are sparse and limited to a few crops, so it cannot yet be concluded whether such concentrations are rare or common in the field.

At low concentrations of neonicotinoids, sublethal effects can occur. Sublethal effects involve modifications of honeybee behaviour and physiology (e.g., immune system). They do not directly cause the death of the individual or the collapse of the colony but may become lethal in time and/or may make the colony more sensitive (e.g., more prone to diseases), which may contribute to its collapse. For instance, an individual with memory, orientation or physiological impairments might fail to return to its hive, dying from hunger or cold. This would not be detected in standard pesticide tests, which focus on acute mortality. A distinction can be made between acute and chronic sublethal effects. Acute sublethal effects are assessed by exposing bees only once to the substance (by ingestion or by contact), and observing them for some time (variable from one laboratory to another, from several minutes to four days). Chronic sublethal effects are assessed by exposing honeybees more than once to neonicotinoids during an extended period of time (e.g., every 24 hours, for 10 days). Both acute and chronic sublethal effects are expressed as NOEC and/or LOEC (No or Lowest Observable Effect Concentration, respectively) [42].

In an extensive review Desneux *et al.* found that sublethal effects of neonicotinoids exist on neurophysiology, larval development, moulting, adult longevity, immunology, fecundity, sex ratio, mobility, navigation and orientation, feeding behaviour, oviposition behaviour, and learning [124]. All these effects have been reported for pollinators and all have the potential to produce colony level, population level and community level impacts on pollinators.

At field realistic concentrations (1 $\mu g\ l^{-1}$) imidacloprid repels pollinating beetles while at concentrations well below the analytical detection limit (0.01 $\mu g\ l^{-1}$) it repels pollinating flies [125]. This implies that imidacloprid pollution may disrupt pollination both in polluted nature and in agricultural lands. On honeybees, imidacloprid has no repelling effect at field realistic concentrations: it starts being repellent at 500 $\mu g\ l^{-1}$ [126]. In some plant protection formulations, neonicotinoids are mixed with bee repellents. However, the persistence of neonicotinoids exceeds that of the repellence and their systemic properties differ. Besides, if bees are effectively repelled and avoid the contaminated flowers, pollination is disrupted because plants are not visited by bees.

Sublethal doses of neonicotinoids impair the olfactory memory and learning capacity of honeybees [127,128,129•,130] and the orientation and foraging activity [131]. The impact of sublethal exposure on the flying behaviour and navigation capacity has been shown through homing flight tests [82,126,132,133]. Exposed to a very low concentration (0.05 $\mu g\ kg^{-1}$) imidacloprid honeybees show an initial slight increase in travel distance. However, with increasing concentration, starting at 0.5 $\mu g\ kg^{-1}$ imidacloprid decreases distance travelled and interaction time between bees, while time in the food zone increases with concentration [134•]. Imidacloprid disrupts honeybee waggle dancing and sucrose responsiveness at doses of 0.21 and 2.16 $ng\ bee^{-1}$ [135].

If honeybee brood is reared at suboptimal temperatures (the number of adult bees is not sufficient to maintain the optimal temperature level), the new workers will be characterised by reduced longevity and increased susceptibility to pesticides (bee-level effect) [136]. This will again result in a number of adult bees insufficient to maintain the brood at the optimal temperature, which may then lead to chronic colony weakening until collapse (colony-level effect).

Sublethal effects seem to be detected more frequently and at lower concentrations when bumblebees (*Bombus terrestris*) have to travel to gather food, even when the distances are tiny. No observable impacts of imidacloprid at field realistic concentrations on micro-colonies of *B. terrestris* provided with food in the nest were found, but when workers had to walk just 20 cm down a tube to gather food, they exhibited significant sublethal effects on foraging activity, with a median sublethal effect concentration (EC_{50}) of 3.7 $\mu g\ kg^{-1}$ [102]. In queenright bumblebee colonies foraging in a glasshouse where food was 3 m away from their nest, 20 $\mu g\ kg^{-1}$ of imidacloprid caused significant worker mortality, with bees dying at the feeder. Significant mortality was also observed at

10 μg kg^{-1}, but not at 2 μg kg^{-1} [102]. Bumblebees exhibit concentration-dependent sublethal responses (declining feeding rate) to imidacloprid starting at 1 μg l^{-1} in syrup, while honeybees seemed unaffected [137].

Field-relevant concentrations of imidacloprid, used alone or in mixture with λ-cyhalothrin, were shown to impair pollen foraging efficiency in bumblebee colonies [138•]. In an attempt to fulfill colony needs for pollen, more workers were recruited to forage instead of taking care of brood. This seemed to affect brood development resulting in reduced worker production [138•]. Bumblebee colonies have been exposed to field realistic levels of imidacloprid (0.7 μg kg^{-1} in nectar, 6 μg kg^{-1} in pollen) for two weeks in the laboratory. When subsequently placed back in the field and allowed to develop naturally for the following six weeks, treated colonies showed an 85% reduction in queen production and a significantly reduced growth rate [81••]. Effects on bumblebee reproduction occur at imidacloprid concentrations as low as 1 μg l^{-1} [139•] which is highly field-realistic.

It has also been shown that pesticides like imidacloprid act on the hypopharyngeal glands of honeybee nurses by degenerating the tissues [140,141,142••], which induces a shift from nest to field activities. In the native stingless bee *Melipona quadrifasciata anthidioides*, imidacloprid causes impairment of the mushroom bodies which are involved in learning [143]. Imidacloprid and clothianidin have been shown to be potent neuromodulators of the honeybee brain, causing mushroom body neuronal inactivation in honeybees, which affect honeybee cognition and behaviour at concentrations that are encountered by foraging honeybees and within the hive [8]. Sublethal doses of imidacloprid were also found to have cytotoxic activity in the Malpighian tubules in honeybees that make up the excretory and osmoregulatory system [144]. Exposure to thiamethoxam has also been shown to result in morphological impairment of the bee brain and bee midgut [116].

Exposure to neonicotinoid residues leads to a delayed development of honeybee larvae, notably in the early stages (day 4 to day 8) [145]. This can favour the development of the *Varroa destructor* parasitic mite within the colony. Likewise, the life span of adult bees emerging from the exposed brood proved to be shorter.

Short-term and mid-term sublethal effects on individuals or age groups result in long-term effects at the colony level, which follow weeks to months after the exposure, such as honeybee colony depopulation and bumblebee colony queen production [76,81••,123,138•]. As it has recently been acknowledged, the field tests on which the marketing authorisation of the use of neonicotinoids is essentially based were not developed to detect sublethal nor long-term effects on the colony level, and the observation of the performances of colonies after experimental exposure do not last long enough [85]. Major weaknesses of existing field studies are the small size of the colonies, the very small distance between the hives and the treated field and the very low surface of the test field. As a consequence of these weaknesses, the real exposures of the honey bees during these field tests are highly uncertain and may in reality be much smaller than what has been assumed in these field studies. [85]

In addition, the meta-analysis [146•] demonstrates that field tests published until now on which European and North American authorizations are based, lack the statistical power required to detect the reduction in colony performance predicted from the dose–response relationship derived from that meta-analysis. For this purpose, the tests were wrongly designed, there were too few colonies in each test group, and the follow up time monitoring the long term colony level impacts were too short to detect many of the effects described above. Nonetheless, these field studies have been the basis for granting the present market authorizations by national and European safety agencies. The meta-analyses combined data from 14 previous studies, and subsequently demonstrated that, at exposure to field realistic doses, imidacloprid does have significant sublethal effects, even at authorised levels of use, impairs performance and thus weakens honeybee colonies [146•].

A further limitation of field studies is their limited reproducibility due to the high variability in environmental conditions in the foraging area of honeybees, which extends up to a 9 km radius around the hive. Observations made in a particular field experiment might not be representative of the range of effects that could occur in real conditions. Owing to the large variability of factors that cannot be controlled (e.g. other stressors, soil structure, climate, combination of plants attractive to bees etc.), current field experiments only give information about the particular situation in which they were done.

The challenges of field studies became also clear in the debates over the highly contested field study recently conducted by the Food and Environment Research Agency (FERA) which resorts under the UK Department for Environment, Food and Rural Affairs (DEFRA). This study was set up in response to the *Science* publication that showed that a short term exposure of bumblebees to field realistic imidacloprid concentrations causes a long term 85% reduction in queen production [81••]. At three sites 20 bumblebee colonies were exposed to crops grown from untreated, clothianidin-treated or imidacloprid-treated seeds. The agency concluded that 'no clear consistent relationships' between pesticide levels and harm to the insects could be found [FERA: URL: http://www.fera.defra.gov.uk/scienceResearch/scienceCapabilities/chemicalsEnvironment/documents/reportPS2371V4a.pdf].

However, it turned out that the control colonies themselves were contaminated with the pesticides tested [147]. Further, thiamethoxam was detected in two out of the three bee groups tested, even though it was not used in the experiment. The major studies that have measured neonicotinoid residues in pollen collected by honeybees clearly show that neonicotinoids are found in pollen all over the year and in all studied regions, not only after the sowing or during the flowering period [89,91,96]. With the present scale of use, it will be very difficult to find a control site where bees cannot come into contact with neonicotinoids.

Given all the major limitations to the reliability of outcomes of field studies, it is recommendable to give more weight in the risk assessment to reproducible results from controlled lab studies and use the ratio between the environmental concentration and the no effect concentration as the main risk indicator [40,42]. It could perhaps be linked to modelling to explore how, and to what the degree, the various well-known sublethal effects on individual bees can weaken the colony [148].

A key aspect in honeybee biology is that the colony behaves as a 'superorganism' [149]. In a colony, sufficient membership, so that the number of organisms involved in the various tasks to maintain that colony, is critical, not the individual quality of a task performed by an individual bee. Varying between winter and summer, the 10,000–60,000 honeybees that typically form a colony function as a cooperative unit, maintaining intraorganismic homeostasis as well as food storage, nest hygienic, defence of the hive, rearing of brood etc. Hence, sublethal effects affecting the number of individuals that perform specific functions, can influence the functioning of the whole colony. In a simplified theoretical modelling approach, colony failure can be understood in terms of observed principles of honeybee population dynamics [150]. A colony simulation model predicts a critical threshold forager death rate above which rapid population decline is predicted and colony failure is inevitable. High forager death rates draw hive bees towards the foraging population at much younger ages than normal, which acts to accelerate colony failure [150].

Synergistic effects: pesticide–pesticide and pesticide–infectious agents

A synergy occurs when the effect of a combination of stressors is higher than the sum of the effect of each stressor alone. When neonicotinoids are combined with certain fungicides (azoles, such as prochloraz, or anilides, such as metalaxyl) or other agrochemicals that block cytochrome P450 detoxification enzymes, their toxicity increases by factor from 1.52 to 1141 depending on the combination [151,152]. The strongest synergism has been found for triflumizole making thiacloprid 1141 times more acutely toxic to honeybees [151]. This synergistic effect is the subject of patents by agrochemical companies [152,153].

Synergy has also been demonstrated for neonicotinoids and infectious agents. Prolonged exposure to a non-lethal dose of neonicotinoids renders beehives more susceptible to parasites such as *Nosema ceranae* infections [39••,154••, 155•,156]. This can be explained either by an alteration of the immune system or by an impairment of grooming and allogrooming that leads to reduced hygiene at the individual level and in the nest, which gives the pathogens more chances to infect the bees. The same mechanism, where the balance between an insect and its natural enemies is disturbed by sublethal exposures to neonicotinoids that impairs grooming, is well known and often used in pest management of target insects [157–161].

Conclusion and prospects

In less than 20 years, neonicotinoids have become the most widely used class of insecticides. Being used in more than 120 countries in more than 1000 different crops and applications, they now account for at least one quarter of the world insecticide market. For pollinators, this has transformed the agrochemical landscape to one in which most flowering crops and an unknown proportion of wild flowers contain varying concentrations of neonicotinoids in their pollen and nectar. Most neonicotinoids are highly persistent in soil, water and sediments and they accumulate in soil after repeated uses. Severe surface water pollution with neonicotinoids is common. Their systemic mode of action inside plants means phloemic and xylemic transport that results in translocation to pollen and nectar. Their wide application, persistence in soil and water and potential for uptake by succeeding crops and wild plants make neonicotinoids bioavailable to pollinators in sublethal concentrations for most of the year. This results in the frequent presence of neonicotinoids in honeybee hives. Neonicotinoids are highly neurotoxic to honeybees and wild pollinators. Their capacity to cross the ion-impermeable barrier surrounding the central nervous system (BBB, blood–brain barrier) [7•] and their strong binding to nAChR in the bee's central nervous system are responsible for a unique chronic and sublethal toxicity profile. Neonicotinoid toxicity is reinforced by exposure time. Some studies indicate a non-monotonic [162•] dose–response curve at doses far below the LD50. Mass bee dying events in spring from acute intoxication have occurred in Germany, Italy, Slovenia and France during pneumatic sowing of corn seeds coated with neonicotinoids. Bees that forage near corn fields during sowing get exposed to acute lethal doses when crossing the toxic dust cloud created by the sowing machine.

At field realistic exposure levels, neonicotinoids produce a wide range of adverse sublethal effects in honeybee colonies and bumblebee colonies, affecting colony performance through impairment of foraging success, brood

and larval development, memory and learning, damage to the central nervous system, susceptibility to diseases, hive hygiene etc. Neonicotinoids synergistically reinforce infectious agents such as *N. ceranae* and exhibit synergistic toxicity with other agrochemicals. The large impact of short term field realistic exposure of bumblebee colonies on long term bumblebee queen production (85% reduction) could be a key factor contributing to the global trends of bumblebee decline. Only a few studies assessed the toxicity to other wild pollinators, but the available data suggest that they are likely to exhibit similar toxicity to all wild insect pollinators. The worldwide production of neonicotinoids is still increasing. In view of the vital importance of the service insect pollinators provide to both natural ecosystems and farming, they require a high level of protection. Therefore a transition to pollinator-friendly alternatives to neonicotinoids is urgently needed for the sake of the sustainability of pollinator ecosystem services. The recent decision by the European Commission to temporary ban the use of imidacloprid, thiamethoxam and clothianidin in crops attractive to bees is a first step in that direction [163].

Acknowledgements

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

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Tomizawa M, Casida JE: **Neonicotinoid insecticides: highlights of a symposium on strategic molecular designs**. *J Agric Food Chem* 2011, **59**:2883-2886 http://dx.doi.org/10.1021/jf103856c.

2. Bromilow RH, Chamberlain K, Evans AA: **Physicochemical aspects of phloem translocation of herbicides**. *Weed Sci* 1990, **38**:305-314.

3. Buckingham SD, Lapied B, Le Corronc H, Grolleau F, Sattelle DB: **Imidacloprid actions on insect neuronal acetylcholine receptors**. *J Exp Biol* 1997, **200**:2685-2692.

4. Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Sattelle DB: **Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors**. *Trends Pharmacol Sci* 2001, **22**:573-580.

5. Matsuda K, Shimomura M, Ihara M, Akamatsu M, Sattelle DB: **Neonicotinoids show selective and diverse actions on their nicotinic receptor targets: electrophysiology, molecular biology, and receptor modeling studies**. *Biosci Biotechnol Biochem* 2005, **69**:1442-1452.

6. •• Belzunces LP, Tchamitchian S, Brunet JL: **Neural effects of insecticides in the honey bee**. *Apidologie* 2012, **43**:348-370.
Excellent review of neural impacts of field exposure of honeybees to sublethal residues of neurotoxic insecticides in pollen and nectar. These impair: firstly cognitive functions, including learning and memory, habituation, olfaction and gustation, navigation and orientation; secondly behaviour, including foraging and thirdly physiological functions, including thermoregulation and muscle activity. Time is a key factor in insecticide toxicity. Combination toxicity of joint exposure to multiple pesticides urgently requires attention.

7. • Tomizawa M: **Chemical biology of the nicotinic insecticide receptor**. *Adv Insect Physiol* 2013, **44**:63-99.
Introduction into the molecular basis of binding site interaction and explanation of different binding affinity of neonicotinoids with insect and vertebrate nicotinic acetyl choline receptor. Another key factor explaining the high insect toxicity is its capacity (stemming from hydrophobicity) to penetrate the ion-impermeable barrier surrounding the insect nervous system.

8. •• Palmer MJ, Moffat C, Saranzewa N, Harvey J, Wright GA, Connolly CN: **Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees**. *Nat Commun* 2013, **4**:1634 http://dx.doi.org/10.1038/ncomms2648.
Using recordings from mushroom body Kenyon cells in acutely isolated honeybee brain, it is shown that the neonicotinoids imidacloprid and clothianidin, and the organophosphate miticide coumaphos oxon, cause a depolarization-block of neuronal firing and inhibit nicotinic responses. These effects are observed at concentrations that are encountered by foraging honeybees and within the hive, and are additive with combined application. Exposure to multiple pesticides that target cholinergic signalling will cause enhanced toxicity to pollinators.

9. Tomizawa M, Casida JE: **Neonicotinoid insecticide toxicology: mechanism of selective action**. *Annu Rev Pharmacol Toxicol* 2005, **45**:247-268.

10. Suchail S, Guez D, Belzunces LP: **Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera***. *Environ Toxicol Chem* 2001, **20**:2482-2486.

11. Suchail S, Debrauwer L, Belzunces LP: **Metabolism of imidacloprid in *Apis mellifera***. *Pest Manag Sci* 2004, **60**:291-296.

12. Casida JE: **Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance**. *J Agric Food Chem* 2010, **59**:2923-2931 http://dx.doi.org/10.1021/jf102438c.

13. Liu GY, Ju XL, Cheng J: **Selectivity of Imidacloprid for fruit fly versus rat nicotinic acetylcholine receptors by molecular modeling**. *J Mol Model* 2010, **16**:993-1002.

14. Liu Z, Yao X, Zhang Y: **Insect nicotinic acetylcholine receptors (nAChRs): important amino acid residues contributing to neonicotinoid insecticides selectivity and resistance**. *Afr J Biotechnol* 2008, **7**:4935-4939.

15. Cutler P, Slater R, Edmunds JF, Maienfisch P, Hall RG, Earley GP, Pitterna T, Pal S, Paul V-L, Goodchild J, Blacker M, Hagmann L, Crossthwaite AJ: **Investigating the mode of action of sulfoxaflor: a fourth-generation neonicotinoid**. *Pest Manag Sci* 2013, **69**:607-619 http://dx.doi.org/10.1002/ps.3413.

16. Casida JE, Durkin K: **Neuroactive insecticides: targets, selectivity, resistance, and secondary effects**. *Annu Rev Entomol* 2013, **58**:99-117 http://dx.doi.org/10.1146/annurev-ento-120811-153645.

17. Pollak P: *Fine Chemicals: The Industry and the Business*. John Wiley & Sons; 2011.

18. Shao X, Liu Z, Xu X, Li Z, Qian X: **Overall status of neonicotinoid insecticides in China: production, application and innovation**. *J Pest Sci* 2013, **38**:1-9 http://dx.doi.org/10.1584/jpestics.D12-037.

19. **European Food Safety Authority: statement on the findings in recent studies investigating sub-lethal effects in bees of some neonicotinoids in consideration of the uses currently authorised in Europe**. *EFSA J* 2012, **10**:2752 http://dx.doi.org/10.2903/j.efsa.2012.2752.

20. Sur R, Stork A: **Uptake, translocation and metabolism of imidacloprid in plants**. *Bull Insectol* 2003, **56**:35-40.

21. Van de Meent D, Hollander A, Peijnenburg W, Breure T: **Fate and transport of contaminants**. In *Ecological Impacts of Toxic Chemicals*. Edited by Sánchez-Bayo F, Van den Brink P, Mann RM. Bentham; 2011.

22. Gupta S, Gajbhiye V, Kalpana T, Agnihotri NP: **Leaching behavior of imidacloprid formulations in soil**. *Bull Environ Contam Toxicol* 2002, **68**:502-508.

23. Haith DA: **Ecological risk assessment of pesticide runoff from grass surfaces**. *Environ Sci Technol* 2010, **44**:6496-6502.

24. Selim HM, Jeong CY, Elbana TA: **Transport of imidacloprid in soils: miscible displacement experiments**. *Soil Sci* 2010, **175**:375-381.

25. Miranda GRB, Raetano CG, Silva E, Daam MA, Cerejeira MA: **Environmental fate of neonicotinoids and classification of their potential risks to hypogean, epygean, and surface water ecosystems in Brazil**. *Hum Ecol Risk Assess* 2011, **17**:981-995.

26. Kurwadkar ST, Dewinne D, Wheat R, McGahan DG, Mitchell FL: **Time dependent sorption behavior of dinotefuran, imidacloprid and thiamethoxam**. *J Environ Sci Health B* 2013, **48**:237-242 http://dx.doi.org/10.1080/03601234.2013.742412.

27. Van Dijk T, Van Staalduinen M, Van der Sluijs JP: **Macro-invertebrate decline in surface water polluted with imidacloprid**. *PLoS ONE* 2013, **8**:e62374 http://dx.doi.org/10.1371/journal.pone.0062374.

28. Starner K, Goh KS: **Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010–2011**. *Bull Environ Contam Toxicol* 2012, **88**:316-321 http://dx.doi.org/10.1007/s00128-011-0515-5.

29. Kreuger J, Graaf S: *Pesticides in Surface Water in Areas with Open Ground and Greenhouse Horticultural Crops in Sweden 2008*. 2010.

30. Anderson TA, Salicea CJ, Ericksona RA, McMurry ST, Coxa SB, Smith LM: **Effects of land use and precipitation on pesticides and water quality in playa lakes of the southern high plains**. *Chemosphere* 2013, **92**:84-90 http://dx.doi.org/10.1016/j.chemosphere.2013.02.054.

31. Doering J, Maus C, Schoening R: *Residues of Imidacloprid WG 5 in Blossom Samples of Rhododendron sp (variety Nova Zembla) after Soil Treatment in the Field Application: 2003, Sampling 2003 and 2004, Bayer Cropscience AG. Report No G201806/32*. 2004.

32. Rexrode M, Barrett M, Ellis J, Gabe P, VaughanA, Felkel J, Melendez J: *EFED Risk Assessment for the Seed Treatment of Clothianidin 600FS on Corn and Canola*. United States Environmental Protection Agency; 20 February 2003.

33. NPIC (National Pesticde Information Center): *Imidacloprid Technical Fact Sheet*. 2013:. http://www.npic.orst.edu/factsheets/imidacloprid.pdf (accessed 17.02.13).

34. Bonmatin JM, Moineau I, Charvet R, Colin ME, Fleche C, Bengsch ER: **Behaviour of imidacloprid in fields. Toxicity for honey bees**. *Environmental Chemistry – Green Chemistry and Pollutants in Ecosystems*. Berlin: Springer; 2005, 483-494.

35. Greatti M, Sabatini AG, Barbattini R, Rossi S, Stravisi A: **Risk of environmental contamination by the active ingredient imidacloprid used for corn seed dressing. Preliminary results**. *Bull Insectol* 2003, **56**:69-72.

36. •• Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K: **Multiple routes of pesticide exposure for honey bees living near agricultural fields**. *PLoS ONE* 2012, **7**:e29268 http://dx.doi.org/10.1371/journal.pone.0029268.

Bees are exposed in many ways to neonicotinoids in agricultural fields throughout the foraging period. During spring, extremely high levels of clothianidin and thiamethoxam were found in planter exhaust material produced during the planting of treated maize seed. Neonicotinoids were found in soil also of unplanted fields. Dandelions visited by foraging bees growing near these fields contained neonicotinoids: 1.1–9.4 $\mu g\ kg^{-1}$ clothinidin and 1.1–2.9 $\mu g\ kg^{-1}$ thiamethoxam.

37. Bonmatin JM, Marchand PA, Charvet R, Moineau I, Bengsch ER, Colin ME: **Quantification of imidacloprid uptake in maize crops**. *J Agric Food Chem* 2005, **53**:5336-5341 http://dx.doi.org/10.1021/jf0479362.

38. Maini S, Medrzycki P, Porrini C: **The puzzle of honey bee losses: a brief review**. *Bull Insectol* 2010, **63**:153-160.

39. •• Vidau C, Diogon M, Aufauvre J, Fontbonne R, Viguès B, Brunet J-L, Texier C, Biron DG, Blot N, El Alaoui H *et al.*: **Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae***. *PLoS ONE* 2011, **6**:e21550 http://dx.doi.org/10.1371/journal.pone.0021550.

Synergism was found between neurotoxic insecticides and *Nosema ceranae* infections: Sublethal doses of fipronil and thiacloprid make honeybees more prone to *Nosema ceranae* infections and induces increased mortality of nosema-infected bees. This effect does not seem to be linked to a decrease of the bee detoxification system. A step forward in understanding the interactions of factors jointly producing colony depopulation.

40. Maxim L, Van der Sluijs JP: **Uncertainty: cause or effect of stakeholders' debates? Analysis of a case study: the risk for honey bees of the insecticide Gaucho®**. *Sci Total Environ* 2007, **376**:1-17.

41. Suryanarayanan S, Kleinman DL: **Disappearing bees and reluctant regulators**. *Issues Sci Technol* 2011, **27**:33.

42. •• Maxim L, Van der Sluijs JP: **Seed-dressing systemic insecticides and Honeybees**. *Late Lessons from Early Warnings: Science, Precaution, Innovation*. European Environment Agency (EEA); 2013:. p. 401–438.

Using the bans of imidacloprid in France for sunflower (1999) and maize (2004) as a case study, the social processes that ultimately lead to application of the precautionary principle are analyzed in a very insightful way. The analysis focuses on the ways in which scientific findings were used by stakeholders and decision-makers to influence policy during the controversy. Eight lessons are drawn about governance of controversies related to chemical risks.

43. •• Suryanarayanan S, Kleinman D: **Be(e)coming experts the controversy over insecticides in the honey bee colony collapse disorder**. *Soc Stud Sci* 2013, **43**:215-240 http://dx.doi.org/10.1177/0306312712466186.

This paper explores the politics of expertise in an ongoing controversy in the United States over the role of neonicotinoids in colony collapse disorder. A set of research norms and practices from agricultural entomology came to dominate the investigation of the links between pesticides and honey bee health. The epistemological dominance of these norms and practices served to marginalize the knowledge claims and policy positions of bee-keepers in the colony collapse disorder controversy.

44. Buchmann SL, Nabhan GP: *The Forgotten Pollinators*. Island Press; 1997.

45. Klein AM, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T: **Importance of pollinators in changing landscapes for world crops**. *Proc Biol Sci* 2007, **274**:303-313 http://dx.doi.org/10.1098/rspb.2006.3721.

46. Brussaard L, Caron P, Campbell B, Lipper L, Mainka S, Rabbinge R, Babin D, Pulleman M: **Reconciling biodiversity conservation and food security: scientific challenges for a new agriculture**. *Curr Opin Environ Sustain* 2010, **2**:34-42.

47. Settele J, Penev L, Georgiev T, Grabaum R, Grobelnik V, Hammen V, Klotz S, Kühn I (Eds): *Atlas of Biodiversity Risk*. Sofia: Pensoft; 2010.

48. •• Lautenbach S, Seppelt R, Liebscher J, Dormann CF: **Spatial and temporal trends of global pollination benefit**. *PLoS ONE* 2012, **7**:e35954 http://dx.doi.org/10.1371/journal.pone.0035954.

The first spatially explicit map of pollination demands at world level identifies the areas that benefit and depend the most on pollination. On the basis of this maps the dependency of agriculture on pollination is much higher than what has been so far described. Increases in the price of pollination dependent crops were considered as an early warning tool on the abundance of pollinators. Still the effects of international trade of pollination-dependent crops have not been taken into account, in which case the dependency of developed countries would be higher than shown.

49. Gallai N, Salles JM, Settele J, Vaissière BE: **Economic valuation of the vulnerability of world agriculture confronted with pollinator decline**. *Ecol Econ* 2009, **68**:810-821 http://dx.doi.org/10.1016/j.ecolecon.2008.06.014.

50. Winfree R, Gross BJ, Kremen C: **Valuing pollination services to agriculture**. *Ecol Econ* 2011, **71**:80-88.

51. Maes J, Hauck J, Paracchini ML, Ratamäki O, Hutchins M, Termansen M, Furman E, Pérez-Soba M, Braat L, Bidoglio G: **Mainstreaming ecosystem services into EU policy**. *Curr Opin Environ Sustain* 2013, **5**:128-134 http://dx.doi.org/10.1016/j.cosust.2013.01.002.

52. Kevan PG, Menzel R: **The plight of pollination and the interface of neurobiology, ecology and food security**. *Environmentalist* 2012, **32**:300-310 http://dx.doi.org/10.1007/s10669-012-9394-5.

53. Ollerton J, Winfree R, Tarrant S: **How many flowering plants are pollinated by animals?** *Oikos* 2011, **120**:321-326 http://dx.doi.org/10.1111/j.1600-0706.2010.18644.x.

54. • Eilers EJ, Kremen C, Smith Greenleaf S, Garber AK, Klein AM: **Contribution of pollinator-mediated crops to nutrients in the human food supply**. *PLoS ONE* 2011, **6**:e21363 http://dx.doi.org/10.1371/journal.pone.0021363.

Pollinator mediated crops account for >90% of vitamin C, 100% of lycopene, almost 100% of the antioxidants β-cryptoxanthin and β-tocopherol, the majority of the lipid, vitamin A and related carotenoids, calcium and fluoride, and a large portion of folic acid. Ongoing pollinator decline may thus put the provision of a nutritionally adequate diet for the global human population at risk.

55. Dams LR: **Bees and honey-hunting scenes in the Mesolithic rock art of eastern Spain**. *Bee World* 1978, **59**:43-53.

56. Pattinson D: **Pre-modern beekeeping in China: a short history**. *Agric Hist* 2012, **86**:235-255.

57. Bloch G, Francoy TM, Wachtel I, Panitz-Cohen N, Fuchs S, Mazar A: **Industrial apiculture in the Jordan valley during Biblical times with Anatolian honeybees**. *Proc Natl Acad Sci U S A* 2010, **107**:11240-11244.

58. Ebert A: **Nectar for the taking: the popularization of scientific bee culture in England, 1609–1809**. *Agric Hist* 2011, **85**:322-343.

59. Breeze TD, Bailey AP, Balcombe KG, Potts SG: **Pollination services in the UK: How important are honeybees?** *Agric Ecosyst Environ* 2011, **142**:137-143 http://dx.doi.org/10.1016/j.agee.2011.03.020.

60. Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, Bartomeus I, Benjamin F *et al.*: **Wild pollinators enhance fruit set of crops regardless of honey bee abundance**. *Science* 2013, **339**:1608-1611 http://dx.doi.org/10.1126/science.1230200.

61. Banskota AH, Tezuka Y, Kadota S: **Recent progress in pharmacological research of propolis**. *Phytother Res* 2001, **15**:561-571.

62. Jull AB, Rodgers A, Walker N: **Honey as a topical treatment for wounds**. *Cochrane Database Syst Rev* 2009, **4** art.no. CD005083.

63. Srinivasan MV: **Honeybees as a model for the study of visually guided flight, navigation, and biologically inspired robotics**. *Physiol Rev* 2011, **91**:413-460.

64. Rogers J, Sleigh C: **Here is my honey-machine: Sylvia plath and the mereology of the Beehive**. *Rev Engl Stud* 2012, **63**:293-310.

65. Biesmeijer JC, Roberts SPM, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers P *et al.*: **Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands**. *Science* 2006, **313**:351-354 http://dx.doi.org/10.1126/science.1127863.

66. Holden C: **Ecology: report warns of looming pollination crisis in North America**. *Science* 2006, **314**:397 http://dx.doi.org/10.1126/science.314.5798.397.

67. Goulson D, Lye GC, Darvill B: **Decline and conservation of bumblebees**. *Ann Rev Entomol* 2008, **53**:191-208.

68. Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE: **Global pollinator declines: trends, impacts and drivers**. *Trends Ecol Evol* 2010, **25**:345-353 http://dx.doi.org/10.1016/j.tree.2010.01.007.

69. Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, Griswold TL: **Patterns of widespread decline in North American bumble bees**. *Proc Natl Acad Sci U S A* 2010, **108**:662-667.

70. Burkle LA, Marlin JC, Knight TM: **Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function**. *Science* 2013, **339**:1611-1615 http://dx.doi.org/10.1126/science.1232728.

71. UNEP: *Global Honey Bee Colony Disorders and Other Threats to Insects*. Agriculture United Nations Environmental Program; 2010

72. • van der Zee R, Pisa L, Andonov S, Brodschneider R, Chlebo R, Coffey MF, Crailsheim K, Dahle B, Gajda A, Gray A *et al.*: **Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008–9 and 2009–10**. *J Apic Res* 2012, **51**:100-114 http://dx.doi.org/10.3896/IBRA.1.51.1.12.

This publication gathers the to-date most harmonised information on colony losses worldwide. Colony bee losses are voluntarily communicated by beekeepers through a common worldwide questionnaire designed by the scientific network COLOSS.

73. VanEngelsdorp D, Meixner M: **A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them**. *J Invertebr Pathol* 2010, **103**:S80-S95 http://dx.doi.org/10.1016/j.jip.2009.06.011.

74. VanEngelsdorp D, Caron D, Hayes J, Underwood R, Henson M, Spleen A, Andree M, Andree M, Snyder R, Lee K, Roccasecca K, Wilson M, Wilkes J, Lengerich E, Pettis J: **A national survey of managed honey bee 2010–11 winter colony losses in the USA: results from the Bee Informed Partnership**. *J Apic Res* 2012, **51**:115-124 http://dx.doi.org/10.3896/IBRA.1.51.1.14.

75. • Taniguchi T, Kita Y, Matsumoto T, Kimura K: **Honeybee colony losses during 2008–2010 caused by pesticide application in Japan**. *J Apic* 2012, **27**:15-27.

A survey carried out to beekeepers over three consecutive years identified large numbers of bee losses due to acute intoxications to putatively neonicotinoids. The colonies around rice and orange fields were the most affected.

76. Yamada T, Yamada K, Wada N: **Influence of dinotefuran and clothianidin on a bee colony**. *Jpn J Clin Ecol* 2012, **21**:10-23.

77. Maxim L, Van der Sluijs JP: **Expert explanations of honeybee® losses in areas of extensive agriculture in France: Gaucho® compared with other supposed causal factors**. *Environ Res Lett* 2010, **5**:014006 http://dx.doi.org/10.1088/1748-9326/5/1/014006.

78. Runckel C, Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R, DeRisi JL: **Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, nosema, and crithidia**. *PLoS ONE* 2011, **6**:e20656 http://dx.doi.org/10.1371/journal.pone.0020656.

79. • Farooqui T: **A potential link among biogenic amines-based pesticides, learning and memory, and colony collapse disorder: a unique hypothesis**. *Neurochem Int* 2012, **62**:122-136 http://dx.doi.org/10.1016/j.neuint.2012.09.020.

An insightful discussion of a hypothetical link among biogenic amines-based pesticides (neonicotinoids and formamidines) and their disruptive effects on biogenic amine signaling causing olfactory dysfunction in honeybees. The hypothesis that chronic exposure disrupts neural cholinergic and octopaminergic signaling in honeybees is supported by the fact that abnormality in biogenic amines-mediated neuronal signaling impairs their olfactory learning and memory. This explains why foragers exposed to neonicotinoids fail to return to their hive — a possible cause of CCD.

80. Stokstad E: **Agriculture Field research on bees raises concern about low-dose pesticides**. *Science* 2012, **335**:1555 http://dx.doi.org/10.1126/science.335.6076.1555.

81. •• Whitehorn PR, O'Connor S, Wackers FL, Goulson D: **Neonicotinoid pesticide reduces bumble bee colony growth and queen production**. *Science* 2012:351 http://dx.doi.org/10.1126/science.1215025.

Colonies of bumble bees (*Bombus terrestris*) exposed to field relevant doses of imidacloprid showed significantly lower growth rate and a reduction of 85% of new queen production. This means a severe negative impact on the bumblebees' population.

82. •• Henry M, Béguin M, Requier F, Rollin O, Odoux JF, Aupinel P, Aptel J, Tchamitchian S, Decourtye A: **A common pesticide decreases foraging success and survival in honey bees**. *Science* 2012, **336**:348-350 http://dx.doi.org/10.1126/science.1215039.

Bees exposed to doses of thiamethoxam at 1.34 ng/bee significantly decrease their ability to carry out their homing flight up to levels triggering depopulation dynamics in their colony. RFID tagging technology is used to register the activity of foragers leaving and entering the hive.

83. Stoner KA, Eitzer BD: **Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (Cucurbita pepo)**. *PLoS ONE* 2012, **7**:e39114 http://dx.doi.org/10.1371/journal.pone.0039114.

84. Girolami V, Mazzon L, Squartini A, Mori N, Marzaro M, Di Bernardo A, Greatti M, Giorio C, Tapparo A: **Translocation of neonicotinoid insecticides from coated seeds to seedling guttation drops: a novel way of intoxication for bees**. *J Econ Entomol* 2009, **102**:1808-1815.

85. • European Food Safety Authority: **Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, Bombus spp. and solitary bees)**. *EFSA J* 2012, **10**:2668 http://dx.doi.org/10.2903/j.efsa.2012.2668.

This non-comprehensive, but rather complete review, includes information about many of the following subjects: first, a definition of specific protection goals linked to pollinators; second, exposure paths of bees to pesticides defined in scientific literature; third, toxicological effects observed; fourth, synergistic effects of pesticides and pathologies and among pesticide molecules; fifth, an analysis of the different toxicological tests currently carried out for risk assessment. On the basis of the previous points, a new proposal for pesticide risk assessment on bees is proposed.

86. Beekman M, Ratnieks FLW: **Long-range foraging by the honey-bee, *Apis mellifera* L.**. *Funct Ecol* 2000, **14**:490-496.

87. Goulson D, Lepais O, O'Connor S, Osborne JL, Sanderson RA, Cussans J, Goffe L, Darvill B: **Effects of land use at a landscape scale on bumblebee nest density and survival**. *J Appl Ecol* 2010, **46**:1207-1215.

88. Rortais A, Arnold G, Halm M-P, Touffet-Briens F: **Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees'**. *Apidologie* 2005, **36**:71-83.

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

90. Chauzat MP, Carpentier P, Martel AC, Bougeard S, Cougoule N, Porta P, Lachaize J, Madec F, Aubert M, Faucon JP: **Influence of pesticide residues on honey bee (Hymenoptera: Apidae) colony health in France**. *Environ Entomol* 2009, **38**:514-523.

91. Chauzat M-P, Martel A-C, Cougoule N, Porta P, Lachaize J, Zeggane S, Aubert M *et al.*: **An assessment of honeybee colony matrices, *Apis mellifera* (Hymenoptera: Apidae) to monitor pesticide presence in continental France**. *Environ Toxicol Chem* 2011, **30**:103-111 http://dx.doi.org/10.1002/etc.361.

92. Blacquière T, Smagghe G, Van Gestel CM, Mommaerts V: **Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment**. *Ecotoxicology* 2012, **21**:973-992 http://dx.doi.org/10.1007/s10646-012-0863-x.

93. Dively GP, Kamel A: **Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators**. *J Agric Food Chem* 2012, **60**:4449-4456 http://dx.doi.org/10.1021/jf205393x.

94. Tapparo A, Giorio C, Marzaro M, Marton D, Soldà L, Girolami V: **Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds**. *J Environ Monit* 2011, **13**:1564-1568 http://dx.doi.org/10.1039/c1em10085h.

95. Hoffmann EJ, Castle SJ: **Imidacloprid in melon guttation fluid: a potential mode of exposure for pest and beneficial organisms**. *J Econ Entomol* 2012, **105**:67-71.

96. Mullin C, Frazier M, Frazier JL, Ashcraft S, Simonds R, Vanengelsdorp D, Pettis JS: **High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health**. *PLoS ONE* 2010, **5**:e9754 http://dx.doi.org/10.1371/journal.pone.0009754.

97. Yáñez KP, Bernal JL, Nozal MJ, Martín MT, Bernal J: **Determination of seven neonicotinoid insecticides in beeswax by liquid chromatography coupled to electrospray–mass spectrometry using a fused-core column**. *J Chromatogr A* 2013, **1285**:110-117 http://dx.doi.org/10.1016/j.chroma.2013.02.032.

98. Laurino D, Porporato M, Patetta A, Manino A: **Toxicity of neonicotinoid insecticides to honey bees: laboratory tests**. *Bull Insectol* 2011, **64**:107-113.

99. Abbott V, Nadeau JL, Higo H, Winston ML: **Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on Megachile rotundata (Hymenoptera: Megachilidae)**. *J Econ Entomol* 2008, **101**:784-796.

100. Scott-Dupree CD, Conroy L, Harris CR: **Impact of currently used or potentially useful insecticides for canola agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymentoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae)**. *J Econ Entomol* 2009, **102**:177-182.

101. Gradish AE, Scott-Dupree CD, Shipp L, Harrisa CR, Ferguson G: **Effect of reduced risk pesticides for use in greenhouse vegetable production on *Bombus impatiens* (Hymenoptera: Apidae)**. *Pest Manag Sci* 2010, **66**:142-146.

102. Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G, Smagghe G: **Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior**. *Ecotoxicology* 2010, **19**:207-215 http://dx.doi.org/10.1007/s10646-009-0406-2.

103. Jeyalakshmi T, Shanmugasundaram R, Saravanan M, Geetha S, Mohan SS, Goparaju A, Balakrishna Murthy R: **Comparative toxicity of certain insecticides against *Apis cerana indica* under semi field and laboratory conditions**. *Pestology* 2011, **35**:23-26.

104 •• Tapparo A, Marton D, Giorio C, Zanella A, Solda L, Marzaro M, Vivan L, Girolami V: **Assessment of the Environmental Exposure of Honeybees to Particulate Matter Containing Neonicotinoid Insecticides Coming from Corn Coated Seeds**. *Environ Sci Technol* 2012, **46**:2592-2599 http://dx.doi.org/10.1021/es2035152.

Thanks to a quick analytical method, new potential sources of exposure to pesticides (clothianidin and thiamethoxam) in the environment have been proved: significant amounts of coating particles are emitted by pneumatic drilling machines during corn sowing. Coarse particles seem to sediment closer to the sown field, while fine particles cover longer distances owing to the wind. The amount of toxic compound released is toxic to bees, and this toxicity is facilitated by humidity.

105. Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D, Tapparo A: **Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication**. *J Appl Entomol* 2012, **136**:17-26 http://dx.doi.org/10.1111/j.1439-0418.2011.01648.x.

106. Girolami V, Marzaro M, Vivan L, Mazzon L, Giorio C, Marton D, Tapparo A: **Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers**. *J Appl Entomol* 2013, **137**:35-44 http://dx.doi.org/10.1111/j.1439-0418.2012.01718.x.

107. ApeNet: *Effects of Coated Maize Seed on Honey Bees – Report Based on Results Obtained from the Second Year (2010) Activity of the APENET Project*. 2012.

108. Pochi D, Biocca M, Fanigliulo R, Pulcini P, Conte E: **Potential exposure of bees, *Apis mellifera* L., to particulate matter and pesticides derived from seed dressing during maize sowing**. *Bull Environ Contam Toxicol* 2012, **89**:354-361 http://dx.doi.org/10.1007/s00128-012-0664-1.

109. Tapparo A, Giorio C, Soldà L, Bogialli S, Marton D, Marzaro M, Girolami V: **UHPLC-DAD method for the determination of neonicotinoid insecticides in single bees and its relevance in honeybee colony loss investigations**. *Anal Bioanal Chem* 2013, **405**:1007-1014 http://dx.doi.org/10.1007/s00216-012-6338-3.

110. Gross M: **Pesticides linked to bee deaths**. *Curr Biol* 2008, **18**:684.

111. Sgolastra F, Renzi T, Draghetti S, Medrzycki P, Lodesani M, Maini S, Porrini C: **Effects of neonicotinoid dust from maize seed-dressing on honey bees**. *Bull Insectol* 2012, **65**:273-280.

112. European Commission: **Commission Directive 2010/21/EU amending Annex I to Council Directive 91/414/EEC as regards the specific provisions relating to clothianidin, thiamethoxam, fipronil and imidacloprid**. *Off J* 2010, **L 65**.

113. Biocca M, Conte E, Pulcini P, Marinelli E, Pochi D: **Sowing simulation tests of a pneumatic drill equipped with systems aimed at reducing the emission of abrasion dust from maize dressed seed**. *J Environ Sci Health B: Pest Food Contam Agric Wastes* 2011, **46**:438-448 http://dx.doi.org/10.1080/03601234.2011.583825.

114. Marzaro M, Vivan L, Targa A, Mazzon L, Mori N, Greatti M, Petrucco Toffolo E, Di Bernardo A, Giorio C, Marton D *et al.*: **Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat**. *Bull Insectol* 2011, **64**:119-126.

115. *Austria Investigations in the incidence of bee losses in corn and oilseed rape growing areas of Austria and possible correlations with bee diseases and the use of insecticidal plant protection products (MELISA)*. Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH, Institut für Pflanzenschutzmittel; 2012.

116. Oliveira RA, Roat TC, Carvalho SM, Malaspina O: **Side-effects of thiamethoxam on the brain and midgut of the africanized honeybee *Apis mellifera* (Hymenopptera: Apidae)**. *Environ Toxicol* 2013. (in press).

117. Sánchez-Bayo F: **From simple toxicological models to prediction of toxic effects in time**. *Ecotoxicology* 2009, **18**:343-354 http://dx.doi.org/10.1007/s10646-008-0290-1.

118. Tennekes HA: **The significance of the Druckrey–Küpfmüller equation for risk assessment — the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time**. *Toxicology* 2010, **276**:1-4 http://dx.doi.org/10.1016/j.tox.2010.07.005.

119. Maus C, Nauen R: **Response to the publication: Tennekes, H.A. (2010): the significance of the Druckrey–Küpfmüller equation for risk assessment — the toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time**. *Toxicology* 2011, **280**:176-177 http://dx.doi.org/10.1016/j.tox.2010.11.014.

120. Tennekes HA: **The significance of the Druckrey–Küpfmüller equation for risk assessment—The toxicity of neonicotinoid insecticides to arthropods is reinforced by exposure time: Responding to a Letter to the Editor by Drs. C. Maus and R. Nauen of Bayer CropScience AG**. *Toxicology* 2011, **280**:173-175 http://dx.doi.org/10.1016/j.tox.2010.11.015.

121 • Tennekes HA, Sánchez-Bayo F: **Time-dependent toxicity of neonicotinoids and other toxicants: implications for a new approach to risk assessment**. *J Environ Anal Toxicol* 2011, **S4**:001 http://dx.doi.org/10.4172/2161-0525.S4-001.
Neonicotinoids have an irreversible impact on nAChR which implies that a time-to-event model of toxicity is the adequate description of their chronic toxicity profile. Because of time depends of the toxic effect, the standard risk assessment procedures are not valid in case of exposure to sublethal concentrations of neonicotinoids for long periods of time, so LC50 is a misleading indicator for harm.

122. Mason R, Tennekes H, Sánchez-Bayo F, Uhd Jepsen P: **Immune suppression by neonicotinoid insecticides at the root of global wildlife declines**. *J Environ Immunol Toxicol* 2013, **1**:2-12 http://dx.doi.org/10.7178/jeit.1.

123. Lu C, Warchol KM, Callahan RA: **In situ replication of honey bee colony collapse disorder**. *Bull Insectol* 2012, **65**:99-106.

124. Desneux N, Decourtye A, Delpuech JM: **The sublethal effects of pesticides on beneficial arthropods**. *Annu Rev Entomol* 2007, **52**:81-106 http://dx.doi.org/10.1146/annurev.ento.52.110405.091440.

125. Easton AH, Goulson D: **The neonicotinoid insecticide imidacloprid repels pollinating flies and beetles at field-realistic concentrations**. *PLoS ONE* 2013, **8**:e54819 http://dx.doi.org/10.1371/journal.pone.0054819.

126. Bortolotti L, Montanari R, Marcelino J, Medrzycki P, Maini S, Porrini C: **Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees**. *Bull Insectol* 2003, **56**:63-67.

127. Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, Cluzeau S, Pham-Delègue MH: **Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera***. *Arch Environ Contam Toxicol* 2005, **48**:242-250 http://dx.doi.org/10.1007/s00244-003-0262-7.

128. Gauthier M: **State of the art on insect nicotinic acetylcholine receptor function in learning and memory**. In *Insect Nicotinic Acetylcholine Receptors*. Edited by Thany SH. Berlin: Springer; 2010:97-115.

129 • Yang EC, Chang HC, Wu WY, Chen YW: **Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval stage**. *PLoS ONE* 2012, **7**:e49472 http://dx.doi.org/10.1371/journal.pone.0049472.
A short term sublethal dosage of imidacloprid given to honeybee larvae has a long term effect: it renders the olfactory associative behavior of the adult bees impaired. This may affect the survival condition of the entire colony, even though the larvae survive to adulthood. Also, the brood-capped, pupation, and eclosion rates of the larvae decrease significantly with dose.

130. Williamson SM, Wright GA: **Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees**. *J Exp Biol* 2013, **216**:1799-1807 http://dx.doi.org/10.1242/jeb.083931.

131. Yang EC, Chuang YC, Chen YL, Chang LH: **Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae)**. *J Econ Entomol* 2008, **101**:1743-1748 http://dx.doi.org/10.1603/0022-0493-101.6.1743.

132. Decourtye A, Devillers J, Aupinel P, Brun F, Bagnis C, Fourrier J, Gauthier M: **Honeybee tracking with microchips: a new methodology to measure the effects of pesticides**. *Ecotoxicology* 2011, **20**:429-437 http://dx.doi.org/10.1007/s10646-011-0594-4.

133. Schneider CW, Tautz J, Grünewald B, Fuchs S: **RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera***. *PLoS ONE* 2012, **7**:e30023 http://dx.doi.org/10.1371/journal.pone.0030023.

134 • Teeters BS, Johnson RM, Ellis MD, Siegfried BD: **Using video-tracking to assess sublethal effects of pesticides on honey bees (*Apis mellifera* L.)**. *Environ Toxicol Chem* 2012, **31**:1349-1354 http://dx.doi.org/10.1002/etc.1830.
Video-tracking technology has been used to measure effects on behaviour of two pesticides: tau-fluvalinate (topical) and imidacloprid (oral). While at very low dose (0.05 $\mu g\ kg^{-1}$) imidacloprid showed an activating effect, starting at 0.5 $\mu g\ kg^{-1}$ imidacloprid triggered a dose dependent negative effect: an increase in time spent at feeder, while distance travelled and interaction time between bees decrease with dose.

135. Eiri DM, Nieh JC: **A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing**. *J Exp Biol* 2012, **215**:2022-2029 http://dx.doi.org/10.1242/jeb.068718.

136. Medrzycki P, Sgolastra F, Bortolotti L, Bogo G, Tosi S, Padovani E, Porrini C, Sabatini AG: **Influence of brood rearing temperature on honey bee development and susceptibility to intoxication by pesticides**. *J Apic Res* 2010, **49**:52-59.

137. Cresswell JE, Page CJ, Uygun MB, Holmbergh M, Li Y, Wheeler JG, Laycock I, Pook CJ, de Ibarra NH, Smirnoff N *et al.*: **Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid)**. *Zoology* 2012, **115**:365-371 http://dx.doi.org/10.1016/j.zool.2012.05.003.

138 • Gill RJ, Ramos-Rodriguez O, Raine NE: **Combined pesticide exposure severely affects individual- and colony-level traits in bees**. *Nature* 2012, **491**:105-108 http://dx.doi.org/10.1038/nature11585.
Chronic exposure of bumble bees to a neonicotinoid (imidacloprid) and a pyrethroid (λ-cyhalothrin) at field-relevant concentrations reduced the foraging performance and increased worker mortality. This leads to a reduction of brood production and colony success. A synergistic effect of both pesticides is clearly observed.

139 • Laycock I, Lenthall KM, Barratt AT, Cresswell JE: **Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (*Bombus terrestris*)**. *Ecotoxicology* 2012, **21**:1937-1945 http://dx.doi.org/10.1007/s10646-012-0927-y/.

Environmental relevant concentrations of imidacloprid negatively affects ovary development and fecundity in bumble bees. Dietary imidacloprid at 1 $\mu g\ l^{-1}$ is capable to reduce brood production by one third. The causal speculations include a feeding reduction linked to toxic exposure and/or alteration of the social communication among bumblebee workers.

140. Smodis Skerl MIS, Gregorc A: **Heat shock proteins and cell death in situ localisation in hypopharyngeal glands of honeybee (*Apis mellifera* carnica) workers after imidacloprid or coumaphos treatment**. *Apidologie* 2010, **41**:73-86.

141. Heylen K, Gobin B, Arckens L, Huybrechts R, Billen J: **The effects of four crop protection products on the morphology and ultrastructure of the hypopharyngeal gland of the European honeybee, *Apis mellifera***. *Apidologie* 2011, **42**:103-116 http://dx.doi.org/10.1051/apido/2010043.

142 • Hatjina F, Papaefthimiou C, Charistos L, Dogaroglu T, Bouga M, Emmanouil C, Arnold G: **Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo**. *Apidologie* 2013 http://dx.doi.org/10.1007/s13592-013-0199-4.
Imidacloprid administered under laboratory conditions to honeybees at 2 $\mu g\ kg^{-1}$ in sugar solution and 3 $\mu g\ kg^{-1}$ in pollen pastry has sublethal effects on the development of the hypopharyngeal glands (HPGs) and respiratory rhythm. The acini, the lobes of the HPGs of imidacloprid-treated honeybees, were 14.5% smaller in diameter in 9-day-old honeybees and 16.3% smaller in 14-day-old honeybees than in the same-aged untreated honeybees. Imidacloprid also significantly affected the bursting pattern of abdominal ventilation movements (AVM) by causing a 59.4% increase in the inter-burst interval and a 56.99% decrease in the mean duration of AVM bursts.

143. van Tome HV, Martins GF, Lima MAP, Campos LAO, Guedes RNC: **Imidacloprid-induced impairment of mushroom bodies and behavior of the native stingless bee melipona quadrifasciata anthidioides**. *PLoS ONE* 2012, **7**:e38406 http://dx.doi.org/10.1371/journal.pone.0038406.

144. De Almeida Rossi C, Roat TC, Tavares DA, Cintra-Socolowski P, Malaspina O: **Effects of sublethal doses of imidacloprid in malpighian tubules of africanized *Apis mellifera* (Hymenoptera, Apidae)**. *Microsc Res Tech* 2013, **76**:552-558 http://dx.doi.org/10.1002/jemt.22199.

145. Wu JY, Anelli CM, Sheppard WS: **Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity**. *PLoS ONE* 2011, **6**:e14720 http://dx.doi.org/10.1371/journal.pone.0014720.

146 • Cresswell JE: **A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees**. *Ecotoxicology* 2010, **20**:149-157 http://dx.doi.org/10.1007/s10646-010-0566-0.
This meta-analyses shows that dietary imidacloprid at field realistic doses is able to induce sublethal effects at colony level under either acute or chronic regimes (between 6% and 20% performance reduction). The statistical power of the existing field tests that claim to show no effects of neonicotinoids on honeybee colonies is insufficient to support a claim that such effects do not exist.

147. Cressey D: **Europe debate risks to bees**. *Nature* 2013, **496**:408 http://dx.doi.org/10.1038/496408a.

148. Becher MA, Thorbek P, Kennedy PJ, Osborne J, Grimm V: **Towards a systems approach for understanding honeybee decline: a stock-taking and synthesis of existing models**. *J Appl Ecol* 2013. (in press).

149. Moritz RFA, Southwick EE: *Bees as Superorganisms: An Evolutionary Reality*. Verlag: Springer; 1992, .

150. Khoury DS, Myerscough MR, Barron AB: **A quantitative model of honey bee colony population dynamics**. *PLoS ONE* 2012, **6**:e18491 http://dx.doi.org/10.1371/journal.pone.0018491.

151. Iwasa T, Motoyama N, Ambrose JT, Roe RM: **Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera***. *Crop Prot* 2004, **23**:371-378 http://dx.doi.org/10.1016/j.cropro.2003.08.018.

152. Krohn EA: **Synergistic mixtures exhibiting insecticidal and fungicidal action**. *US Patent US2008/0261811*. 2008.

153. Jamet EA: **Pesticidal mixtures.** *US Patent US2011/0046123*. 2011.

154 •• Alaux C, Brunet JL, Dussaubat C, Mondet F, Tchamitchan S, Cousin M, Brillard J, Baldy A, Belzunces LP, Le Conte Y: **Interactions between Nosema microspores and a neonicotinoid weaken honeybees (*Apis mellifera*)**. *Environ Microbiol* 2010, **12**:774-782 http://dx.doi.org/10.1111/j.1462-2920.2009.02123.x.
This study demonstrates the joint effect of a pathogen microsporidia (*Nosema ceranae*) and imidacloprid on honeybee health. A synergistic effect appears whenever honeybees are exposed to environmental relevant doses of the latter. Significant reduction of the enzyme glucose oxidase, enzyme linked to the social immunity of the colony, was observed in the groups exposed to both stressors.

155 • Pettis JS, Vanengelsdorp D, Johnson J, Dively G: **Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema**. *Naturwissenschaften* 2012, **99**:153-158 http://dx.doi.org/10.1007/s00114-011-0881-1.
Nosema infection increases significantly whenever bee colonies are exposed chronically to sublethal levels of the neonicotinoid, imidacloprid. 5 and 20 $\mu g\ kg^{-1}$ imidacloprid were administered to pollen patties and provided to bee colonies over 5 and 8 weeks.

156. Aufauvre J, Biron DG, Vidau C, Fontbonne R, Roudel M, Diogon M, Viguès B, Belzunces LP, Delbac F, Blot N: **Parasite–insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee**. *Nat Sci Rep* 2012, **2**:326.

157. Paula AR, Carolino AT, Paula CO, Samuels RI: **The combination of the entomopathogenic fungus Metarhizium anisopliae with the insecticide Imidacloprid increases virulence against the dengue vector Aedes aegypti (Diptera: Culicidae)**. *Paras Vec* 2011, **4**:8 http://dx.doi.org/10.1186/1756-3305-4-8.

158. Boucias DG, Stokes C, Storey G, Pendland JC: **The effects of imidacloprid on the termite Reticulitermes flavipes and its interaction with the mycopathogen Beauveria bassiana**. *Pflan Nachr Bayer* 1996, **49**:103-144.

159. Quintella ED, McCoy CW: **Pathogenicity enhancement of Metarhizium anisopliae and Beauveria bassiana to first instars of Diaprepes abbreviatus (Coleoptera: Curculionidae) with sublethal doses of imidacloprid**. *Environ Entomol* 1997, **26**:1173-1182.

160. Koppenhöfer AM, Grewal PS, Kaya HK: **Synergism of imidacloprid and entomopathogenic nematodes against white grubs: the mechanism**. *Entomol Exp Appl* 2000, **94**:283-293.

161. James RR, Xu J: **Mechanisms by which pesticides affect insect immunity**. *J Invertebr Pathol* 2012, **109**:175-182 http://dx.doi.org/10.1016/j.jip.2011.12.005.

162 • Fagin D: **The learning curve**. *Nature* 2012, **490**:5-8.
Researchers say that some chemicals have unexpected and potent effects at very low doses. The complex interplay of receptor binding and gene reprogramming can generate bizarre dose–response relationships, many of which are still being mapped out. Regulators are not convinced.

163. Stokstad E: **Pesticides under fire for risks to pollinators**. *Science* 2013, **340**:674-675.

Environ Chem Lett (2015) 13:269–291
DOI 10.1007/s10311-015-0513-7

REVIEW

Chlorpyrifos: pollution and remediation

Elizabeth Mary John[1] · **Jisha Manakulam Shaike**[1]

Received: 25 May 2015 / Accepted: 17 June 2015 / Published online: 2 July 2015

Abstract The widespread use of pesticides in modern agriculture is of increasing concern due to environmental contamination and subsequent biodiversity loss. Chlorpyrifos is a toxic organophosphate pesticide. Repeated applications of chlorpyrifos modify the soil microbial community structure and pose potential health risks to the other nontargets. Chlorpyrifos has been reported as the second most commonly detected pesticide in food and water. Extensive use of chlorpyrifos in agriculture and persistence in the environment have raised public concern and demand for safe technologies to overcome the pollution and toxicity problems. Here, we review pollution and toxicity issues associated with chlorpyrifos use and discuss strategies to solve pesticide contamination. Chlorpyrifos, previously shown to be resistant to enhanced degradation, has now been proved to undergo enhanced microbe-mediated decay. Here, special emphasis is given to degradation methods such as ozonation, Fenton treatment, photodegradation, and advanced oxidation processes along with microbial degradation. Finally, we focus on degradation process at enzyme and molecular levels which will enable us to elucidate the exact degradative pathway involved in biodegradation.

Keywords Chlorpyrifos · Pesticide pollution · Bioremediation · Physicochemical treatments · Organophosphorus hydrolase enzymes · Trichloropyridinol

✉ Jisha Manakulam Shaike
jishams@mgu.ac.in

[1] School of Biosciences, Mahatma Gandhi University, Kottayam 685 560, Kerala, India

Introduction

Pesticides indeed have become an inevitable part of modern agricultural practices. Organophosphates (OP), a group of synthetic pesticides developed during the Second World War, are being used as insecticides and nerve agents. Since the removal of organochlorine insecticides from use, organophosphates have become the most widely used pesticides (Cortina-Puig et al. 2010) and are used in most countries. Chlorpyrifos is a broad-spectrum chlorinated organophosphate insecticide, nematicide, and acaricide used for pest control on various crops as well as lawns and ornamental plants. It is a degradable compound, and hence despite its high toxicity, it is being increasingly used worldwide since its introduction in 1965. Today, chlorpyrifos is registered in most of the countries worldwide. Although its use in certain applications has been restricted in several countries, producers easily find market in most of the developing and non-developing countries. Low economic price and easy access make public choose chlorpyrifos over the other pesticides (Panuwet et al. 2009; Lu 2011; Munoz-Quezada et al. 2012; Murphy et al. 2012; Phung et al. 2012). It was reported as one among the most commonly used organophosphate pesticide in the USA in 2007, with an estimated 7–9 million pounds applied (Grube et al. 2011). According to China's market review, an annual growth of 10 % in the global demand for chlorpyrifos is expected, and by 2015, this will exceed 200,000 tonnes (AgroNews 2013). Even after withdrawing chlorpyrifos products from indoor/outdoor domestic, garden, and industrial uses because of toxicity concerns in children, pets, wildlife, and the environment (Dow AgroSciences 2013), its production and consumption are drastically increasing every year.

A single application may not have any long-lasting impact on environmental health, but in practice pesticides

are usually applied repeatedly leading to its environmental accumulation. This in turn has already led to the contamination of a wide range of environmental compartments, ultimately posing threats to the sustainability of agricultural soils (Johnsen et al. 2001; Singh et al. 2002; Hua et al. 2009). The extent of toxicity increases for degradation products such as chlorpyrifos oxon, 3,5,6-trichloro-2-pyridinol, and the secondary metabolite 3,5,6-trichloro-2-pyridinol (TCP) which are more hazardous pollutants than the parent. The environmental fate of chlorpyrifos and biodegradation are the subject of an extensive review (Racke 1993). Hence, the environmental persistence, toxicity, and remediation of chlorpyrifos are detailed in this review, with special emphasis given to physical, chemical, and biological remediation strategies rather than microbial decay. Information available on biodegradation mechanism at molecular and enzyme levels is also discussed. Thus, this review is an attempt to summarize the current status of research in remediation of chlorpyrifos.

Chemistry of chlorpyrifos

Chlorpyrifos was first registered for use in the USA in 1965 by Dow Chemical Company for the control of foliage and soil-borne insect pests. It is formulated and marketed as liquid, gel, granular, soluble, emulsifiable and flowable concentrates, microencapsulated material, pellets, tablets, impregnated materials, baits, wettable powders, dusts, and ready-to-use formulations. Owing to its structure and physicochemical properties (Table 1), chlorpyrifos persists for relatively long period. Because of its nonpolar nature, chlorpyrifos has low solubility in water and great partition from aqueous into organic solvents. Its hydrolytic stability decreases with increasing pH. Relatively low volatilization and degradation under aerobic conditions also make it more persistent in environment (NFMS 2008).

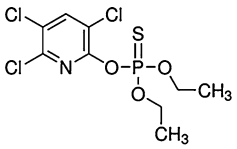
Environmental persistence

Chlorpyrifos enters the soil environment by direct pesticide application, through spray drift or foliar wash off (Fig. 1). Adsorption, desorption, and mobility of chlorpyrifos in soil and water are greatly dependent on the nature of the adsorbents and water solubility (Sharom et al. 1980a). Adsorption tends to reduce chlorpyrifos mobility and increases persistence in the soil by reducing its availability to degradative forces. Chlorpyrifos adsorbs to soil (with a soil adsorption coefficient (K_{oc}) of greater than 5000), soil particles, organic matter, clay minerals, and sediments to differing degrees, and the extent of soil adsorption is greater with organic soils than sandy loams (Gebremariam et al. 2012). The half life of chlorpyrifos in soil varies ranging from a few days to 4 years, greatly depending on application rate, the ecosystem type, soil microorganisms, and climatic conditions. Application level is important that chlorpyrifos shows a greater persistence at higher concentrations. The typical 1–2 months aerobic soil degradation half lives at normal agricultural rates seem to increase to 6 months to 4 years at higher application rates. Both biotic degradation (microbial) and abiotic degradation (hydrolysis and photolysis) bring its dissipation in soil. The dissipative half life is significantly equal to or slightly faster under tropical conditions than temperate conditions (Chai et al. 2009) and longer in organic soils than mineral soils (Gebremariam et al. 2012). Soil moisture content and pH play major role, and dissipation is especially predominant under higher moisture content (Wang et al. 2013a) and alkalinity (Singh et al. 2003b). Shading appears to reduce photodegradation (Chai et al. 2009), and hence, persistence will be greater in dark conditions. Prevailing environmental conditions alter the rate of decay where lower the temperature lower the loss of chlorpyrifos from the soil by vaporization and photodegradation (Mugni et al. 2012; Watts 2012).

Generally, adsorption prevents leaching of chlorpyrifos but constitutes a major off-site migration route to water bodies when bound to suspended sediments and particulates (Gebremariam et al. 2012). Though chlorpyrifos is reported to undergo dissipation quickly by hydrolytic and photolytic dissipation under neutral pH, its persistence has been observed in natural water even after 8 weeks, indicating the importance of microbial action to occur (Sharom et al. 1980b). It binds to aquatic sediments, and the dissipation rates in sediment are similar to those observed in soil. However, a slow microbial action is expected to occur under lower temperature, anaerobic condition (Bondarenko and Gan 2004), and salinity (Bondarenko et al. 2004). Like in the case of soil, alkalinity reduces the half life in water bodies to around two weeks. The average DT50 for chlorpyrifos in sediment under wetland and anaerobic ponds varies between 100 and 200 days (NRA 2000; Budd et al. 2011).

Measureable levels of chlorpyrifos have been reported in air (Hill 2006; Mills and Kegley 2006). Residues reported in Arctic media indicate that chlorpyrifos is expected to undergo long-range transport (Watts 2012). It is considered to be degraded more quickly in air with a shorter atmospheric residence time (Hayward et al. 2009). The degradation half life of chlorpyrifos in air due to photolysis is 6.34 h (Howard 1991) and that due to volatilization is 72 h (Lyman et al. 1990).

Table 1 Identity, structure, and physicochemical characteristics of chlorpyrifos

Identity	
ISO common name	Chlorpyrifos
Chemical class	Organophosphate
Chemical names	
IUPAC name	*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate
CA	*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate
CAS no.	2921-88-2
Molecular formula	$C_9H_{11}Cl_3NO_3PS$
Relative molecular mass	350.6
Trade names include	Lorsban, Dursban, Suscon Green, Empire, Whitmire PT 270, Brdan, Chlorpyrifos-ethyl, Detmol UA, Dowco 179, Eradex, Paqeant, Piridane, Scout, Stipend, Tricel, etc.
Structure	
	
Physicochemical properties	
State	Crystalline solid
Color	Colorless to white
Odor	Mild mercaptan (thiol) odor
Vapor pressure	3.35×10^{-3} Pa at 25 °C[a]
Melting point	41.5–42.5 °C
Solubility (water)	0.0014 g L^{-1} at 25 °C[b]
Partition coefficient (octanol/water)	Log $K_{ow} = 4.96$–5.11[c]
	Log $K_{ow} = 5.2$–5.267[c]
Henry's law constant	0.478 Pa × m^3 × mol^{-1}[a]
Soil sorption coefficient, K_{oc}	360–31000[d]
Aquatic sediment sorption, K_{oc}	3000–25,565 L kg^{-1}[c]; Mean = 13,439 L kg^{-1}
	Median = 15,500 L kg^{-1}
Half life	7 and 120 days
Regulatory status	Restricted use for liquid formulations (agricultural crops)
Identity tests	HPLC retention time, GC retention time, mass spectrum (from GC-MS)

[a] EFSA (2005)

[b] Tomlin (2009)

[c] Gebremariam et al. (2012)

[d] Smegal (2000)

Toxicity concerns

Toxicity on microorganisms

Soil microbes have different responses to different types of pesticides. The effect is dependent on many factors including the mechanism of action of the pesticide, bioavailability and physicochemical properties of the pesticide in the soil, environmental factors and concentration, dose, and frequency at which they are being applied. In general, microbial population in soil often remains unaffected by pesticide applications at recommended filed dose. Low amount of pesticides applied to soil is unlikely to have detrimental effects on soil microbes and the enzymes important to soil fertility (Tu 1981). However, concentrations greater than recommended levels exert deleterious effect on total microbial population and their biological activity (Moorman 1989). Also, repeated application of pesticides over many years can have increased impacts on soil microbial populations. Though the null effect of chlorpyrifos on soil microbial biomass had been reported (Adesodun et al. 2005), impacts of chlorpyrifos on soil microbial characteristics including microbial biomass, microbial populations, microbial respiration, enzymatic activities, and mineral cycling have been frequently studied. Findings indicated statistically

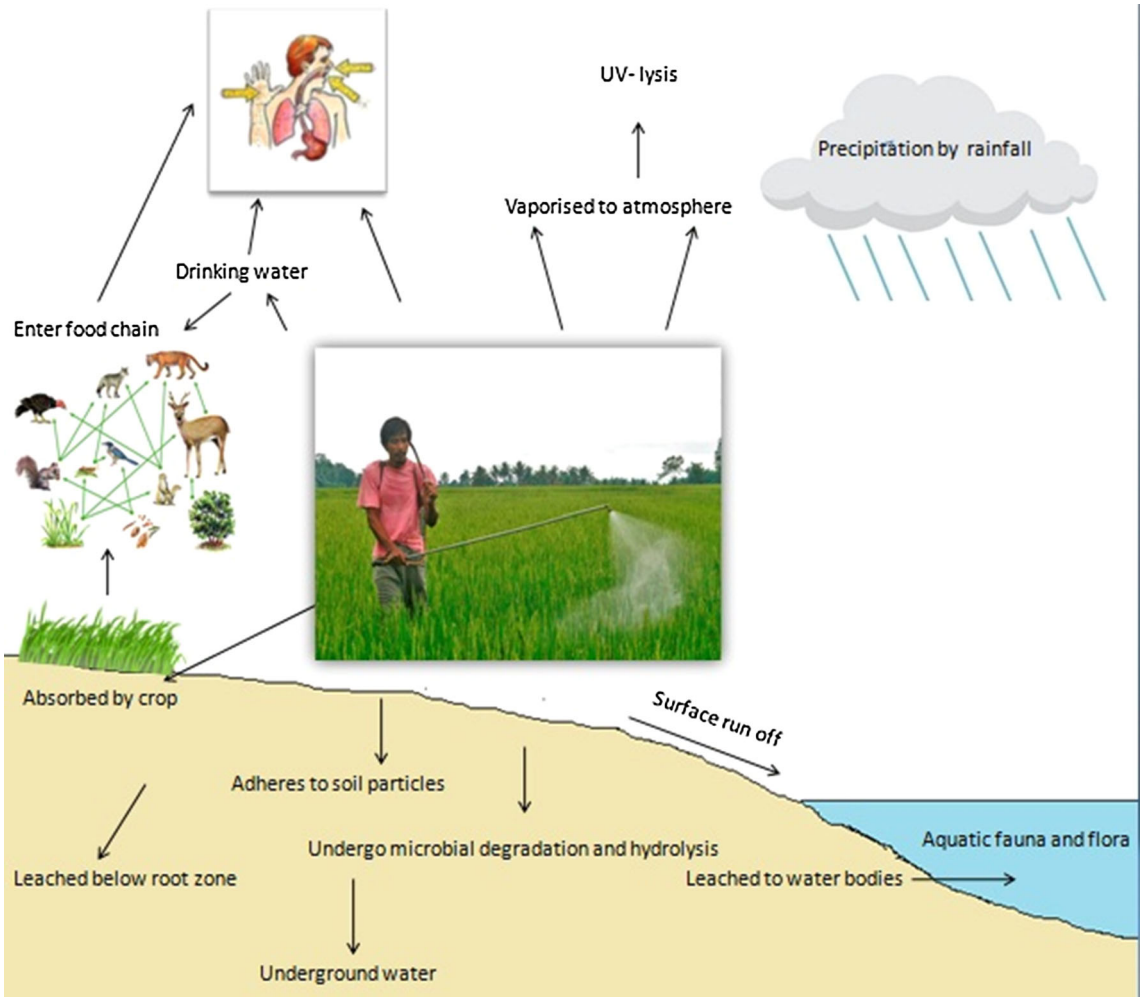


Fig. 1 Pollution problems and toxicity concerns associated with different trophic levels due to indiscriminate use of chlorpyrifos

significant reduction in soil microbial biomass (Vischetti et al. 2007), and soil enzyme activities (Singh et al. 2003a) resulted from repeated application of chlorpyrifos and accumulation of 3,5,6-trichloro-2-pyridinol. There are contradictory evidences on changes in microbial population due to chlorpyrifos application. Researchers have reported the non-inhibitory effect on total viable count of any kind of bacteria (Sarnaik et al. 2006) or fungal populations, nitrifying bacteria, and denitrifying bacteria (Pozo et al. 1995) as well as the short term inhibitory effect on the total bacterial population (Pandey and Singh 2004; Ahmed and Ahmad 2006; Hindumathy and Gayathri 2013), fungal and actinomycete populations (Shan et al. 2006). On the other hand, some studies showed significant increase in the same after chlorpyrifos treatment (Sivasithamparam 1970; Pozo et al. 1995; Pandey and Singh 2004; Pankhurst 2006).

Chlorpyrifos at recommended field dose is not likely to be detrimental, but at higher than that poses a significant inhibitory effect on the soil microbial metabolic activities also (Dutta et al. 2010). There exist reports on inhibitory effect of chlorpyrifos on dinitrogen fixing bacteria and dinitrogen fixation, and N, P, and K mineralization (Martinez-Toledo et al. 1992; Pozo et al. 1995; Sardar and Kole 2005). The arginine ammonification activity of rhizospheric microbes was also inhibited after chlorpyrifos treatment (Menon et al. 2004). However, this inhibitory effect on available N, P, K and ammonification was attributed to 3,5,6-trichloro-2-pyridinol, the major metabolite of chlorpyrifos rather than chlorpyrifos itself (Racke et al. 1990).

Time dependent increased activity, followed by progressive decrease in soil, gave statistically significant evidence for the influence of chlorpyrifos on soil phosphatase enzyme (Pozo et al. 1995; Madhuri and Rangaswamy 2002). An overall inhibition (Menon et al. 2005) and reduced activity of dehydrogenase, urease, and alkaline phosphatase to a greater extent (Jastrzebska 2011) proves the detrimental effect of chlorpyrifos on microorganisms. Madhaiyan et al. (2006) have shown the potential of chlorpyrifos to alter cell morphology of *Gluconacetobacter diazotrophicus* resulting in large number of pleomorphic

cells. United States Environmental Protection Agency (US EPA 2009) stated that chlorpyrifos is not mutagenic in bacteria but did cause slight genetic alterations in yeast and DNA damage to bacteria.

Toxicity on plants

According to Worthing (1979), chlorpyrifos has no systemic action, and at insecticidal concentrations, it is not phytotoxic. However, literature is rich in information referring to the phytotoxic and inhibitory effects of chlorpyrifos. Data indicate that chlorpyrifos and its metabolites can accumulate in various terrestrial and aquatic plants, and the extent of toxicity depends on chlorpyrifos concentration. The effects were assessed based on changes in germination pattern, morphological traits (root and shoot length, fresh and dry weight of roots and shoots), biochemical functions, and metabolism. Chlorpyrifos was found to have inhibitory effect on germination in *Pinus halepensis* seeds and seedlings (Olofinboba and Kozlowski 1982), annual grass and annual forb (Gange et al. 1992), ryegrass (*Lolium multiflorum*) (Korade and Fulekar 2009), different vegetables (Zhang et al. 2013), *Cenchrus setigerus* and *Pennisetum pedicellatum* (Dubey and Fulekar 2011), white mustard (*Sinapis alba* L.), maize (*Zea mays* L.) (Gvozdenac et al. 2013), and soybean (*Glycine max*) (Bassey et al. 2015). The intensity of toxicity on seed germination energy, seed germination, survival, and subsequent growth increases with chlorpyrifos concentration (Dubey and Fulekar 2011; Gvozdenac et al. 2013). It has also been reported to exert detrimental effect on the root biomass, root appearance and disappearance, and root density (Dawson et al. 2003; Singh et al. 2003a). Even though it did not affect the in vitro growth of *Rhizobium*, reduced nodulation (Bassey et al. 2015) and nitrogen fixation (C_2H_2 reduction) were observed in alfalfa sweet clover and red clover associated with the application of chlorpyrifos (Smith et al. 1978; Parween et al. 2011). The Pesticide Action Network (PAN) toxicity study data give a clear picture of severity of accumulation and population, biochemical, and physiological effects of chlorpyrifos in various aquatic plants (Kegley et al. 2014).

In contrast to these reports, chlorpyrifos is being suggested as useful tool at lower concentration regardless of its insecticidal properties, to increase the seed quality and quantity as all the growth parameters and yield parameters tested were found to be increased in seedling exposed to chlorpyrifos (Kashyap and Kumar 2013). And one surprising finding to be noted is lower dose of chlorpyrifos proved as stimulant for root and shoot length, activities of nitrate reductase (NR), content of nitrate, and sugar soluble protein, whereas at higher concentration it proved detrimental for the same (Parween et al. 2011).

Toxicity on aquatic system

The acute and chronic toxicities of pesticides to aquatic fauna have been well studied. Organophosphates are found to be more toxic to fishes such as blue gill sunfish *Lepomis macrochiru*, rainbow trout, channel catfish, *Ictalurus punctatus*, fathead minnow *Pimephales promelas*, golden shiner (Barron and Woodburn 1995), and juvenile and adult of *Oreochromis niloticus* (Oruc 2010). A number of previous works have confirmed the toxicity of chlorpyrifos to freshwater, estuarine, and marine vertebrates and invertebrates. It is known to cause acetylcholinesterase (AChE) and aliesterases (AliE) (Carr et al. 1997) inhibition and several prominent developmental, behavioral, neurological, oxidative, histopathological (Fig. 2), and endocrine effects in aquatic fauna. Chlorpyrifos toxicity data were available for several fish species (Table 2). Freshwater LC_{50} values were found to be similar between most of the species ranging from 1.8 to 280 μg L^{-1} chlorpyrifos. The lowest LC_{50} reported for fish was 1.8 μg L^{-1} chlorpyrifos for bluegill sunfish, where highest was reported in channel catfish (280 μg L^{-1}). Extent of toxicity increases in the presence of atrazine (Wacksman et al. 2006) and for chlorpyrifos oxon, one major by-product, 10–100 times more than the parent chlorpyrifos (Sparling and Fellers 2007).

The primary visualized changes include exhibition of unusual swimming behavior (Sharbidre et al. 2011), mortality, paralysis, and histological abnormalities including loss and shortening of secondary lamellae (De Silva and Samayawardhena 2002). Long lasting neurobehavioral deficits during early development, persistent hatching, larval morphological deformities, decrease in whole brain activity (Levin et al. 2003, 2004; Eddins et al. 2009; Richendrfer et al. 2012; Sledge et al. 2011), reduced survival, adult length and body weight, and reproductive failure are the other primary effects likely to result from chronic chlorpyrifos exposures. Moreover, life cycle study has provided enough data to show that second generations are more sensitive than the first generation (Corbin et al. 2009). It is found highly effective in inducing structural alteration and biochemical changes such as depletion in protein content and decrease in different metabolic enzyme levels (Kavitha and Rao 2008), altering the ion transport mechanism and lipid peroxidation, and causing hormonal changes (Oruc 2010). Data show that chlorpyrifos is mutagenic and genotoxic to fishes. For example, significant micronucleus induction and DNA damage were observed in freshwater fish *Channa punctatus* upon exposure to chlorpyrifos (Porichha et al. 1998; Ali et al. 2009).

Chlorpyrifos is highly toxic to amphibians with larval stages appeared to be more sensitive than older life stages (Howard et al. 2003). The high acute toxicity and

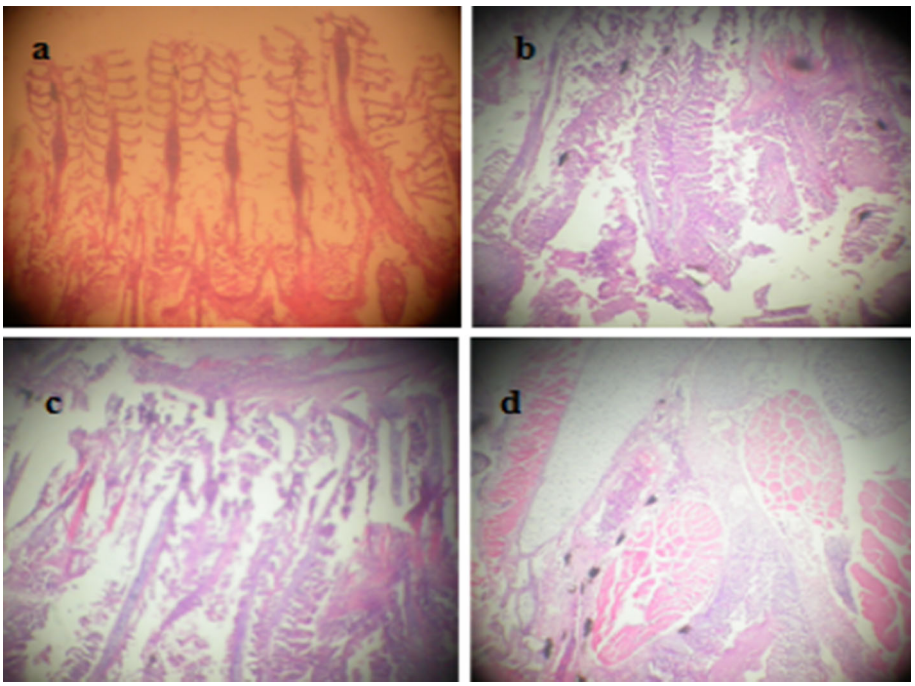


Fig. 2 Histopathologic analysis of *Oreochromis mossambicus* gills exposed to chlorpyrifos (96 h). **a** Control. **b**, **c** Degenerated gill lamellae. **d** Lamellar edema (unpublished data from our laboratory)

persistence in soil sediments represent a hazard to the sea bottom dwellers (Schimmel et al. 1983), marine and freshwater crustaceans (Key and Fulton 1993; Palma et al. 2008; Eamkamon et al. 2012), water flea (Caceres et al. 2007), marine infaunal copepod (Green et al. 1996), midges (Jin-Clark et al. 2002), and phytoplankton (DeLorenzo and Serrano 2003). Acute and chronic effects of chlorpyrifos on various amphibians as well as aquatic vertebrates and invertebrates have been well reviewed by Corbin et al. (2009). Ephyra stage of the Scyphozoan jellyfish *Aurelia aurita* was recently reported susceptible to dose dependent effect of chlorpyrifos (Costa et al. 2015). All these together indicate apprehension about the potential hazards of chlorpyrifos to aquatic life.

Toxicity on higher vertebrates

Age and sex (Levin et al. 2001) related differences in sensitivity to chlorpyrifos can vary both quantitatively and qualitatively with exposure conditions, doses, and frequency of exposure. Like in lower vertebrates in all higher vertebrates also chlorpyrifos target mainly AChE activity (Schuh et al. 2002; Srebocan et al. 2003; Reiss et al. 2012). Previous studies have shown that subtoxic doses of chlorpyrifos are capable of affecting brain development by inhibiting mitosis, inducing apoptosis, and altering neuronal activity and reactivity. Its ability to exert reproductive toxicity is evident from fetotoxic (Farag et al. 2003) and teratogenic effects (Tian et al. 2005)). Chlorpyrifos is a potent developmental neurotoxin (Slotkin 2004; Flaskos 2012) and an endocrine disruptor also (Slotkin et al. 2005; De Angelis et al. 2009; Haviland et al. 2010). Apart from that, the genotoxicity, mutagenicity, carcinogenicity, and immune toxicity are under investigation. Laboratory studies showed that both acute and chronic exposures result in genotoxicity and mutagenicity in human (Sobti et al. 1982; Sandal and Yilmaz 2011), rat (Ojha et al. 2013) and mouse (Rahman et al. 2002; Tian et al. 2005; Cui et al. 2011). It is considered moderately to extremely toxic to birds, and no such toxicity studies are available on reptiles (Watts 2012).

In humans, chlorpyrifos is proved to be anti-androgenic (Usmani et al. 2003; Viswanath et al. 2010) and estrogenic (Meeker et al. 2008; Ventura et al. 2012). It induces alterations in thyroid (Fortenberry et al. 2012) and adrenal glands, thereby reducing serum levels of respective hormones. Children exposed to higher chlorpyrifos levels are significantly more likely to experience psychomotor development index and mental development index delays, attention problems, attention deficit or hyperactivity disorder, and pervasive developmental disorder problems (Rauh et al. 2006). The carcinogenicity of chlorpyrifos is not confirmed in human; yet, there are a considerable number of epidemiological studies indicating increased risk of various cancers in pesticide applicators, especially colorectal (Lee et al. 2007), prostate (Alavanja et al. 2003), breast (Engel et al. 2005), lymphoma (Karunanayake et al. 2012), hematopoietic, leukemia, and brain cancers (Lee et al. 2004). There is evidence of immune toxicity, including the effects on lymphocytes (Blakley et al. 1999), thymocytes (Prakash et al. 2009), T cells (Thrasher et al.

Table 2 Acute toxicities of chlorpyrifos to several species of fishes (LC_{50})

Fish	LC_{50}	Concentration (ppb)	Remarks-chlorpyrifos	Reference
Bluegill sun fish (*Lepomis macrochirus*)		2–10[a]		Tomlin (2009)
Rainbow trout, *Oncorhynchus mykiss*		7–51[a]	Technical grade	Tomlin (2009)
	Freshwater, static	15[d]	97 %	WISER
	Juvenile	11.4[c]	99.9 %	WISER
	Freshwater, flow through	8.0[b]		
Channel catfish, *Ictalurus punctatus*	Freshwater, flow through	806[b]	98.7 %	WISER
Lake trout		98[a]	97.0 %	Johnson and Finley (1980)
Salvelinus namaycush				
Cutthroat trout	pH 7.5	18.4[a]	97.0 %	Barron and Woodburn (1995)
Salmo clarki	pH 9.9	5.4[a]		
Fathead minnow	Larva	122[a]	Technical grade	Jarvinen et al. (1988)
Pimephales promelas	Mature	120–540[a]		Tomlin (2009)
		542[a]		Phipps and Holcombe (1985)
Golden shiner *Notemigonus crysoleucas*		35[a]	99.0 %	Barron and Woodburn (1995)
Mosquito fish *Gambusia affinis*		340[d]		Karim et al. (1985)
		297[a]	Technical grade	Kavitha and Rao (2008)
	Adult female	150[d]	95 %	WISER
	Freshwater, static			
	Gravid female	1400[d]	Formulated product	WISER
	Freshwater, static	440[c]		
	Adult	520[c]	93.99 %	WISER
	Saltwater	540[b]		
Oreochromis niloticus	Juvenile	98.67[a]	Dursban (480 g L^{-1} chlorpyrifos)	Oruc (2010)
	Adult	154.01[a]		
Guppy		176[a]		Sharbidre et al. (2011)
Poecila reticulata				
Gulf toadfish		520[a]		Clark et al. (1985)
Opsanus beta				
Carp		580[a]		Xing et al. (2012)
Oreochromis mossambicus	Juveniles	26[a]	Analytical grade	Rao et al. (2003)
		82[a]	Technical grade	Aniladevi Kunjamma et al. (2008)
		16.812[a]	Pesticide formulation 20 % EC	(Unpublished data from our laboratory)
Gold fish		806[a]	Technical (98.7 %)	Phipps and Holcombe (1985)
Carasslus auratus				

Values are in μg chlorpyrifos per liter (ppb) of medium fatal to 50 percent (LC_{50}) in [a] 96 h, [b] 72 h, [c] 48 h, [d] 24 h

2002), tumor necrosis factor (Rowsey and Gordon 1999), and autoimmunity. In humans, chlorpyrifos exposure is associated with reproductive issues such as birth weight and length problems, DNA damage in sperm and decreased sperm fluid, sperm concentration, sperm motility, cervical fluid, cord blood, meconium, and breast milk (Watts 2012). However, ultimate evaluation of the toxicity and behavioral effects due to chlorpyrifos would require long term assessments during discrete developmental and age periods (Dam et al. 2000).

Solutions to chlorpyrifos toxicity

The first priority for any waste management strategies should be practices that prevent or at least minimize the generation of waste and promote recycling (Felsot et al. 2003). Solutions to chlorpyrifos pollution also begin with avoiding and reducing its use as alternatives are available for all or most of its purposes. These include cultural practices, mechanical techniques, biological controls, and use of other chemicals. Reuse and recycle are other options for minimizing the generation of waste. However, the present scenario of excessive use of chlorpyrifos for domestic and agriculture purposes and resulted environment contamination demand special treatment technologies. Though a variety of remediation technologies are practiced for pesticide contaminated materials, soil and water, the selection of the most appropriate is often a quiet tough task. As a single technology may found inappropriate, sometimes number of technologies should be combined together to accomplish satisfactory results.

Physical and chemical remediation strategies

For the treatment of water, sediments, and soils polluted with pesticides, many physicochemical technologies have been investigated. When exposed directly to UV or sunlight, chlorpyrifos undergoes fission of the phosphate esters with the preferential liberation of trichloropyridinol, which undergoes further photodehalogenation and oxidative ring cleavage (Walia et al. 1988). Most of the researchers use artificial solar sources of irradiation (xenon arc lamps or low- or medium-pressure mercury lamps) or ultraviolet (UV) light (Kralj et al. 2007). During photodegradation on soil surfaces with UV light (254 nm), three different photochemical processes—hydrolysis, dechlorination, and oxidation—take place simultaneously, and the oxidative and dehalogenated products formed undergo further photolysis to form chloropyridinols and O, O-diethyl phosphorothioic acid which are unstable and hydrolyze more rapidly without any accumulation in the soil (Walia et al. 1988). The UV lysis appears to be less effective compared to other processes where it can be accelerated by the combined use of hydrogen peroxide, ozone or homogeneous or heterogeneous photocatalyst. Among the chemical methods, ozonation is considered as the most promising since a relatively fast reaction of ozone occurs with chlorpyrifos. Ozone mediated oxidation of thiophosphoryl bond of chlorpyrifos as well as that of chlorpyrifos-methyl has been reported (Ohashi et al. 1993). Other chemical oxidation methods including chlorine (Duirk and Collette 2005), chlorine dioxide, potassium permanganate, and hydrogen peroxide (H_2O_2) used are not effective enough to degrade highly refractory synthetic organic chemicals such as pesticides (Ikehata and El-Din 2006). Moreover, such methods seem to be expensive and lead to the contamination of medium with other toxic pollutants.

On the other hand, combinations of the same chemical oxidants (e.g., O_3 and H_2O_2) with iron salts, semiconductors (e.g., titanium dioxide, TiO_2), and/or ultraviolet–visible light (UV–Vis) irradiation yield better results. For example, the Fenton reagent (H_2O_2/Fe^{2+}) has been proven to be effective in degradation and mineralization of chlorpyrifos (Ikehata and El-Din 2006). These physicochemical approaches were collectively referred as advanced oxidation processes (AOPs). These are considered high efficiency physical–chemical processes due to their thermodynamic viability and capability to produce deep changes in the chemical structure of the contaminants (Domenech et al. 2004) and hence gain much attention nowadays. The basic mechanism underlying advanced oxidation processes is the formation of strong oxidizing intermediates (mainly $OH^{\bullet}$ radicals), and the reaction of these with organic contaminants not only leads to their destruction, but also gives sufficient conditions for their complete mineralization (Salama and Osman 2013). These are short lived, powerful oxidizing agents, which follow second-order kinetics with low selectivity (Esplugas et al. 2002). $OH^{\bullet}$ can be produced in situ by chemical, electrochemical, and/or photochemical reactions in advanced oxidation process systems. Most of the AOPs use a combination of strong oxidizing agents (e.g., hydroxyl radicals, ozone, fluorine, atomic oxygen, hydrogen peroxide perhydroxyl radical, permanganate, hupobromous acid, hypochlorous acid, and chlorine dioxide) with catalysts (e.g., transition metal ions) and irradiation (e.g., ultraviolet, visible).

Photocatalytic degradation is considered as a promising technology for the elimination of pesticides from aqueous media as it is one of the most effective methods for wastewater treatment. It is a rapidly growing field of research and has been studied in different types of natural waters such as lake, river, and groundwater as well as in distilled and drinking water under natural sun light and simulated irradiation sources (Muhamad 2010). It simply denotes the acceleration of photoreaction with the help of a catalyst (Table 3). These advanced oxidation processes can be classified either as homogeneous or as heterogeneous.

Homogeneous photocatalysis refers to those photocatalytic processes where the reactants and the photocatalysts exist in the same phase during the reaction (Quiroz et al. 2011). The most commonly used homogeneous photocatalysts include ozone and photo-Fenton systems (Fe^{2+} and Fe^{2+}/H_2O_2).

H_2O_2/UV The combination of UV radiation and hydrogen peroxide seems a promising option as it has proved to be a simple and effective method that can totally

Table 3 Reactions underlying the physicochemical methods used for chlorpyrifos degradation

Method	Reaction	Reference
Direct UV lysis	$R + hv \rightarrow$ Intermediates	Sharma (2015)
	Intermediates $+ hv \rightarrow CO_2 + H_2O + R^-$	
Fenton	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$ acidic aqueous solution	Samet et al. (2012)
Homogeneous photocatalysis (general reaction)	$C + hv \rightarrow C_*$	Domenech et al. (2004)
	$C_* + R \rightarrow R_* + C$	
	$R_* \rightarrow P$	
H_2O_2/UV	$H_2O_2 + hv$ ($\lambda = 250$–254 nm) $\rightarrow 2OH^\bullet$	Ikehata and El-Din (2006)
Photocatalytic ozonation	$H_2O + O_3 + hv \rightarrow 2OH^\bullet + O_2$	Sharma (2015)
	$2OH^\bullet \rightarrow H_2O_2$	
Photo-Fenton	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$	Samet et al. (2012)
	$Fe(OH)^{2+} + hv \rightarrow Fe^{2+} + OH^\bullet$ ($\lambda < 450$ nm)	
	$Fe(RCO_2)^{2+} + hv \rightarrow Fe^{2+} + R^\bullet + CO_2$ ($\lambda < 500$ nm)	
Heterogeneous photocatalysis	$TiO_2 + hv \rightarrow e^- + h^+$	Pelizzetti et al. (1993)
TiO_2	O_2 (ads) $+ e^- \rightarrow O_2^{-\bullet}$ (ads)	
	Ti(IV)-OH^- $+ h^+ \rightarrow$ Ti(IV)-$OH^\bullet$/Ti(IV)-$H_2O + h^+ \rightarrow$Ti(IV)-$OH^\bullet + H^+$	
	$R + OH^\bullet/O_2^{-\bullet} \rightarrow R^\bullet + H_2O$	

mineralize chlorpyrifos in water (Ikehata and El-Din 2006). This technique where hydrogen peroxide molecule is cleaved into two hydroxyl radicals by UV photolysis requires a relatively high dose of H_2O_2 and/or a much longer UV exposure time. The process is greatly pH dependent, the rate of photolysis of hydrogen peroxide increases with alkaline conditions (de Oliveira et al. 2014). 93 % conversions of chlorpyrifos have been achieved in 20 min with H_2O_2 initial concentration of 450 mg L^{-1} (0.16 mg of chlorpyrifos degraded per mg of H_2O_2 consumed) (Femia et al. 2013).

Fenton's oxidation The degradation velocity of Fenton oxidation can be enhanced when ultraviolet radiation and visible light (UV–Vis) or solar radiation were added to the reaction, and this modified Fenton is called photo-Fenton treatment processes (combination of H_2O_2, Fe^{2+}, and UV–Vis irradiation) (Oppenlander 2003). The solar photo-Fenton process is found to be 50 % more efficient than the Fenton process (Samet et al. 2012). The degradation rate in both methods is strongly dependent on pH, temperature, H_2O_2 dosing rate, and initial concentrations of the insecticide and Fe^{2+}. The acidification of the medium is must for photo-Fenton, and the pH range between 2.6 and 3 gives the best performance of the system. Yu (2002) has reported the treatment of concentrated solution of chlorpyrifos along with many other organophosphates via a Fenton process at pH 2.8 and was able to show more than 95 % reduction in COD with no degradation products detected. A comparative study on different effective remediation technologies for chlorpyrifos reveals nano photo-Fenton-like reagent (Fe_2O_3(nano)/H_2O_2/UV) as the most effective treatment for chlorpyrifos removal in drinking water followed by ZnO(nano)/H_2O_2/UV, Fe^{3+}/H_2O_2/UV, and ZnO/H_2O_2/UV, respectively (Derbalah et al. 2013). According to Yu (2002), a combination of supercritical carbon dioxide extraction (SC–CO_2) and subsequent degradation by Fenton's reagent may provide an alternative water purification strategy for treating organophosphate pesticides in agro-wastewater.

Ultrasound energy/ozone (US/O_3) Apart from the use of UV energy, ultrasound energy (US) is also used coupled with oxidants. Oxidative degradation of standard chlorpyrifos by individual and combined effects of ultrasonic irradiation and ozone showed that US/O_3 combined treatment had a synergistic effect that significantly lowered the toxicity of chlorpyrifos with increased production of chloride, nitrate, and sulfate. However, decrease in pH (from 7.46 to 4.49) observed during the degradation process points towards the release of HCl which is unacceptable (Pengphol et al. 2012).

Heterogeneous photocatalysis degradation concept involves the use of a solid semiconductor (e.g., metallic oxides and sulfur such as TiO_2, ZnO, CdS, ZnS) to generate a colloidal suspension stable under radiation to stimulate a reaction in the solid–liquid (or solid–gas) interface, and charge transference occurs along the interface to balance chemical potentials between the two faces (Quiroz et al. 2011). The term heterogeneous refers to the fact that the

contaminants are present in the aqueous phase, while the catalyst is in the solid phase. Titanium dioxide (TiO_2) coupled with UV radiation of photon wavelength less than 400 nm is the most frequently used semiconductor for chlorpyrifos treatment (Devipriya and Yesodharan 2005; Schulman 2013). TiO_2 photocatalytic degradation of chlorpyrifos in aqueous phase in the presence of artificial UV light and sunlight was reported by Verma and Dixit (2012). The authors suggest treatment under natural solar conditions will give better results as compared to UV treatment. Similar photocatalytic degradation of chlorpyrifos in aqueous suspensions under solar light using nanocrystals of ZnO has also been demonstrated (Kanmoni et al. 2012). However, many investigators reported the ability of TiO_2 to exhibit a better photocatalytic activity on the degradation of chlorpyrifos than that of ZnO under same photocatalytic reaction conditions (Kanmoni et al. 2012; Fadaei and Kargar 2013). Enhancement of degradation and improvement in biodegradability index of TiO_2 by the addition of H_2O_2 has been evaluated and found $UV/TiO_2/H_2O_2$ photocatalysis is very effective in degradation of chlorpyrifos in aqueous solution (Miguel et al. 2012; Affam and Chaudhuri 2013). $UV/TiO_2/H_2O_2$ photocatalysis may be applied as pre-treatment of a chlorpyrifos, cypermethrin, and chlorothalonil pesticide contaminated wastewater at pH 6, for biological treatment. Photocatalysis with $FeCl_3$ also reported to enhance chlorpyrifos degradation (Penuela and Barcelo 1997). Similarly, the application of $O_3/H_2O_2/TiO_2$ process found to give better chlorpyrifos degradation at an average degradation yield of 36 % than that of ozone, O_3/H_2O_2 and O_3/TiO_2 (Ormad et al. 2010). However, doping with transition metal ions such as V^{5+}, Mo^{6+}, and Th^{4+} was found to decrease the photocatalytic activity of TiO_2 (Devi et al. 2009).

Radiation process with a beam of accelerated electrons or gamma radiation is considered one of the most powerful advanced oxidation processes for decomposition of various pollutants. Gamma irradiation is reported to have the potential to degrade chlorpyrifos and could be utilized in combination with the conventional method sunlight to clean up environmental samples contaminated with chlorpyrifos (Hossain et al. 2013). A dose dependent pseudo-first-order (decay) radiolysis of chlorpyrifos by ^{60}Co γ-ray in aqueous solution was observed in a laboratory study, and the results depicted 100 % degradation of 500 μg L^{-1} solution at an absorbed dose of 575 Gy (the dose rate was 300 Gy h^{-1}) (Ismail et al. 2013). An advanced oxidation process system that combines microwave radiation, ultraviolet radiation, TiO_2, and H_2O_2 solutions ($MO/UV/TiO_2/H_2O_2$) was reported as the most efficient to remove chlorpyrifos from aqueous media, with 100 % removal reached within 6 min reaction time in diluted concentrations of the oxidizing agents (Barros et al. 2013).

Degradation of chlorpyrifos by a combination of immobilized hemoglobin and in situ generated hydrogen peroxide is reported for the first time by Tang et al. (2011). This electrochemical–enzyme system under the optimal conditions could give more than 98 % of the chlorpyrifos degradation with low power and high efficiency and hence suggested as a possible alternative for expensive natural enzymes used for chlorpyrifos contaminated wastewater treatment.

Biodegradation

Biotic degradation is a common process for the removal of any organic pollutants. Several species of bacteria and fungi have been demonstrated to exhibit considerable capacity for the metabolism of chlorpyrifos in soil and liquid media (Table 4). Microbes can degrade chlorpyrifos either catabolically (John et al. 2014; Singh et al. 2004) or co-metabolically (Anwar et al. 2009), former refers to mineralization by direct use as a source of carbon and energy, while latter is the incidental metabolism where the compound is transformed without any benefit to the microbe. A reasonably good number of bacteria were reported to mineralize chlorpyrifos including *Flavobacterium* sp. ATCC 27551, *Arthrobacter* sp. (Mallick et al. 1999), and *Enterobacter* (Singh et al. 2004). Chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading *Alcaligenes faecalis* strain have been reported (Yang et al. 2005) but was not efficient enough for chlorpyrifos removal. *Klebsiella* sp. obtained from activated sludge sample of wastewater treatment plant in Damascus was reported to degrade 92 % chlorpyrifos in 4 days (Ghanem et al. 2007). 100 % degradation of 100 mg L^{-1} chlorpyrifos has been observed using different bacteria including *Sphingomonas*, *Stenotrophomonas*, *Bacillus* sp., *Brevundimonas*, and *Pseudomonas* sp. isolated from polluted water samples of chlorpyrifos manufacturing industry, China, and enriched in mineral salts medium (Li et al. 2008). Similarly, a *Paracoccus* sp. isolated from activated sludge sample from pesticide manufacturers and enriched in TYC medium found to be degrading 100 % of chlorpyrifos (50 mg L^{-1}) and 3,5,6-trichloro-2-pyridinol in four days (Xu et al. 2008). Growth studies revealed that a soil bacterium designated MS09, capable of utilizing chlorpyrifos as the sole carbon source isolated by selective enrichment, which later identified as a strain of *Providencia stuartii* is able to utilize different concentrations of chlorpyrifos varying from 50 to 700 mg L^{-1} (Rani et al. 2008). A *Pseudomonas* sp. (ChlD), isolated from agricultural soil by enrichment culture technique, was capable of producing biosurfactant (rhamnolipids) and degrading chlorpyrifos (Singh et al. 2009). Many *Bacillus* sp. including *Bacillus pumilus* (Anwar et al. 2009), *Bacillus licheniformis* (Zhu et al. 2010),

Table 4 Organisms capable of degrading or removing chlorpyrifos

Organisms	Reference
Bacteria	
Acinetobacter calcoaceticus	Zhao et al. (2014)
Agrobacterium	Maya et al. (2011), Chishti and Arshad (2012)
Alcaligenes faecalis	Yang et al. (2005)
Arthrobacter sp.	Mallick et al. (1999)
Bacillus cereus	Lakshmi et al. (2009), Liu et al. (2012)
Bacillus firmus	Sabdono (2013)
Bacillus licheniformis	Zhu et al. (2010)
Bacillus pumilus	Anwar et al. (2009)
Bacillus subtilis	Lakshmi et al. (2008), El-Helow et al. (2013)
Brucella melitensis	Lakshmi et al. (2008)
Brevundimonas sp.	Li et al. (2008)
Cupriavidus sp.	Lu et al. (2013)
Cupriavidus taiwanensis	Zhu et al. (2013)
Enterobacter sp.	Singh et al. (2004), Chishti and Arshad (2012)
Escherichia coli	Wang et al. (2002)
Flavobacterium sp.	Mulbry et al. (1986) Mallick et al. (1999)
Micrococcus sp.	Guha et al. (1997)
Klebsiella sp.	Ghanem et al. (2007), Lakshmi et al. (2009), Gilani et al. (2010), John et al. (2014)
Paracoccus sp.	Xu et al. (2008)
Plesiomonas sp.	Yang et al. (2006)
Providencia stuartii	Rani et al. (2008)
Pseudomonas sp.	
Pseudomonas fluorescence	Lakshmi et al. (2008)
Pseudomonas aeruginosa	Sasikala et al. (2012), Latifi et al. (2012), John et al. (2014)
Pseudomonas desmolyticum	Rokade and Mali (2013)
Pseudomonas nitroreducens	Latifi et al. (2012)
Pseudomonas putida	Ajaz et al. (2009), Sasikala et al. (2012)
Pseudomonas stutzeri	Sasikala et al. (2012)
Pseudomonas pseudoalcaligenes	Wu et al. (2004)
Serratia sp.	Xu et al. (2008), Lakshmi et al. (2009)
Sphyngomonas	Li et al. (2007)
Stenotrophomonas sp.	Yang et al. (2006), Li et al. (2008)
Cyanobacterium	
Anabaena sp.	Park (2010)
Phormidium valderianum	Palanisami et al. (2009)
Spirulina platensis	Thengodkar and Sivakami (2010)
Synechocystis sp.	Singh et al. (2011)
Actinomycetes	
Streptomyces sp	Briceno et al. (2012)
Streptomyces thermocarboxydus	Eissa et al. (2014)
Fungus	
Acremonium sp.	Kulshrestha and Kumari (2011)
Aspergillus	Liu et al. (2002), Maya et al. (2012)
Aspergillus niger	Mukherjee and Gopal (1996)
Cladosporium cladosporioides	Gao et al. (2012)
Emericella sp.	Maya et al. (2012)

Table 4 continued

Organisms	Reference
Eurotium sp.	Maya et al. (2012)
Fusarium	Wang et al. (2005)
Ganoderma sp.	Silambarasan and Abraham (2013)
Mucor sp.	Hastings and Coster 1981)
Penicillium	Maya et al. (2012)
Penicillium vermiculatum	Hastings and Coster (1981)
Phanerochaete chrysosporium	Bumpus et al. (1993)
Saccharomyces cerevisiae	Lal and Lal (1987)
Trichoderma	
Trichoderma harzianum	Hastings and Coster (1981)
Trichoderma viride	Mukherjee and Gopal (1996)
Verticillium sp.	Yu et al. (2006), Fang et al. (2008)
Algae	
Chlorella vulgaris	Mukherjee et al. (2004)
Phytoremediation (remove chlorpyrifos)	
Chenopodium amaranticolor	Garg et al. (2010)
Lemna minor L.	Prasertsup and Ariyakanon (2011)
Plantago major L.	Romeh and Hendawi (2013)
Pistia stratiotes L.	Prasertsup and Ariyakanon (2011)

and *Bacillus subtilis* strain Y242 (El-Helow et al. 2013) were also demonstrated to show almost complete degradation in soil contaminated with chlorpyrifos. Biodegradation of chlorpyrifos by *Pseudomonas* sp. was investigated in batch as well as continuous bioreactors (Yadav et al. 2014). Though chlorpyrifos removal efficiency of more than 91 % was observed up to 300 mg L^{-1} day^{-1}, accumulation of 3,5,6-trichloro-2-pyridinol and flow fluctuations were found to affect the reactor performance.

There are many fungal species known to cause mineralization of chlorpyrifos in liquid medium, including *Saccharomyces cerevisiae* (Lal and Lal 1987). In earlier studies, biodegradation of chlorpyrifos has been investigated using *Aspergillus* sp. (Liu et al. 2002), *Trichoderma* (Mukherjee and Gopal 1996), and *Fusarium* (Wang et al. 2005) fungal communities with varying degrees of success. Chlorpyrifos degradation by fungi, such as *Phanerochaete chrysosporium*, *Aspergillus terreus*, and *Verticillium* sp. DSP, has also been studied (Bumpus et al. 1993; Omar 1998; Yu et al. 2006). Kulshrestha and Kumari (2011) have reported that pure fungal strain *Acremonium* sp. could utilize chlorpyrifos (83.9 %) as a source of C and N. Apart from bacteria and fungi, a few cyanobacteria (Singh et al. 2011; Thengodkar and Sivakami 2010), actinomycetes, and algae have also been exploited for their potential to degrade chlorpyrifos. And the results of Eissa et al. (2014) using *Bacillus* sp. SMF5, *Penicillium* sp. F09-T10-1, and *Streptomyces thermocarboxydus* strain A-B depict that bacteria show the maximum degradation followed by fungi and actinomycetes respectively.

Studies have shown the efficacy of microbial consortium to co-metabolize chlorpyrifos which can be explained by taking into account the compatibility and the physiological relationship between individual members of the consortium. Three aerobic bacterial consortia developed from pesticide contaminated soils of Punjab comprising *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella* sp., and *Serratia marscecens* were able to degrade chlorpyrifos (50 mg L^{-1}) in soil after 30 days by 50, 56, and 64 %. (Lakshmi et al. 2009). A consortium consisting of *Klebsiella* sp., *Pseudomonas aeruginosa*, *Pseudomonas stutzeri,* and *Pseudomonas putida* isolated from chlorpyrifos contaminated agricultural soil, which could degrade chlorpyrifos even at 500 mg L^{-1}, highlights the potential use of consortia to clean up contaminated sites (Sasikala et al. 2012). Degradation efficiency and optimal conditions for efficient chlorpyrifos degradation by co-culture of chlorpyrifos degrading bacterium *Serratia* sp. and 3,5,6-trichloro-2-pyridinol mineralizing *Trichosporon* sp. in liquid medium have also been investigated (Xu et al. 2007). Complete mineralization by co-culture of *Cellulomonas fimi* that could transform chlorpyrifos to 3,5,6-trichloro-2-pyridinol and a 3,5,6-trichloro-2-pyridinol utilizing fungal strain *Phanerochaete chrysosporium* within 16 h showed not only the potential to clean up contaminated environment, but also the advantage of consortia over single cultures (Barathidasan et al. 2014).

Degradation of chlorpyrifos using a biomix and biobed system has been studied in detail. Tortella et al. (2010) have recommended biobeds constructed with biomix prepared with Andisol soil and biostimulated with NPK nutrient as a viable alternative of chlorpyrifos dissipation avoiding soil and water contamination. Recently, our study using microbial consortium consisting of *Klebsiella* sp. and two different strains of *Pseudomona aeruginosa* also has reported the enhanced degradation of chlorpyrifos when amended with NPK nutrients (John et al. 2014). Study to evaluate the efficacy of different selected organic amendments for improving the microbial activity has showed that the biostimulating potential was higher for mushroom spent followed by vermicompost, biogas slurry, soil spiked with 10 ppm chlorpyrifos and farmyard manure, and vermicompost (37 %), mushroom spent (24 %) and farmyard manure (1.9 %), respectively (Kadian et al. 2012).

Evidences from soil and laboratory degradation studies clearly indicate the cleavage and mineralization of the heterocyclic ring due to the activities of microorganisms resulting in the production of 3,5,6-trichloro-2-pyrinidinol and 3,5,6-trichloro-2-methoxypyridine as the major metabolites. The crucial step in the complete degradation of chlorpyrifos is the metabolism and mineralization of 3,5,6-trichloro-2-pyridinol and 3,5,6-trichloro-2-methoxypyridine. Some studies have noted that the metabolites are also degraded and mineralized by soil microorganisms. For example, in a study using seven selected bacteria for chlorpyrifos and 3,5,6-trichloro-2-pyridinol, degradation potential has been found to be higher in *Pseudomonas* followed by *Agrobacterium* and *Bacillus* with all the seven isolates more efficient in degrading 3,5,6-trichloro-2-pyridinol compared to chlorpyrifos (Maya et al. 2011). Inoculation of chlorpyrifos contaminated soil with *Cupriavidus* sp. DT-1 strain has resulted in a degradation rate of 100 and 94.3 %, for chlorpyrifos and 3,5,6-trichloro-2-pyridinol, respectively (Lu et al. 2013). A new fungal strain *Cladosporium cladosporioides* Hu-01 was reported for possessing the metabolic pathway for the complete detoxification of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by significantly reducing the half life of both compounds.

Most of the studies primarily focus on microorganisms and their use in bioremediation of contaminated sites. In addition to microorganisms, some plants also can either stimulate degradation or remove chlorpyrifos by the process referred as phytoremediation (Table 4). Plant roots in rhizosphere soil significantly influence soil microbial population, thereby facilitating degradation of chlorpyrifos by microorganisms. For example, in pot culture experiment study, chlorpyrifos was found to undergo rapid degradation under the influence of ryegrass mycorrhizosphere. The authors also observed 100 % degradation of all the tested concentrations in the inoculated rhizospheric soil compared to the non-inoculated soil during bioaugmentation with the bacterium *Pseudomonas nitroreducens* PS-2 which survived in spiked soil (Korade and Fulekar 2009). Rhizosphere associated chlorpyrifos degradation was also reported by Dubey and Fulekar (2012) who observed better survival of a novel strain *Stenotrophomonas maltophilia* MHF ENV20 in remediated rhizosphere soil of *Pennisetum pedicellatum*. During time course pot experiments conducted in greenhouse, 100, 50, and 33.3 % degradation at 50, 100, and 150 mg kg^{-1} concentrations of chlorpyrifos within 48, 72, and 120 h, respectively, were recorded by this strain in remediated rhizosphere soil. Hence, the authors suggest that high chlorpyrifos tolerance and rhizospheric degradation capability of *Pennisetum pedicellatum* make this plant suitable for decontamination and remediation of contaminated sites. Though relative inhibition in the growth rate has been reported, Wang et al. (2013b) recommended the use of aquatic plant *Iris pseudacorus* against chlorpyrifos in natural water body.

Biodegradation at enzyme and molecular levels

Pesticide biodegradation process is a series of complex reactions that involves three phases, each carried out by different enzymes (Hatzios 1991). Phase I process involves oxidation, reduction, or hydrolysis, which solubilizes the compound in water and increases bioavailability. Oxygenation in the metabolism of pesticides and other organic xenobiotics are manifested by oxidative enzymes, including cytochrome P450s, peroxidases, and polyphenol oxidases. During the second phase conjugation, the pesticide or pesticide metabolites are conjugated to sugar, amino acid, or glutathione, and the products formed with increased water solubility and reduced toxicity compared to parent compound can be stored in cellular organelles. Glutathione S-transferase is the major enzyme responsible for phase II which catalyzes the nucleophilic attack of the sulfur atom of GSH by the electrophilic center of the substrate (Marrs 1996). The last phase involves conversion of phase II metabolites into nontoxic secondary conjugates. Organophosphate hydrolyzing enzymes are collectively known as aryldialkylphosphatase/organophosphorus hydrolase/phosphotriesterase. Chlorpyrifos degrading microorganisms are capable of producing either of these enzymes such as organophosphorus hydrolase (OPH) (Gao et al. 2012), phosphotriesterase (PTE) methyl parathion hydrolase (MPH) (Chino-Flores et al., 2012), and organophosphorus acid anhydrolase (OPAA) (Cheng et al. 1993; Theriot and Grunden 2011). These enzymes differ in

protein sequence, 3D structure, and catalytic mechanism, but they also share several common features. All these enzymes are metal dependent hydrolases that contain a hydrophobic active site with three discrete binding pockets to accommodate the substrate ester groups. Activation of the substrate phosphorus center is achieved by a direct interaction between the phosphoryl oxygen and a divalent metal in the active site (Bigley and Raushel 2013). Biodegradation by organophosphorus hydrolase or phosphotriesterase that catalyzes the first step of the degradation (Singh and Walker 2006) has been studied extensively. Microbial organophosphorus hydrolase effectively cleaves P–O in the phosphotriesters bond, P–S linkage in the phosphothiolesters, P–CN or P–F of organophosphate pesticides (Ang et al. 2005), the former two being the case of chlorpyrifos and yields two major metabolites such as 3,5,6-trichloro-2-pyridinol and diethylphosphate (DETP) (Singh et al. 2004) (Fig. 3). Some other metabolites such as desethyl chlorpyrifos, chlorpyrifos oxon, desethyl chlorpyrifos oxon, and 3,5,6-trichloro-2 methoxypyrimidine are also produced during the degradation in very minute quantities. Chlorpyrifos oxon, the oxidized form of chlorpyrifos, is further hydrolyzed either enzymatically or spontaneously to form diethylphosphate and 3,5,6-trichloro-2-pyridinol. The 3,5,6-trichloro-2-pyridinol can be further degraded to 3,5,6-trichloro-2-methoxy-pyridine and carbon dioxide (Racke 1993). For example, *Cladosporium cladosporioides* Hu-01 was reported to mineralize 3,5,6-trichloro-2-pyridinol in a liquid medium (Chen et al. 2012) and an *Enterobacter* sp. that could mineralize chlorpyrifos to diethylphosphate which was further utilized for its growth and energy (Singh et al. 2004). A *Paracoccus* sp. was reported to be able to completely mineralize chlorpyrifos with no accumulation of 3,5,6-trichloro-2-pyridinol or diethylphosphate (Xu et al. 2008).

The organophosphorus hydrolase is a zinc containing homo-dimeric enzyme that can degrade a broad spectrum of organophosphorus pesticides such as paraoxon, parathion, and diazinon and is believed to be the enzyme produced in chlorpyrifos degrading microbes. Organophosphorus hydrolase activity has been reported in cytoplasm of many microorganisms (Latifi et al. 2012; Mulbry 2000). On the other hand, membrane bound organophosphorus hydrolase was observed in *Flavobacterium* sp. strain ATCC 27551 and *Brevundimonas diminuta* MG (Brown 1980; Dumas et al. 1989). Organophosphorus hydrolase catalyzes hydrolysis of most of phosphorus ester bonds in organophosphates via the addition of an activated water molecule at the phosphorus center (Lewis et al. 1988). This enzyme mediated cleavage of bonds occurs with different efficiencies, where the hydrolytic efficiency of organophosphorus hydrolase towards the P–S bond class of organophosphates is very poor. For example, organophosphorus hydrolase catalyzes

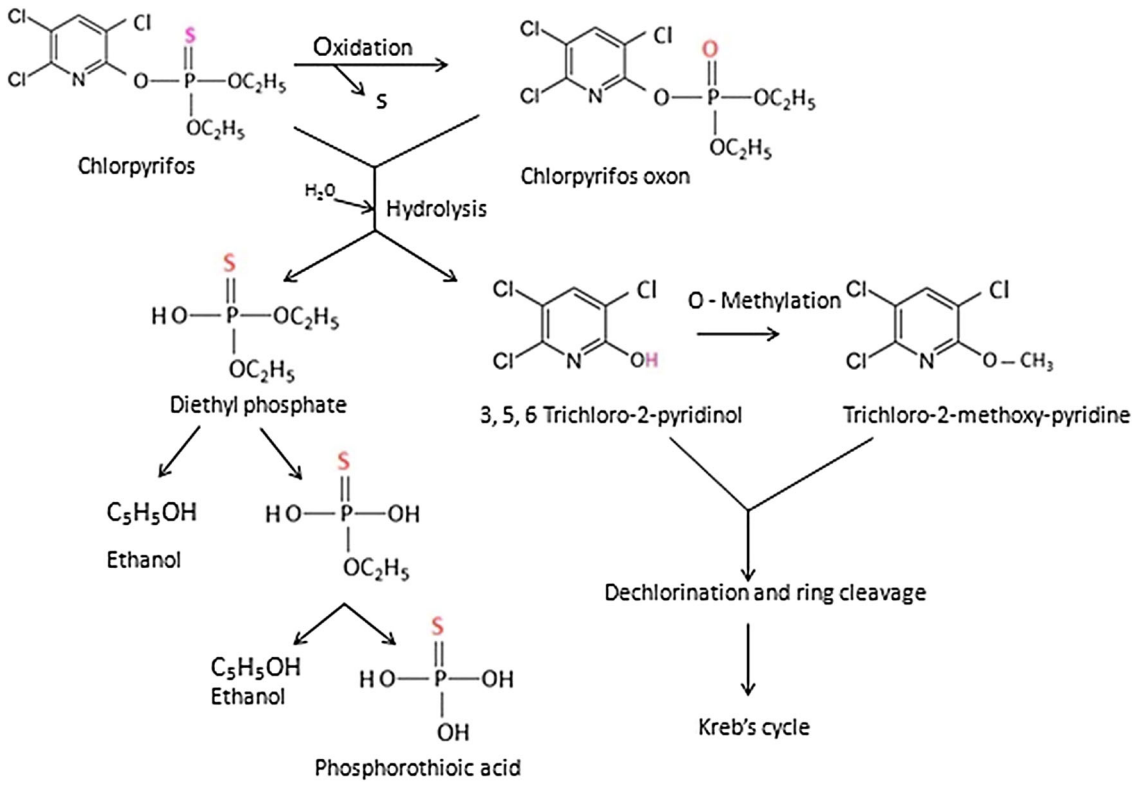


Fig. 3 Proposed degradation pathway of chlorpyrifos

the P–O bond of paraoxon with a k_{cat} of 2280 s^{-1}, but the reaction is very slow in the case of P–S bonds of demeton-S, malathion, phosalone, and acephate, with k_{cats} of 0.63–13.16 s^{-1} (Di Sioudi et al. 1999). Similar is the case of chlorpyrifos also, where it is hydrolyzed almost 1000 fold slower than the preferred substrate, paraoxon (Cho et al. 2004). The authors have demonstrated that wild type organophosphorus hydrolase had minimal degradatory activity for chlorpyrifos, and directed evolution can be used to improve a 725 fold increase in the k_{cat}/K_m value for this very poorly hydrolyzed compound. A novel organophosphorus hydrolase hydrolyzing methyl parathion, parathion, paraoxon, coumaphos, demeton-S, phosmet, and malathion has been purified and characterized in *Penicillium lilacinum* BP303. This amonomeric enzyme had a molecular mass of 60,000 Da, a pI of 4.8, and optimum enzyme activity at 45 °C, and pH 7.5. Its activity was strongly inhibited by Hg^{2+}, Fe^{3+}, *ρ*-chloromercuribenzoate, iodoacetic acid, and *N*-ethylmaleimide, while Cu^{2+}, mercaptoethanol, dithiothreitol, dithioerythritol, glutathione, and detergents slightly activated the enzyme (Liu et al. 2004). A novel chlorpyrifos hydrolase from cell extract of *Cladosporium cladosporioides* Hu-01 having a monomeric structure with a molecular mass of 38.3 kDa was purified 35.6 fold to apparent homogeneity. This enzyme which shared no similarity with any reported organophosphorus hydrolase had a pI value estimated to be 5.2 and showed optimal pH and temperature of 6.5 and 40 °C, respectively. Chlorpyrifos was the preferred substrate with K_m and V_{max} values of 6.7974 μM and 2.6473 μmol min^{-1}, respectively. No cofactors were required for the hydrolysis activity and was able to hydrolyze various organophosphorus insecticides with P–O and P–S bond (Gao et al. 2012).

Methyl parathion hydrolase (MPH), the enzyme that catalyzes the turnover of methyl parathion to *p*-nitrophenol (pNP), also shares the broad substrate range like organophosphorus hydrolase. Many microbial esterases have also been studied for pesticide hydrolysis. For example, esterase activity involved in malathion degradation was determined in culture filtrate of *Bacillus thuringiensis* MOS-5 (Zeinat Kamal et al. 2008). Increase in activity of pesticide metabolizing enzymes such as polyphenol oxidase, catalase, superoxide dismutase, esterase, and glutathione S-transferase, and metabolization of chlorpyrifos by the use of esterase A has been observed in marine cyanobacterium *Phormidium valderianum* BDU 20041 upon exposure to chlorpyrifos (Palanisami et al. 2009). Interaction of bacterial phosphotriesterase with organophosphates has long been accepted as a useful biosensor system for decontamination of real samples of pesticides with concentrations up to 20 μg L^{-1} (Istamboulie et al. 2010). Chlorpyrifos induced organophosphorous phosphatase (OPP) production and degradation of chlorpyrifos was reported by Harishankar et al. (2013) in the intestinal bacteria *Lactobacillus lactis*, *L. fermentum*, and *Escherichia coli* which were able to grow even at higher concentration of chlorpyrifos (greater than 1400 μg mL^{-1}).

The molecular basis of degradation of organophosphates has been extensively studied. The organophosphorus hydrolase enzymes are encoded by the *opd* (organophosphate degradation) and *mpd* (methyl parathion hydrolase) genes. *opd* genes have been isolated from different microorganisms from different geographical regions, and some of them have been shown to hydrolyze chlorpyrifos. Most of the *opd* genes were found to be plasmid based (Serdar et al. 1982) with similar sequences. For example, *Flavobacterium* sp. strain ATCC 27551 and *B. diminuta* MG contain identical *opd* genes that are on very different plasmids (Brown 1980; Dumas et al. 1989). In contrast to this, Horne et al. (2002) isolated chromosome located *opd* gene from *Agrobacterium radiobacter*, which had a similar sequence to the *opd* gene from other bacteria. However, study conducted by Ajaz et al. (2009) demonstrated chlorpyrifos degradation in *Pseudomonas putida* MAS-1 as a function of combined action of plasmid and chromosomal genes. A gene (*ophB*) encoding a protein involved in chlorpyrifos degradation was isolated from an endophytic bacterial strain, *Pseudomonas* sp. BF1-3 and cloned into *Escherichia coli* DH5α for confirming its enzyme activity. This 1024 bp nucleotide sequence *ophB* encodes OphB protein with an estimated molecular weight of 31.4 kDa having optimal activity at pH 8 and temperature around 35° (Barman et al. 2014).

The *mpd* gene, which is completely different from the *opd* gene, codes for methyl parathion hydrolase enzyme following a separate organophosphate degrading pathway to mineralize methyl parathion, methyl paraoxon, etc. Since chlorpyrifos has a similar chemical structure to parathion-methyl, it is likely to be acted upon by the enzymes coded by *mpd* gene family. The *mpd* gene was first isolated from *Plesiomonas* sp. M6 (Zhongli et al. 2001) which could be effectively expressed in *Escherichia coli*. Researchers reported that the *mpd* gene is chromosome based, as no plasmids were detected in isolates (Li et al. 2007; Yang et al. 2006). However, an exception to this is the plasmid location of *mpd* in *Pseudomonas* sp. WBC-3 (Liu et al. 2005). The *mpd* gene encoding the organophosphorus hydrolase cloned from *Stenotrophomonas* YC1 strain was successfully expressed in *Escherichia coli* DH5α cells, and the results showed this 996 bp *mpd* gene encoded a protein with high similarity to the hydrolase from *Plesiomonas* sp. M6 (Yang et al. 2006). Similarly, the gene encoding the chlorpyrifos hydrolytic enzyme from *Sphingomonas* sp. strain Dsp-2 cloned using a PCR based technique showed 99 % sequence similarity to the *mpd* genes of *Plesiomonas* sp. M6, *Pseudomonas* sp.

WBC-3, and *Ochrobactrum* sp. mp-6. However, at the amino acid level, it had only 92 % similarity to methyl parathion hydrolase from *Plesiomonas* sp. strain M6 with 26 amino acid substitutions, including an insertion which enhanced its hydrolytic efficiency for chlorpyrifos significantly than the wild type *mpd* from strain M6 (Li et al. 2007). Information elucidating the relationships between function and structure of organophosphorus hydrolase and polymorphism in the *mpd* gene has been provided by Li et al. (2008). During the gene amplicon study, they observed several key amino acid substitutions in highly conserved *mpd* genes of chlorpyrifos degrading two *Pseudomonas* sp. (Dsp 1 and Dsp 3), *Sphingomonas* sp. (Dsp 2), and *Stenotrophomonas* sp. (Dsp 4). Three groups of hydrolases were resulted from these substitutions at seven sites where hydrolases of strains Dsp-1 and Dsp-3 were identical. Such variations attributed to the increased hydrolytic efficiency and hydrolysis rate for chlorpyrifos compared to other tested substrates such as profenofos. Zhang et al. (2006) have noticed *mpd* gene cluster that constituted catabolic transposon, which contribute to the dispersion and expression of this gene among a variety of indigenous bacteria in a polluted environment.

There have been reports of organophosphate degradation genes which differed in sequences from the *opd* gene. For example, studies on chlorpyrifos degrading *Enterobacter* strain possessing a novel phosphotriesterase enzyme system revealed a gene system with different sequence from the widely studied *opd* gene. The authors suggested polygenic and chromosome based degradation of chlorpyrifos by microorganisms (Singh et al. 2004). Unique organophosphorus hydrolase activity and the responsible gene distinct from other known genes were reported in *Nocardiodes simplex* NRRL B-24074 (Latifi et al. 2012). A gene called *opdE* (organophosphate degradation from *Enterobacter*) consisting of 753 bp and encoding a protein of 25 kDa was isolated from an *Enterobacter* sp. This gene had no similarity to any genes reported to degrade organophosphates (Chino-Flores et al.).

Conclusion

The global demand for agrochemicals is rapidly increasing. The constant expanding of pesticide industry to a highly competitive sector that resulted from indiscriminate wide spread use makes pesticides the top players of the agrochemical industry. The consumption of organophosphate pesticides including chlorpyrifos is increasing day by day leading to the environmental accumulation and contamination. Statistical data show the presence of pesticides in various environmental compartments to unacceptable levels. The drastic environmental problems and toxicity issues originated from pesticide use have thus resulted in greater public pressure to assess, monitor, and minimize the impacts. Efforts are currently taken to develop safe, convenient, environmental friendly, and economically feasible methods for pesticides detoxification. Physicochemical treatment methods used to detoxify organophosphate are expensive and lead to the contamination of medium. Advanced methods are in demand for the effective treatment of pesticides polluted environment to achieve complete mineralization without the formation of toxic end products. In this scenario, microbiological catabolism and metabolism gains much attention as a route of mineralization of pesticides. Bioaugmentation and biostimulation are considered as reliable treatment methods to achieve the goal. Studies at enzyme and molecular levels provide greater understanding and valuable information of the mechanisms underlying the degradation process. Recombinant expression of these genes and enzymes will be a low-cost technology that enables better degradation and environmental protection.

Acknowledgments The authors gratefully acknowledge the financial support received from Department of Science and Technology (DST/INSPIRE fellowship/2011/IF120107) Government of India, New Delhi.

References

Adesodun JK, Davidson DA, Hopkins DW (2005) Micromorphological evidence for changes in soil faunal activity following application of sewage sludge and biocide. Appl Soil Ecol 29(1):39–45. doi:10.1016/j.apsoil.2004.09.004

Affam AC, Chaudhuri M (2013) Degradation of pesticides chlorpyrifos, cypermethrin and chlorothalonil in aqueous solution by TiO_2 photocatalysis. J Environ Manage 130:160–165. doi:10.1016/j.jenvman.2013.08.058

AgroNews (2013) 2012 China's Major Pesticides Varieties Tracking – chlorpyrifos. http://newsagropagescom/News/NewsDetail%e2%80%948993htm

Ahmed S, Ahmad MS (2006) Effect of insecticides on the total number of soil bacteria under laboratory and field conditions. Pak Entomol 28(2):63–68

Ajaz M, Jabeen N, Ali TA, Rasool SA (2009) Split role of plasmid genes in the degradation of chlorpyrifos by indigenously isolated *Pseudomonas putida* Mas-1. Pak J Bot 41(4):2055–2060

Alavanja MCR et al (2003) Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. Am J Epidemiol 157(9):800–814

Ali D, Nagpure NS, Kumar S, Kumar R, Kushwaha B, Lakra WS (2009) Assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish Channa punctatus (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food Chem Toxicol 47(3):650–656. doi:10.1016/j.fct.2008.12.021

Ang EL, Zhao H, Obbard JP (2005) Recent advances in the bioremediation of persistent organic pollutants via biomolecular engineering. Enzyme Microb Tech 37(5):487–496. doi:10.1016/j.enzmictec.2004.07.024

Aniladevi Kunjamma KP, Philip B, Smitha V, Bhanu SV, Jose J (2008) Histopathological effects on *Oreochromis mossambicus*

(Tilapia) exposed to chlorpyrifos. J Environ Res Develop 2(4):553–559
Anwar S, Liaquat F, Khan QM, Khalid ZM, Iqbal S (2009) Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by *Bacillus pumilus* strain C2A1. J Hazard Mater 168(1):400–405. doi:10.1016/j.jhazmat.2009.02.059
Barathidasan K, Reetha D, Milton DJ, Sriram N, Govindammal M (2014) Biodegradation of chlorpyrifos by co-culture of *Cellulomonas fimi* and *Phanerochaete chrysosporium*. Afr J Microbiol Res 8(9):961–966. doi:10.5897/AJMR2013.6530
Barman DN, Haque MA, Islam SMA, Yun HD, Kim MK (2014) Cloning and expression of *ophB* gene encoding organophosphorus hydrolase from endophytic *Pseudomonas* sp. BF1-3 degrades organophosphorus pesticide chlorpyrifos. Ecotoxicol Environ Saf 108:135–141. doi:10.1016/j.ecoenv.2014.06.023
Barron MG, Woodburn KB (1995) Ecotoxicology of chlorpyrifos. Rev Environ Contam Toxicol 144:1–93
Barros FCF, Barros AL, Silva MA, do Nascimento RF (2013) Use of microwave-assisted oxidation for removal of the pesticide chlorpyrifos from aqueous media. Int J Civil Environ Eng 13(6):16–27
Bassey IY, Effiong EB, Archibong UD, Ita WU (2015) Germination and root nodule formation of soybean (*Glycine max* (L.) Merr.) in ridomil and chlorpyriphos treated soil. American. Journal of Environmental Protection 4(1):17–22. doi:10.11648/j.ajep.20150401.12
Bigley AN, Raushel FM (2013) Catalytic mechanisms for phosphotriesterases. Biochim Biophys Acta Proteins Proteom 1:443–453. doi:10.1016/j.bbapap.2012.04.004
Blakley BR, Yole MJ, Brousseau P, Boermans H, Fournier M (1999) Effect of chlorpyrifos on immune function in rats. Vet Hum Toxicol 41(3):140–144
Bondarenko S, Gan J (2004) Degradation and sorption of selected organophosphate and carbamate insecticides in urban stream sediments. Environ Toxicol Chem 23(3):1809–1814
Bondarenko S, Gan J, Haver DL, Kabashima JN (2004) Persistence of selected organophosphate and carbamate insecticides in waters from a coastal watershed. Environ Toxicol Chem 23(11):2649–2654
Briceno G, Fuentes MS, Palma G, Jorquera MA, Amoroso MJ, Diez MC (2012) Chlorpyrifos biodegradation and 3,5,6-trichloro-2-pyridinol production by actinobacteria isolated from soil. Int Biodeter Biodegr 73:1–7. doi:10.1016/j.ibiod.2012.06.002
Brown KA (1980) Phosphotriesterases of *Flavobacterium* sp. Soil Biol Biochem 12(2):105–112. doi:10.1016/0038-0717(80)90044-9
Budd R, O'geen A, Goh KS, Bondarenko S, Gan J (2011) Removal mechanisms and fate of insecticides in constructed wetlands. Chemosphere 83(11):1581–1587. doi:10.1016/j.chemosphere.2011.01.012
Bumpus JA, Kakar SN, Coleman RD (1993) Fungal degradation of organophosphorous insecticides. Appl Biochem Biotechnol 399(1):715–726
Caceres T, He W, Naidu R, Megharaj M (2007) Toxicity of chlorpyrifos and TCP alone and in combination to Daphnia carinata: the influence of microbial degradation in natural water. Water Res 41(19):4497–4503
Carr RL, Ho LL, Chambers JE (1997) Selective toxicity of chlorpyrifos to several species of fish during an environmental exposure: biochemical mechanisms. Environ Toxicol Chem 16(11):2369–2374. doi:10.1002/etc.5620161124
Chai LK, Mohd-Tahir N, Bruun Hansen HC (2009) Dissipation of acephate, chlorpyrifos, cypermethrin and their metabolites in a humid-tropical vegetable production system. Pest Manag Sci 65(2):189–196. doi:10.1002/ps.1667
Chen S, Liu C, Peng C, Liu H, Hu M, Zhong G (2012) Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by a new fungal strain *Cladosporium cladosporioides* Hu-01. PLoS ONE 7:1–12. doi:10.1371/journal.pone.0047205
Cheng T-C, Harvey SP, Stroup AN (1993) Purification and properties of a highly active organophosphorus acid anhydrolase from *Alteromonas undina*. Appl Environ Microbiol 59(9):3138–3140
Chino-Flores C et al (2012) Isolation of the *opdE* gene that encodes for a new hydrolase of *Enterobacter* sp. capable of degrading organophosphorus pesticides. Biodegradation 23(3):387–397. doi:10.1007/s10532-011-9517-6
Chishti Z, Arshad M (2012) Growth linked biodegradation of chlorpyrifos by *Agrobacterium* and *Enterobacter* spp. Int J Agric Biol 15(1):19–26
Cho CM-H, Mulchandani A, Chen W (2004) Altering the substrate specificity of organophosphorus hydrolase for enhanced hydrolysis of chlorpyrifos. Appl Environ Microbiol 70:4681–4685. doi:10.1128/AEM.70.8.4681-4685.2004
Clark JR, Patrick JM, Middaugh DP, Moore JC (1985) Relative sensitivity of six estuarine fishes to carbophenothion, chlorpyrifos, and fenvalerate. Ecotoxicol Environ Saf 10(3):382–390. doi:10.1016/0147-6513(85)90083-1
Corbin M, Daiss R, Wolf JK, Hurley P, Spatz D, Branch ERA (2009) Risks of chlorpyrifos use to federally threatened & endangered california red-legged frog (*Rana aurora draytonii*), California tiger salamander (*Ambystoma californiense*), San Francisco garter
Cortina-Puig M, Istamboulie G, Marty J-L, Noguer T (2010) Analysis of pesticide mixtures using intelligent biosensors. INTECH Open Access Publisher
Costa E et al (2015) Effect of neurotoxic compounds on ephyrae of *Aurelia aurita* jellyfish. Hydrobiologia. doi:10.1007/s10750-015-2284-3
Cui Y, Guo J, Xu B, Chen Z (2011) Genotoxicity of chlorpyrifos and cypermethrin to ICR mouse hepatocytes. Toxicol Mech Methods 21(1):70–74. doi:10.3109/15376516.2010.529192
Dam K, Seidler FJ, Slotkin TA (2000) Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity. Brain Res Dev Brain Res 121(2):179–187
Dawson LA et al (2003) Influence of pasture management (nitrogen and lime addition and insecticide treatment) on soil organisms and pasture root system dynamics in the field. Plant Soil 255(1):121–130
De Angelis S et al (2009) Developmental exposure to chlorpyrifos induces alterations in thyroid and thyroid hormone levels without other toxicity signs in cd1 mice. Toxicol Sci 108(2):311–319. doi:10.1093/toxsci/kfp017
de Oliveira AG, Ribeiro JP, de Oliveira JT, De Keukeleire D, Duarte MS, do Nascimento RF (2014) Degradation of the pesticide chlorpyrifos in aqueous solutions with UV/H_2O_2: optimization and effect of interfering anions. J Adv Oxid Technol 17(1):133–138
De Silva P, Samayawardhena LA (2002) Low concentrations of lorsban in water result in far reaching behavioral and histological effects in early life stages in guppy. Ecotoxicol Environ Saf 53(2):248–254. doi:10.1002/etc.5620161122
DeLorenzo ME, Serrano L (2003) Individual and mixture toxicity of three pesticides; atrazine, chlorpyrifos, and chlorothalonil to the marine phytoplankton species *Dunaliella tertiolecta*. J Environ Sci Health B 38(5):529–538
Derbalah A, Ismail A, Shaheen S (2013) Monitoring of organophosphorus pesticides and remediation technologies of the frequently detected compound (chlorpyrifos) in drinking water. Pol J Chem Technol 15(3):25–34. doi:10.2478/pjct-2013-0040
Devi LG, Murthy BN, Kumar SG (2009) Photocatalytic activity of V^{5+}, Mo^{6+} and Th^{4+} doped polycrystalline TiO_2 for the degradation of chlorpyrifos under UV/solar light. J Mol Catal A: Chem 308(1):174–181. doi:10.1016/j.molcata.2009.04.007

Devipriya S, Yesodharan S (2005) Photocatalytic degradation of pesticide contaminants in water. Sol Energ Mat Sol C 86(3):309–348. doi:10.1016/j.solmat.2004.07.013

Di Sioudi BD, Miller CE, Lai K, Grimsley JK, Wild JR (1999) Rational design of organophosphorus hydrolase for altered substrate specificities. Chem-Biol Interact 119:211–223. doi:10.1016/S0009-2797(99)00030-7

Domenech X, Jardim WF, Litter MI (2004) Advanced oxidation processes for contaminant removal. In: Blesa MA, Sánchez B (eds) Contaminants removal by heterogeneous photocatalysis. Editorial CIEMAT, Madrid

Dow AgroSciences (2013) Re Organophosphate and Carbamate Reassessment (APP201045) Submission 102675 Appendix A Chlorpyrifos Environmental Protection Authority, Wellington. http://www.epa.govt.nz/search-databases/HSNO%20Application%20Register%20Documents/APP201045_Submission%20102675%20Appendix%20A%20Chlorpyrifos.pdf

Dubey KK, Fulekar MH (2011) Effect of pesticides on the seed germination of *Cenchrus setigerus* and *Pennisetum pedicellatum* as monocropping and co-cropping system: implications for rhizospheric bioremediation. Rom Biotech Letters 16:5908–5918

Dubey KK, Fulekar MH (2012) Chlorpyrifos bioremediation in Pennisetum rhizosphere by a novel potential degrader Stenotrophomonas maltophilia MHF ENV20. W J Microbiol Biotechnol 28(4):1715–1725

Duirk SE, Collette TW (2005) Organophosphate pesticide degradation under drinking water treatment conditions. US Environmental Protection Agency Office of Research and Development, EPA/600/R-05/103, Washington, DC

Dumas DP, Caldwell SR, Wild JR, Raushel FM (1989) Purification and properties of the phosphotriesterase from *Pseudomonas diminuta*. J Biol Chem 264(33):19659–19665

Dutta M, Sardar D, Pal R, Kole RK (2010) Effect of chlorpyrifos on microbial biomass and activities in tropical clay loam soil. Environ Monit Assess 160(1–4):385–391. doi:10.1007/s10661-008-0702-y

Eamkamon T, Klinbunga S, Thirakhupt K, Menasveta P (2012) Acute toxicity and neurotoxicity of chlorpyrifos in black tiger shrimp, *Penaeus monodon*. Environ Asia 5(1):26–31

Eddins D, Cerutti D, Williams P, Linney E, Levin ED (2009) Developmental chlorpyrifos causes behavioral and neurochemical defects in zebrafish. Neurotoxicol Teratol 32(2):99–108. doi:10.1016/j.ntt.2009.02.005

EFSA (2005) Review report for the active substance chlorpyrifos. SANCO/3059/99 - rev. 1.5 European Food Safety Authority. http://eceuropaeu/sanco_pesticides/public/indexcfm?event=activesubstanceselection

Eissa FI, Mahmoud HA, Massoud ON, Ghanem KM, Gomaa IM (2014) Biodegradation of chlorpyrifos by microbial strains isolated from agricultural wastewater. J Am Sci 10(3):98–108

El-Helow ER, Badawy MEI, Mabrouk MEM, Mohamed EAH, El-Beshlawy YM (2013) Biodegradation of chlorpyrifos by a newly isolated *Bacillus subtilis* strain, Y242. Biorem J 17(2):113–123. doi:10.1080/10889868.2013.786019

Engel LS et al (2005) Pesticide use and breast cancer risk among farmer's wives in the agricultural health study. Am J Epidemiol 161(2):121–135

Esplugas S, Gimenez J, Contreras S, Pascual E, Rodríguez M (2002) Comparison of different advanced oxidation processes for phenol degradation. Water Res 36(4):1034–1042. doi:10.1016/S0043-1354(01)00301-3

Fadaei A, Kargar M (2013) Photocatalytic degradation of chlorpyrifos in water using titanium dioxide and zinc oxide. Fresen Environ Bull 22(8A):2442–2447

Fang H, Xiang YQ, Hao YJ, Chu XQ, Pan XD, Yu JQ, Yu YL (2008) Fungal degradation of chlorpyrifos by *Verticillium* sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pakchoi. Int Biodeter Biodegr 61(4):294–303. doi:10.1016/j.ibiod.2007.10.001

Farag AT, El Okazy AM, El-Aswed AF (2003) Developmental toxicity study of chlorpyrifos in rats. Reprod Toxicol 17(2):203–208

Felsot AS, Racke KD, Hamilton DJ (2003) Disposal and degradation of pesticide waste. In: Reviews of environmental contamination and toxicology. Springer, pp 123–200

Femia J, Mariani M, Zalazar C, Tiscornia I (2013) Photodegradation of chlorpyrifos in water by UV/H2O2 treatment: toxicity evaluation. Water Sci Technol 68(10):2279–2286. doi:10.2166/wst.2013.493

Flaskos J (2012) The developmental neurotoxicity of organophosphorus insecticides: a direct role for the oxon metabolites. Toxicol Lett 209(1):86–93

Fortenberry GZ, Hu H, Turyk M, Barr DB, Meeker JD (2012) Association between urinary 3,5,6-trichloro-2-pyridinol, a metabolite of chlorpyrifos and chlorpyrifos-methyl, and serum T4 and TSH in NHANES 1999-2002. Sci Total Environ 424:351–355. doi:10.1016/j.scitotenv.2012.02.039

Gange AC, Brown VK, Farmer LM (1992) Effects of pesticides on the germination of weed seeds: implications for manipulative experiments. J Appl Ecol 29(2):303–310. doi:10.2307/2404499

Gao Y, Chen S, Hu M, Hu Q, Luo J, Li Y (2012) Purification and characterization of a novel chlorpyrifos hydrolase from *Cladosporium cladosporioides* Hu-01. PLoS ONE 7(6):e38137. doi:10.1371/journal.pone.0038137

Garg A, Mehetre S, Sherkhane P, Eapen S, Kale SP (2010) Biodegradation of C-14-chlorpyrifos by hairy root culture of *Chenopodium amaranticolor* Coste & Reynier. Curr Sci India 99(7):896–898

Gebremariam SY, Beutel MW, Yonge DR, Flury M, Harsh JB (2012) Adsorption and desorption of chlorpyrifos to soils and sediments. In: Reviews of environmental contamination and toxicology. Springer, pp 123–175. doi:10.1007/978-1-4614-1463-6_3

Ghanem I, Orfi M, Shamma M (2007) Biodegradation of chlorpyrifos by *Klebsiella* sp. isolated from an activated sludge sample of waste water treatment plant in damascus. Folia Microbiol (Praha) 52(4):423–427

Gilani STS, Ageen M, Shah H, Raza S (2010) Chlorpyrifos degradation in soil and its effect on soil microorganisms. J Anim Pant Sci 20(2):99–102

Green AS, Chandler TG, Piegorsch WW (1996) Life stage specific toxicity of sediment-associated chlorpyrifos to a marine, infaunal copepod. Environ Toxicol Chem 15(7):1182–1188

Grube A, Donaldson D, Kiely T, Wu L (2011) Pesticides industry sales and usage. US EPA, Washington, DC

Guha A, Kumari B, Bora TC, Roy MK (1997) Possible involvement of plasmids in degradation of malathion and chlorpyriphos by Micrococcus sp. Folia Microbiol 42(6):574–576

Gvozdenac S, Indic D, Vukovic S (2013) Phytotoxicity of chlorpyrifos to white mustard (*Sinapis alba* L) and maize (*Zea mays* L): potential indicators of insecticide presence in water. Pesticidi i fitomedicina 28(4):265–271. doi:10.2298/PIF1304265G

Harishankar MK, Sasikala C, Ramya M (2013) Efficiency of the intestinal bacteria in the degradation of the toxic pesticide, chlorpyrifos. Biotech 3(2):137–142. doi:10.1007/s13205-012-0078-0

Hastings FL, Coster JE (1981) Field and laboratory evaluations of insecticides for southern pine beetle control United States Department of Agriculture, Southeastern Experiment Station, Forest Service, General Technical Report SE-21

Hatzios KK (1991) Biotransformations of herbicides in higher plants. In: Grover R, Cessna AJ (eds) Environmental chemistry of, vol 2. CRC Press, Boca Raton, pp 141–185

Haviland JA, Butz DE, Porter WP (2010) Long-term sex selective hormonal and behaviour alterations in mice exposed to low doses of chlorpyrifos in utero. ReproduToxicol 29(1):74–79. doi:10.1016/j.reprotox.2009.10.008

Hayward SJ, Gouin T, Wania F (2009) Levels and seasonal variability of pesticides in the rural atmosphere of Southern Ontario. J Agric Food Chem l 58(2):1077–1084. doi:10.1021/jf902898f

Hill BR (2006) Drift Catcher reveals unacceptable levels of chlorpyrifos in air. Community action, Pesticide News 73. http://wwwpan-ukorg/pestnews/Issue/pn73/pn73p12pdf

Hindumathy CK, Gayathri V (2013) Effect of pesticide (chlorpyrifos) on soil microbial flora and pesticide degradation by strains isolated from contaminated soil. J Bioremed Biodegrad 4(178):1–6. doi:10.4172/2155-6199.1000178

Horne I, Sutherland TD, Harcourt RL, Russell RJ, Oakeshott JG (2002) Identification of an OPD (organophosphate degradation) gene in an *Agrobacterium isolate*. Appl Environ Microbiol 68(7):3371–3376. doi:10.1128/AEM.68.7.3371-3376.2002

Hossain MS, Fakhruddin ANM, Chowdhury MAZ, Alam MK (2013) Degradation of chlorpyrifos, an organophosphorus insecticide in aqueous solution with gamma irradiation and natural sunlight. J Environ Chem Eng 1(3):270–274. doi:10.1016/j.jece.2013.05.006

Howard PH (1991) Handbook of environmental fate and exposure data: for organic chemicals, volume III pesticides, vol 3. CRC Press, Lewis publishers, Chelsea

Howard JH, Julian SE, Ferrigan J (2003) Golf course maintenance: impact of pesticides on amphibians. Golf Course Mon 94–101. doi:10.1007/s00244-005-0264-8

Hua F, Yunlong YU, Xiaoqiang CHU, Xiuguo W, Xiaoe Y, Jingquan YU (2009) Degradation of chlorpyrifos in laboratory soil and its impact on soil microbial functional diversity. J Environ Sci 21(3):380–386. doi:10.1016/S1001-0742(08)62280-9

Ikehata K, El-Din MG (2006) Aqueous pesticide degradation by hydrogen peroxide/ultraviolet irradiation and fenton-type advanced oxidation processes: a review. J Environ Eng Sci 5(2):81–135

Ismail M, Khan HM, Sayed M, Cooper WJ (2013) Advanced oxidation for the treatment of chlorpyrifos in aqueous solution. Chemosphere 93(4):645–651. doi:10.1016/j.chemosphere.2013.06.051

Istamboulie G, Durbiano R, Fournier D, Marty J-L, Noguer T (2010) Biosensor-controlled degradation of chlorpyrifos and chlorfenvinfos using a phosphotriesterase-based detoxification column. Chemosphere 78(1):1–6. doi:10.1016/j.chemosphere.2009.10.037

Jarvinen AW, Tanner DK, Kline ER (1988) Toxicity of chlorpyrifos, endrin, or fenvalerate to fathead minnows following episodic or continuous exposure. Ecotoxicol Environ Saf 15(1):78–95

Jastrzebska E (2011) The effect of chlorpyrifos and teflubenzuron on the enzymatic activity of soil. Pol J Envron Stud 20(4):903

Jin-Clark Y, Lydy MJ, Zhu KY (2002) Effects of atrazine and cyanazine on chlorpyrifos toxicity in *Chironomus tentans* (Diptera: Chironomidae). Environ Toxicol Chem 21(3):598–603

John EM, Rebello S, Jisha MS (2014) Chlorpyrifos degradation using bacterial consortium obtained from soil. Int J Environ Eng–IJEE 1(4):91–94

Johnsen K, Jacobsen CS, Torsvik V, Sorensen J (2001) Pesticide effects on bacterial diversity in agricultural soils—a review. Biol Fert Soils 33(6):443–453. doi:10.1007/s003740100351

Johnson WW, Finley MT (1980) Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. Summaries of toxicity tests conducted at Columbia National Fisheries Research Laboratory, 1965–78 United States Fish and Wildlife Service Resource Publication

Kadian N, Malik A, Satya S, Dureja P (2012) Effect of organic amendments on microbial activity in chlorpyrifos contaminated soil. J Environ Manage 95:S199–S202. doi:10.1016/j.jenvman.2010.10.023

Kanmoni VGG, Daniel S, Raj GAG (2012) Photocatalytic degradation of chlorpyrifos in aqueous suspensions using nanocrystals of ZnO and TiO_2. React Kinet Mech 106(2):325–339. doi:10.1007/s11144-012-0433-5

Karim AA, Haridi AA, El Rayah EA (1985) The environmental impacts of four insecticides on non-target organisms in the Gezira Irrigation Scheme canals of Sudan. J Trop Med Hyg 88(2):161–168

Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH (2012) Hodgkin lymphoma and pesticides exposure in men: a Canadian case–control study. J Agromed 17(1):30–39. doi:10.1080/1059924X.2012.632726

Kashyap V, Kumar M (2013) Studies on the effects of chlorpyrifos on growth and yield in green gram (*Vigna radiata* L.) at different phenological stages. J Biol Chem Res 30(2):734–740

Kavitha P, Rao JV (2008) Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish. Gambusia affinis. Environ Toxicol Pharmacol 26(2):192–198. doi:10.1016/j.etap.2008.03.010

Kegley SE, Hill, BR, Orme S, Choi AH (2014) PAN Pesticide Database, Pesticide Action Network, North America (Oakland, CA).http://wwwpesticideinfoorg/List_AquireAlljsp?Rec_Id=PC33392&Taxa_Group=AquaticPlants

Key PB, Fulton MH (1993) Lethal and sublethal effects of chlorpyrifos exposure on adult and larval stages of the grass shrimp, *Palaemonetes pugio*. J Environ Sci Health Part B 28(5):621–640. doi:10.1080/03601239309372844

Korade DL, Fulekar MH (2009) Effect of organic contaminants on seed germination of *Lolium multiflorum* in soil. Biol Med 1(1):28–34

Kralj MB, Franko M, TrebÅ¡e P (2007) Photodegradation of organophosphorus insecticides investigations of products and their toxicity using gas chromatography-mass spectrometry and AChE-thermal lens spectrometric bioassay. Chemosphere 67(1):99–107. doi:10.1016/j.chemosphere.2006.09.039

Kulshrestha G, Kumari A (2011) Fungal degradation of chlorpyrifos by *Acremonium* sp. strain (GFRC-1) isolated from a laboratory-enriched red agricultural soil. Biol Fertil Soils 47(2):219–225. doi:10.1007/s00374-010-0505-5

Lakshmi CV, Kumar M, Khanna S (2008) Biotransformation of chlorpyrifos and bioremediation of contaminated soil. Int Biodeter Biodegr 62(2):204–209. doi:10.1016/j.ibiod.2007.12.005

Lakshmi CV, Kumar M, Khanna S (2009) Biodegradation of chlorpyrifos in soil by enriched cultures. Curr Microbiol 58(1):35–38. doi:10.1007/s00284-008-9262-1

Lal S, Lal R (1987) Bioaccumulation, metabolism, and effects of DDT, fenitrothion, and chlorpyrifos on *Saccharomyces cerevisiae*. Arch Environ Contam Toxicol 16(6):753–757

Latifi AM, Khodi S, Mirzaei M, Miresmaeili M, Babavalian H (2012) Isolation and characterization of five chlorpyrifos degrading bacteria. Afr J Biotechnol 11(13):3140–3146. doi:10.5897/AJB11.2814

Lee WJ et al (2004) Cancer incidence among pesticide applicators exposed to chlorpyrifos in the Agricultural Health Study. J Natl Cancer Inst 96(23):1781–1789

Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MCR (2007) Pesticide use and colorectal cancer risk in the Agricultural Health Study. J Natl Cancer Inst 121(2):339–346

Levin ED, Addy N, Nakajima A, Christopher NC, Seidler FJ, Slotkin TA (2001) Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. Dev Brain Res 130(1):83–89

Levin ED, Chrysanthis E, Yacisin K, Linney E (2003) Chlorpyrifos exposure of developing zebrafish: effects on survival and long-term effects on response latency and spatial discrimination. Neurotoxicol Teratol 25(1):51–57

Levin ED, Swain HA, Donerly S, Linney E (2004) Developmental chlorpyrifos effects on hatchling zebrafish swimming behavior. Neurotoxicol Teratol 26(6):719–723

Lewis VE, Donarski WJ, Wild JR, Raushel FM (1988) Mechanism and stereochemical course at phosphorus of the reaction catalyzed by a bacterial phosphotriesterase. Biochemistry 27(5):1591–1597. doi:10.1021/bi00405a030

Li X, He J, Li S (2007) Isolation of a chlorpyrifos-degrading bacterium, *Sphingomonas* sp. strain Dsp-2, and cloning of the mpd gene. Res Microbiol 158(2):143–149. doi:10.1016/j.resmic.2006.11.007

Li X, Jiang J, Gu L, Ali SW, He J, Li S (2008) Diversity of chlorpyrifos-degrading bacteria isolated from chlorpyrifos-contaminated samples. Int Biodeter Biodegr 62(4):331–335. doi:10.1016/j.ibiod.2008.03.001

Liu X, You M, Wei Y, Liao J, Ye L, Chen J (2002) Isolation of chlorpyrifos-degrading *Aspergillus* sp. Y and measurement of degradation efficiency. Chin J Appl Environ Biol 9(1):78–80

Liu Y-H, Liu Y, Chen Z-S, Lian J, Huang X, Chung Y-C (2004) Purification and characterization of a novel organophosphorus pesticide hydrolase from *Penicillium lilacinum* BP303. Enzyme Microb Tech 34(3):297–303. doi:10.1016/j.enzmictec.2003.11.009

Liu H, Zhang J-J, Wang S-J, Zhang X-E, Zhou N-Y (2005) Plasmid-borne catabolism of methyl parathion and *p*-nitrophenol in *Pseudomonas* sp. strain WBC-3. Biochem Biophys Res Commun 334(4):1107–1114. doi:10.1016/j.bbrc.2005.07.006

Liu ZY, Chen X, Shi Y, Su ZC (2012) Bacterial degradation of chlorpyrifos by *Bacillus cereus*. Adv Mater Res 356:676–680. doi:10.4028/www.scientific.net/AMR.356-360.676

Lu JL (2011) Farmers' exposure to pesticides and pesticide residues in soils and crops grown in Benguet Philippines. Philipp J Crop Sci 36(3):19–27

Lu P, Li Q, Liu H, Feng Z, Yan X, Hong Q, Li S (2013) Biodegradation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol by *Cupriavidus* sp. DT-1. Bioresour Technol 127:337–342. doi:10.1016/j.biortech.2012.09.116

Lyman WJ, Reehl WF, Rosenblatt DH (1990) Handbook of chemical property estimation methods: environmental behavior of organic compounds. American Chemical Society, Washington, DC

Madhaiyan M, Poonguzhali S, Hari K, Saravanan VS, Sa T (2006) Influence of pesticides on the growth rate and plant-growth promoting traits of *Gluconacetobacter diazotrophicus*. Pest Biochem Physiol 84(2):143–154. doi:10.1016/j.pestbp.2005.06.004

Madhuri RJ, Rangaswamy V (2002) Influence of selected insecticides on phosphatase activity in groundnut (*Arachis hypogeae* L.) soils. J Environ Biol India 23(4):393–397

Mallick K, Bharati K, Banerji A, Shakil NA, Sethunathan N (1999) Bacterial degradation of chlorpyrifos in pure cultures and in soil. Bull Environ Contam Toxicol 62(1):48–54

Marrs KA (1996) The functions and regulation of glutathione *S*-transferases in plants. Annu Rev Plant Biol 47(1):127–158. doi:10.1146/annurev.arplant.47.1.127

Martinez-Toledo MV, Salmeron V, Gonzalez-Lopez J (1992) Effect of the insecticides methylpyrimifos and chlorpyrifos on soil microflora in an agricultural loam. Plant Soil 147(1):25–30

Maya K, Singh RS, Upadhyay SN, Dubey SK (2011) Kinetic analysis reveals bacterial efficacy for biodegradation of chlorpyrifos and its hydrolyzing metabolite TCP. Process Biochem 46(11):2130–2136. doi:10.1016/j.procbio.2011.08.012

Maya K, Upadhyay SN, Singh RS, Dubey SK (2012) Degradation kinetics of chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) by fungal communities. Bioresour Technol 126:216–223. doi:10.1016/j.biortech.2012.09.003

Meeker JD, Ravi SR, Barr DB, Hauser R (2008) Circulating estradiol in men is inversely related to urinary metabolites of nonpersistent insecticides. Reprod Toxicol 25(2):184–191. doi:10.1016/j.reprotox.2007.12.005

Menon P, Gopal M, Prasad R (2004) Influence of two insecticides, chlorpyrifos and quinalphos, on arginine ammonification and mineralizable nitrogen in two tropical soil types. J Agric Food Chem l 52(24):7370–7376

Menon P, Gopal M, Parsad R (2005) Effects of chlorpyrifos and quinalphos on dehydrogenase activities and reduction of Fe^{3+} in the soils of two semi-arid fields of tropical India. Agric Ecosyst Environt 108(1):73–83. doi:10.1016/j.agee.2004.12.008

Miguel N, Ormad MP, Mosteo R, Ovelleiro JL (2012) Photocatalytic degradation of pesticides in natural water: effect of hydrogen peroxide. Int J Photoenergy

Mills K, Kegley SE (2006) Air monitoring for chlorpyrifos in Lindsay, California, June–July 2004 and July–August 2005 Pesticide Action Network, San Francisco

Moorman TB (1989) A review of pesticide effects on microorganisms and microbial processes related to soil fertility. J Prod Agric 2(1):14–23. doi:10.2134/jpa1989.0014

Mugni HN, Demetrio P, Paracampo A, Pardi M, Bulus G, Bonetto C (2012) Toxicity persistence in runoff water and soil in experimental soybean plots following chlorpyrifos application. Bull Environ Contam Toxicol 89(1):208–212. doi:10.1007/s00128-012-0643-6

Muhamad SG (2010) Kinetic studies of catalytic photodegradation of chlorpyrifos insecticide in various natural waters. Arabian J Chem 3(2):127–133. doi:10.1016/j.arabjc.2010.02.009

Mukherjee I, Gopal M (1996) Degradation of chlorpyrifos by two soil fungi *Aspergillus niger* and *Trichoderma viride*. Toxicol Environ Chem 57(1–4):145–151. doi:10.1080/02772249609358383

Mukherjee I, Gopal M, Dhar DW (2004) Disappearance of chlorpyrifos from cultures of *Chlorella vulgaris*. Bull Environ Contam Toxicol 73(2):358–363. doi:10.1007/s00128-004-0436-7

Mulbry W (2000) Characterization of a novel organophosphorus hydrolase from *Nocardiodes simplex* NRRL B-24074. Microbiol Res 154(4):285–288. doi:10.1016/S0944-5013(00)80001-4

Mulbry WW, Karns JS, Kearney PC, Nelson JO, McDaniel CS, Wild JR (1986) Identification of a plasmid-borne parathion hydrolase gene from *Flavobacterium* sp. by southern hybridization with opd from *Pseudomonas diminuta*. Appl Environ Microbiol 51:926–930

Munoz-Quezada MT et al (2012) Predictors of exposure to organophosphate pesticides in schoolchildren in the Province of Talca Chile. Environ Int 47:28–36. doi:10.1016/j.envint.2012.06.002

Murphy MW et al (2012) Type and toxicity of pesticides sold for community vector control use in the Gambia. Epidemiol Res Int. doi:10.1155/2012/387603

NFMS (2008) National Marine Fisheries Service Endangered Species Act Section 7. Consultation, Biological Opinion, Environmental Protection Agency, Registration of Pesticides Containing Chlorpyrifos, Diazinon, and Malathion. http://wwwnmfsnoaagov/pr/pdfs/pesticide_bioppdf

NRA (2000) The NRA review of chlorpyrifos volume 1. National Registration Authority for Agricultural and Veterinary Medicines, Canberra. http://wwwapvmagovau/products/review/docs/chlorpyrifos_summarypdf

Ohashi N, Tsuchiya Y, Sasano H, Hamada A (1993) Screening on reactivity of organic pesticides with ozone in water and their

products. Jpn J Toxicol Environ Health 39(6):522–533. doi:10.1248/jhs1956.39.6_522

Ojha A, Yaduvanshi SK, Pant SC, Lomash V, Srivastava N (2013) Evaluation of DNA damage and cytotoxicity induced by three commonly used organophosphate pesticides individually and in mixture, in rat tissues. Environ Toxicol 28(10):543–552. doi:10.1002/tox.20748

Olofinboba MO, Kozlowski TT (1982) Effects of three systemic insecticides on seed germination and growth of *Pinus halepensis* seedlings. Plant Soil 64(2):255–258

Omar SA (1998) Availability of phosphorus and sulfur of insecticide origin by fungi. Biodegradation 9(5):327–336

Oppenlander T (2003) Photochemical purification of water and air: advanced oxidation processes (AOPs)—principles, reaction mechanisms, reactor concepts. Wiley, New York

Ormad MP, Miguel N, Lanao M, Mosteo R, Ovelleiro JL (2010) Effect of application of ozone and ozone combined with hydrogen peroxide and titanium dioxide in the removal of pesticides from water. Ozone Sci Eng 32(1):25–32

Oruc EO (2010) Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. Pest Biochem Physiol 96(3):160–166. doi:10.1016/j.pestbp.2009.11.005

Palanisami S, Prabaharan D, Uma L (2009) Fate of few pesticide-metabolizing enzymes in the marine cyanobacterium *Phormidium valderianum* BDU 20041 in perspective with chlorpyrifos exposure. Pest Biochem Physiol 94(2):68–72. doi:10.1016/j.pestbp.2009.03.003

Palma P, Palma VL, Fernandes RM, Soares A, Barbosa IR (2008) Acute toxicity of atrazine, endosulfan sulphate and chlorpyrifos to *Vibrio fischeri, Thamnocephalus platyurus* and *Daphnia magna*, relative to their concentrations in surface waters from the Alentejo region of Portugal. Bull Environ Contam Toxicol 81(5):485–489. doi:10.1007/s00128-008-9517-3

Pandey S, Singh DK (2004) Total bacterial and fungal population after chlorpyrifos and quinalphos treatments in groundnut (*Arachis hypogaea* L.) soil. Chemosphere 55(2):197–205

Pankhurst C (2006) Effects of pesticides used in sugarcane cropping systems on soil organisms and biological functions associated with soil health. Sugar Yield Decline Joint Venture, Adelaide

Panuwet P, Prapamontol T, Chantara S, Barr DB (2009) Urinary pesticide metabolites in school students from northern Thailand. Int J Hyg Environ Health 212(3):288–297. doi:10.1016/j.ijheh.2008.07.002

Park BS (2010) Biotransformation of aldrin and chlorpyrifos-methyl by *Anabaena* sp. PCC 7120. Korean J Environ Agric 29(2):184–188

Parween T, Jan S, Fatma T (2011) Evaluation of oxidative stress in *Vigna radiata* L. In response to chlorpyrifos. Int J Environ Sci Tech 9(4):605–612. doi:10.1007/s11738-011-0772-2

Pelizzetti E, Minero C, Piccinini P, Vincenti M (1993) Phototransformations of nitrogen containing organic compounds over irradiated semiconductor metal oxides: nitrobenzene and atrazine over TiO_2 and ZnO. Coord Chem Rev 125(1):183–193

Pengphol S, Uthaibutra J, O-a Arquero, Nomura N, Whangchai K (2012) Oxidative degradation and detoxification of chlorpyrifos by ultrasonic and ozone treatments. J Agric Sci 4(8):164. doi:10.5539/jas.v4n8p164

Penuela GA, Barcelo D (1997) Comparative degradation kinetics of chlorpyrifos in water by photocatalysis with $FeCl_3$, TiO_2 and photolysis using solid-phase disk extraction followed by gas chromatographic techniques. Toxicol Environ Chem 62(1–4):135–147

Phipps GL, Holcombe GW (1985) A method for aquatic multiple species toxicant testing: acute toxicity of 10 chemicals to 5 vertebrates and 2 invertebrates. Environ Pollut A 38(2):141–157. doi:10.1016/0143-1471(85)90073-X

Phung DT, Connell D, Miller G, Chu C (2012) Probabilistic assessment of chlorpyrifos exposure to rice farmers in Viet Nam. J Expo Sci Environ Epidemiol 22(4):417–423. doi:10.1038/jes.2012.32

Porichha SK, Sarangi PK, Prasad R (1998) Genotoxic effect of chlorpyrifos in *Channa punctatu*. Pres Cytol Genet 9:631–638

Pozo C, Martinez-Toledo MV, Salmeron V, Rodelas B, Gonzalez-Lopez J (1995) Effect of chlorpyrifos on soil microbial activity. Environ Toxicol Chem 14(2):187–192. doi:10.1002/etc.5620140201

Prakash A, Khan S, Aggarwal M, Telang AG, Malik JK (2009) Chlorpyrifos induces apoptosis in murine thymocytes. Toxicol Lett 189:S83

Prasertsup P, Ariyakanon N (2011) Removal of chlorpyrifos by water lettuce (*Pistia stratiotes* L.) and duckweed (*Lemna minor* L.). Int J Phytoremediation 13(4):383–395. doi:10.1080/15226514.2010.495145

Quiroz MA, Martinez-Huitle CA, Bandala ER (2011) Advanced oxidation processes (AOPs) for removal of pesticides from aqueous media. INTECH Open Access Publisher. doi:10.5772/13597

Racke KD (1993) Environmental fate of chlorpyrifos. Rev Environl ContamToxicol 131:1–150

Racke KD, Laskowski DA, Schultz MR (1990) Resistance of chlorpyrifos to enhanced biodegradation in soil. J Agric Food Chem l 38(6):1430–1436

Rahman MF, Mahboob M, Danadevi K, Banu BS, Grover P (2002) Assessment of genotoxic effects of chloropyriphos and acephate by the comet assay in mice leucocytes. Mutat Res 516(1):139–147

Rani MS et al (2008) Isolation and characterization of a chlorpyrifos degrading bacterium from agricultural soil and its growth response. Afr J Microbiol Res 2:026–031

Rao JV, Rani CHS, Kavitha P, Rao RN, Madhavendra SS (2003) Toxicity of chlorpyrifos to the fish *Oreochromis mossambicus*. Bull Environ Contam Toxicol 70(5):0985–0992. doi:10.1007/s00128-003-0079-0

Rauh VA et al (2006) Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118(6):e1845–e1859

Reiss R, Neal B, Lamb JC, Juberg DR (2012) Acetylcholinesterase inhibition dose-response modeling for chlorpyrifos and chlorpyrifos-oxon. Regul Toxicol Pharm 63(1):124–131. doi:10.1016/j.yrtph.2012.03.008

Richendrfer H, Pelkowski SD, Colwill RM, Creton R (2012) Developmental sub-chronic exposure to chlorpyrifos reduces anxiety-related behavior in zebrafish larvae. Neurotoxicol Teratol 34(4):458–465. doi:10.1016/j.ntt.2012.04.010

Rokade KB, Mali GV (2013) Biodegradation of chlorpyrifos by *Pseudomonas desmolyticum* NCIM 2112. Int J Pharm Bio Sci 4(2):609–616

Romeh AA, Hendawi MY (2013) Chlorpyrifos insecticide uptake by plantain from polluted water and soil. Environ Chem Lett 11(2):163–170. doi:10.1007/s10311-012-0392-0

Rowsey PJ, Gordon CJ (1999) Tumor necrosis factor is involved in chlorpyrifos-induced changes in core temperature in the female rat. Toxicol Lett 109(1):51–59

Sabdono A (2013) Biodegradation of chlorpyrifos by a marine bacterium *Bacillus firmus* strain by6 associated with branching coral *Acropora* sp. J Coastal Dev 10(2):115–123

Salama AK, Osman KA (2013) Remediation of pesticide-polluted water using ozonation as a safe method. Glob J Hum Soc Sci Res 13(2)

Samet Y, Hmani E, Abdelhedi R (2012) Fenton and solar photo-Fenton processes for the removal of chlorpyrifos insecticide in wastewater. Water Sa 38(4):537–542

Sandal S, Yilmaz B (2011) Genotoxic effects of chlorpyrifos, cypermethrin, endosulfan and 2, 4-D on human peripheral lymphocytes cultured from smokers and nonsmokers. Environ Toxicol 26(5):433–442. doi:10.1002/tox.20569

Sardar D, Kole RK (2005) Metabolism of chlorpyrifos in relation to its effect on the availability of some plant nutrients in soil. Chemosphere 61(9):1273–1280

Sarnaik SS, Kanekar PP, Raut VM, Taware SP, Chavan KS, Bhadbhade BJ (2006) Effect of application of different pesticides to soybean on the soil microflora. J Environ Biol 37(2):423–426

Sasikala C, Jiwal S, Rout P, Ramya M (2012) Biodegradation of chlorpyrifos by bacterial consortium isolated from agriculture soil. World J Microbiol Biotechnol 28(3):1301–1308. doi:10.1007/s11274-011-0879-z

Schimmel SC, Garnas RL, Patrick JM Jr, Moore JC (1983) Acute toxicity, bioconcentration, and persistence of AC 222,705, benthiocarb, chlorpyrifos, fenvalerate, methyl parathion, and permethrin in the estuarine environment. J Agric Food Chem 31(1):104–113. doi:10.1021/jf00115a027

Schuh RA, Lein PJ, Beckles RA, Jett DA (2002) Noncholinesterase mechanisms of chlorpyrifos neurotoxicity: altered phosphorylation of Ca^{2+}/cAMP response element binding protein in cultured neurons. Toxicol Appl Pharm 182(2):176–185

Schulman I (2013) Photocatalytic oxidation for the removal of chlorpyrifos from aqueous solution. University of Nova Gorica. http://www.wpi.edu/Pubs/E-project/Available/Eproject-011314-135820/unrestricted/PhotocatalyticOxidationfortheRemovalofChlorpyrifosfromAqueousSolution2.pdf

Serdar CM, Gibson DT, Munnecke DM, Lancaster JH (1982) Plasmid involvement in parathion hydrolysis by *Pseudomonas diminuta*. Appl Environ Microbiol 44(1):246–249

Shan M, Fang H, Wang X, Feng B, Chu X-Q, Yu Y-L (2006) Effect of chlorpyrifos on soil microbial populations and enzyme activities. J Environ Sci (China) 18(1):4–5

Sharbidre AA, Metkari V, Patode P (2011) Effect of methyl parathion and chlorpyrifos on certain biomarkers in various tissues of guppy fish, *Poecilia reticulata*. Pest Biochem Physiol 101(2):132–141. doi:10.1016/j.pestbp.2011.09.002

Sharma SK (2015) Green Chemistry for dyes removal from waste water: research trends and applications. Wiley, New York

Sharom MS, Miles JRW, Harris CR, McEwen FL (1980a) Behaviour of 12 insecticides in soil and aqueous suspensions of soil and sediment. Water Res 14(8):1095–1100

Sharom MS, Miles JRW, Harris CR, McEwen FL (1980b) Persistence of 12 insecticides in water. Water Res 14(8):1089–1093

Silambarasan S, Abraham J (2013) Efficacy of Ganoderma sp. JAS4 in bioremediation of chlorpyrifos and its hydrolyzing metabolite TCP from agricultural soil. J Basic Microbiol 54(1):44–55. doi:10.1002/jobm.201200437

Singh BK, Walker A (2006) Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev 30(3):428–471. doi:10.1111/j.1574-6976.2006.00018.x

Singh BK, Walker A, Wright DJ (2002) Persistence of chlorpyrifos, fenamiphos, chlorothalonil, and pendimethalin in soil and their effects on soil microbial characteristics. Bull Environ Contam Toxicol 69(2):181–188

Singh BK, Walker A, Grayston SJ, Del Re AAM, Capri E, Padovani L, Trevisan M (2003a) Degradation of chlorpyrifos and its effects on the soil biota. In: Pesticide in air, plant, soil and water system. Proceedings of the XII symposium pesticide chemistry, Piacenza, Italy, La Goliardica Pavese srl, pp 27–34

Singh BK, Walker A, Morgan JAW, Wright DJ (2003b) Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Appl Environ Microbiol 69(9):5198–5206

Singh BK, Walker A, Morgan JAW, Wright DJ (2004) Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soils. Appl Environ Microbiol 70(8):4855–4863. doi:10.1128/AEM.70.8.4855-4863.2004

Singh PB, Sharma S, Saini HS, Chadha BS (2009) Biosurfactant production by *Pseudomonas* sp. and its role in aqueous phase partitioning and biodegradation of chlorpyrifos. Lett Appl Microbiol 49(3):378–383. doi:10.1111/j.1472-765X.2009.02672.x

Singh DP, Khattar JIS, Nadda J, Singh Y, Garg A, Kaur N, Gulati A (2011) Chlorpyrifos degradation by the cyanobacterium *Synechocystis* sp. strain PUPCCC 64. Environl Sci Pol Res 18(8):1351–1359. doi:10.1007/s11356-011-0472-x

Sivasithamparam K (1970) Some effects of an insecticide ("Dursban") and a weedicide ("Linuron") on the microflora of a submerged soil. Riso 19:339–346

Sledge D et al (2011) Critical duration of exposure for developmental chlorpyrifos-induced neurobehavioral toxicity. Neurotoxicol Teratol 33(6):742–751. doi:10.1016/j.ntt.2011.06.005

Slotkin TA (2004) Guidelines for developmental neurotoxicity and their impact on organophosphate pesticides: a personal view from an academic perspective. Neurotoxicology 25(4):631–640

Slotkin TA, Brown KK, Seidler FJ (2005) Developmental exposure of rats to chlorpyrifos elicits sex-selective hyperlipidemia and hyperinsulinemia in adulthood. Environ Health Persp 113(10):1291–1294

Smegal DC (2000) Human health risk assessment chlorpyrifos US Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, Health Effects Division, US Government Printing Office: Washington, DC, pp 1–131

Smith CR, Funke BR, Schulz JT (1978) Effects of insecticides on acetylene reduction by alfalfa, red clover and sweetclover. Soil Biol Biochem 10(6):463–466. doi:10.1016/0038-0717(78)90037-8

Sobti RC, Krishan A, Pfaffenberger CD (1982) Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. Mutat 102(1):89–102

Sparling DW, Fellers G (2007) Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylii*. Environ Pollt 147(3):535–539. doi:10.1016/j.envpol.2006.10.036

Srebocan E, Hrlec G, Grabarevic Z, Pompe-Gotal J (2003) Poisoning with acetylcholinesterase inhibitors in dogs: two case reports. Vet Med-Czech 48(6):175–176

Tang T, Dong J, Ai S, Qiu Y, Han R (2011) Electro-enzymatic degradation of chlorpyrifos by immobilized hemoglobin. J Hazard Mater 188(1):92–97. doi:10.1016/j.jhazmat.2011.01.080

Thengodkar RRM, Sivakami S (2010) Degradation of chlorpyrifos by an alkaline phosphatase from the cyanobacterium *Spirulina platensis*. Biodegradation 21(4):637–644. doi:10.1007/s10532-010-9331-6

Theriot CM, Grunden AM (2011) Hydrolysis of organophosphorus compounds by microbial enzymes. Appl Microbiol Biotechnol 89(1):35–43. doi:10.1007/s00253-010-2807-9

Thrasher JD, Heuser G, Broughton A (2002) Immunological abnormalities in humans chronically exposed to chlorpyrifos. Arch Environ Health 57(3):181–187. doi:10.1080/00039890209602934

Tian Y, Ishikawa H, Yamaguchi T, Yamauchi T, Yokoyama K (2005) Teratogenicity and developmental toxicity of chlorpyrifos: maternal exposure during organogenesis in mice. Reprod Toxicol 20(2):267–270

Tomlin CDS (2009) The pesticide manual: a world compendium. British Crop Production Council, Alton

Tortella GR, Rubilar O, Cea M, Wulff C, Martinez O, Diez MC (2010) Biostimulation of agricultural biobeds with NPK fertilizer on chlorpyrifos degradation to avoid soil and water contamination. J Soil Sci Plant Nutr 10(4):464–475. doi:10.4067/S0718-95162010000200007

Tu CM (1981) Effects of pesticides on activities of enzymes and microorganisms in a clay soil. J Environ Sci Health, Part B 16(2):179–191. doi:10.1080/03601238109372250

USEPA (2009) Memorandom, chlorpyrifos. Revised Human Health Assessment Scoping Document in support of registration review from Drew D, Britton W, Doherty J, Risk Assessment Branch V. Health Effects Division to Santora K, Myers M, Regregistration Branch 2, Special Review and Reregistration Division (7508P) Office of Pesticide Programs, United States Environmental Protection Agency, Washington, DC

Usmani KA, Rose RL, Hodgson E (2003) Inhibition and activation of the human liver microsomal and human cytochrome P450 3A4 metabolism of testosterone by deployment-related chemicals. Drug Metab Dispos 31(4):384–391. doi:10.1124/dmd.31.4.384

Ventura C et al (2012) Differential mechanisms of action are involved in chlorpyrifos effects in estrogen-dependent or-independent breast cancer cells exposed to low or high concentrations of the pesticide. Toxicol Lett 213(2):184–193

Verma A, Dixit D (2012) Photocatalytic degradability of insecticide chlorpyrifos over UV irradiated titanium dioxide in aqueous phase. Int J Environ Sci 3(2):743–755. doi:10.6088/ijes.2012030132001

Vischetti C, Coppola L, Monaci E, Cardinali A, Castillo MdP (2007) Microbial impact of the pesticide chlorpyrifos on Swedish and Italian biobeds. Agron Sustain Dev 27(3):267–272. doi:10.1051/agro:2007020

Viswanath G, Chatterjee S, Dabral S, Nanguneri SR, Divya G, Roy P (2010) Anti-androgenic endocrine disrupting activities of chlorpyrifos and piperophos. J Steroid Biochem Mol 120(1):22–29. doi:10.1016/j.jsbmb.2010.02.032

Wacksman MN, Maul JD, Lydy MJ (2006) Impact of atrazine on chlorpyrifos toxicity in four aquatic vertebrates. Arch Environ Contam Toxicol 51(4):681–689. doi:10.1007/s00244-005-0264-8

Walia S, Dureja P, Mukerjee SK (1988) New photodegradation products of chlorpyrifos and their detection on glass, soil, and leaf surfaces. Arch Environ Contam Toxicol 17(2):183–188

Wang AA, Mulchandani A, Chen W (2002) Specific adhesion to cellulose and hydrolysis of organophosphate nerve agents by a genetically engineered Escherichia coli strain with a surface-expressed cellulose-binding domain and organophosphorus hydrolase. Appl Environ Microbiol 68(4):1684–1689. doi:10.1128/AEM.68.4.1684-1689.2002

Wang JH, Zhu LS, Wang J, Qin K (2005) Degradation characteristics of three fungi to chlorpyrifos. Chin J Appl Environ Biol 11(2):211–214

Wang L, Jiao W, Jiang X, Liang Y (2013a) Abiotic control on the dissipation of chlorpyrifos spiked in an udic ferrisol at a lower dosage. Chem Ecol 29(8):724–732. doi:10.1080/02757540.2013.817565

Wang Q, Yang J, Li C, Xiao B, Que X (2013b) Influence of initial pesticide concentrations in water on chlorpyrifos toxicity and removal by *Iris pseudacorus*. Water Sci Technol 67(9):1908–1915. doi:10.2166/wst.2013.071

Watts M (2012) Chlorpyrifos as a possible global POP Pesticide Action Network North America, Oakland, CA. www pan-europe info/News/PR/121009_Chlorpyrifos_as_POP_final pdf

WISER WirelessInformation System for Emergency Response, Chlorpyrifos (alias of Danusban) CAS RN:2921-88-2, U.S. National Library of Medicine. http://webwisernlmnihgov/getSubstanceDatado?substanceId=280&displaySubstanceName=Danusban&STCCID=&UNNAID=&selectedDataMenuItemID=79

Worthing CR (1979) The pesticide manual: a world compendium. British Crop Protection Council, Croydon

Wu N, Deng M, Shi X, Liang G, Yao B, Fan Y (2004) Isolation, purification and characterization of a new organphosphorus hydrolase OPHC2. Chin Sci Bull 49(3):268–272. doi:10.1360/03wc0453

Xing H, Wang X, Sun G, Gao X, Xu S, Wang X (2012) Effects of atrazine and chlorpyrifos on activity and transcription of glutathione S-transferase in common carp (*Cyprinus carpio* L.). Environ Toxicol Pharmacol 33(2):233–244. doi:10.1016/j.etap.2011.12.014

Xu G, Li Y, Zheng W, Peng X, Li W, Yan Y (2007) Mineralization of chlorpyrifos by coculture of *Serratia* and *Trichosporon* spp. Biotechnol Lett 29(10):1469–1473. doi:10.1007/s10529-007-9444-0

Xu G, Zheng W, Li Y, Wang S, Zhang J, Yan Y (2008) Biodegradation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol by a newly isolated *Paracoccus* sp. strain TRP. Int Biodeter Biodegr 62(1):51–56. doi:10.1016/j.ibiod.2007.12.001

Yadav M, Srivastva N, Singh RS, Upadhyay SN, Dubey SK (2014) Biodegradation of chlorpyrifos by *Pseudomonas* sp. in a continuous packed bed bioreactor. Bioresour Technol 165:265–269. doi:10.1016/j.biortech.2014.01.098

Yang L, Zhao Y-h, Zhang B-x, Yang C-H, Zhang X (2005) Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading bacterium. FEMS Microbiol Lett 251(1):67–73. doi:10.1016/j.femsle.2005.07.031

Yang C, Liu N, Guo X, Qiao C (2006) Cloning of *mpd* gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil. FEMS Microbiol Lett 265(1):118–125. doi:10.1111/j.1574-6968.2006.00478.x

Yu J-J (2002) Removal of organophosphate pesticides from wastewater by supercritical carbon dioxide extraction. Water Res 36(4):1095–1101. doi:10.1016/S0043-1354(01)00293-7

Yu YL, Fang H, Wang X, Wu XM, Shan M, Yu JQ (2006) Characterization of a fungal strain capable of degrading chlorpyrifos and its use in detoxification of the insecticide on vegetables. Biodegradation 17(5):487–494. doi:10.1007/s10532-005-9020-z

Zeinat Kamal M, Nashwa AH, Mohamed AI, Sherif E-N (2008) Biodegradation and detoxification of malathion by of *Bacillus thuringiensis* MOS-5. Aus J Basic Appl Sci 2(3):724–732

Zhang R, Cui Z, Zhang X, Jiang J, Gu J-D, Li S (2006) Cloning of the organophosphorus pesticide hydrolase gene clusters of seven degradative bacteria isolated from a methyl parathion contaminated site and evidence of their horizontal gene transfer. Biodegradation 17(5):465–472. doi:10.1007/s10532-005-9018-6

Zhang Q, Liao C, Yan Y, Lin S, Yang H, Tan H (2013) Toxicity of chlorpyrifos on seed germination of different vegetables. Guizhou Sci 6:46–49

Zhao L, Wang F, Zhao J (2014) Identification and functional characteristics of chlorpyrifosdegrading and plant growth promoting bacterium *Acinetobacter calcoaceticus*. J Basic Microbiol 54(5):457–463. doi:10.1002/jobm.201200639

Zhongli C, Shunpeng L, Guoping F (2001) Isolation of methyl parathion-degrading strain M6 and cloning of the methyl parathion hydrolase gene. Appl Environ Microbiol 67(10):4922–4925. doi:10.1128/AEM.67.10.4922-4925.2001

Zhu J, Zhao Y, Qiu J (2010) Isolation and application of a chlorpyrifos-degrading *Bacillus licheniformis* ZHU-1. Afr J Microbiol Res 4(24):2716–2719

Zhu B, Cao Y, Wang D, Tang X, Hua R, Shi T, Sun L (2013) Survival and chlorpyrifos degradation of strain *Cupriavidus taiwanensis* Lux-X1 in different type soils. J Food Agric Environ 11(2):873–876

Food and Chemical Toxicology 47 (2009) 650–656

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Assessment of genotoxic and mutagenic effects of chlorpyrifos in freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis

Daoud Ali[a], N.S. Nagpure[a,*], Sudhir Kumar[b], Ravindra Kumar[a], B. Kushwaha[a], W.S. Lakra[a]

[a] National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkusha, Lucknow 226 002, India
[b] Department of Zoology, University of Lucknow, Lucknow 226 007, India

ARTICLE INFO

Article history:
Received 24 August 2008
Accepted 17 December 2008

Keywords:
DNA damage
Chlorpyrifos
Comet assay
Micronucleus assay
Genotoxic
Channa punctatus

ABSTRACT

Chlorpyrifos (CPF) is the single largest selling agrochemical that has been widely detected in surface waters in India. The studies on long-term genotoxic effects of CPF in different tissues of fish using genotoxic biomarkers are limited. Therefore, in the present study DNA damage by CPF in freshwater fish *Channa punctatus* using micronucleus (MN) and comet assays was investigated. The LC_{50} – 96 h of CPF was estimated for the fish in a semi-static system. On this basis of LC_{50} value sublethal and nonlethal concentrations were determined. The DNA damage was measured in lymphocytes and gill cells as the percentage of DNA in comet tails and micronuclei were scored in erythrocytes of fishes exposed to above concentrations of CPF. In general, significant effects for both the concentrations and time of exposure were observed in treated fish. It was found that MN induction in the blood was highest on day 14 at 203.0 μg/l of CPF. The highest DNA damage was observed on day 5, followed by a gradual non-linear decline in the lymphocytes and gill cells. The study indicated MN and comet assays to be sensitive and rapid methods to detect mutagenicity and genotoxicity of CPF and other pollutants in fishes.

1. Introduction

Occupational exposure to pesticides is a common and alarming worldwide phenomenon. Approximately, 3 million cases of acute poisoning and 0.22 million deaths from pesticide exposure have been reported annually (Marrs, 1993; USDA, 1994; Yasmashita et al., 1997). Chlorpyrifos (O,O-diethylO-3,5,6-trichloro-2-pyridyl phosphorothioate) is a broad-spectrum organophosphate pesticide used heavily throughout the world for agriculture and domestic purposes. The main target of the pesticides is acetylcholinesterase, which hydrolyses acetylcholine in cholinergic synapses at neuromuscular junctions (Ecobichon, 1991; Amitai et al., 1998). Chlorpyrifos (CPF) elicits a number of other effects including hepatic dysfunction, immunological abnormalities, embryo toxicity, genotoxicity, teratogenicity, neurochemical, and neurobehavioral changes (Muscarella et al., 1984; Muto et al., 1992; Thrasher et al., 1993; Bagchi et al., 1995; Song et al., 1998; Dam et al., 1999; Gomes et al., 1999). It is a widely used against fire ants, ornamental plant insects, cockroaches, mosquitoes, termites, horn flies, lice and fleas (EPA, 1986). Its persistence in sediments is hazardous to sea bottom dwellers (Schimmel et al., 1983). The high concentration of CPF immediately inhibits both DNA and protein syntheses without regional selectivity in 1-day old rats (Whitney et al., 1995).

In India, CPF is classified as an extremely hazardous pesticide (ITRC, 1989); its residue has been found in scented roses and their products (Kumar et al., 2004). Its maximum concentration has been reported to be 88.6 μg/g in tissues of fishes *Channa striata* and *Catla catla* from Kolleru Lake, India (Amaraneni and Pillai, 2001) and 198.5 μg/g in sediment, prawn and water samples from prawn ponds near Kolleru Lake wetland (Amaraneni, 2006).

Surprisingly, the soft drinks also contain CPF in a concentration of 4.8 ppb, which is 47 times higher than permissible limit (CSE, 2006). It has been reported to be genotoxic in *C. punctatus* and mice leukocytes (Porichha et al., 1998; Rahman et al., 2002) and root meristem cells of *Crepis capillaris* (Dimitrov and Gadeva, 1997). The exposure to 0.08 μg/l of CPF caused reproductive impairment in *Daphnia magna* (EPA, 1985).

Since there is growing a concern over the presence of genotoxins in the aquatic environment, the development of sensitive biomarkers for detection of genotoxic effects in aquatic organisms has gained importance (Hayashi et al., 1998). The micronucleus (MN) and comet assays are two sensitive, rapid and extensively used tools for detecting the mutagenic and genotoxic effects of chemicals in the environment (Tucker and Preston, 1996; Kassie

* Corresponding author. Tel.: +91 522 2442440/2442441; fax: +91 522 2442403.
E-mail address: nagpurens@yahoo.co.in (N.S. Nagpure).

0278-6915/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2008.12.021

et al., 2000). The genotoxic effects of environmental pollutants can be monitored using a broad range of both the *in vitro* and *in vivo* biomarker assays but the comet assay is gaining popularity over others since its advantages include sensitivity for detecting low levels of DNA damage (0.1 DNA break/10^9 Da) (Gedic et al., 1992) and the short time needed to complete a study. The micronucleus (MN) assay is another useful and popular technique for showing clastogenic and aneugenic effect (Norppa and Falck, 2003) and has been extensively used *in situ* (Al-Sabti and Metcalfe, 1995).

Although the toxic effects of CPF on fish have been studied earlier (Johnson and Finley, 1980), the information regarding the genotoxic and mutagenic nature of CPF in aquatic organisms is rare, especially the data pertaining to the long-term genotoxic effect of CPF in fishes. Therefore, the present study investigates the mutagenic and genotoxic effects of CPF using MN assay in erythrocytes and comet assay in lymphocytes and gill cells of *C. punctatus* exposed *in vivo*.

2. Materials and methods

2.1. Experimental fish specimens and chemicals

Healthy specimens of freshwater fish *C. punctatus* (Bloch, Family: Channidae and Order: Channiformes) were procured from the local outlets. The specimens had an average wet weight and length of 30 ± 2.0 g and 14 ± 3.0 cm, respectively. The specimens were given prophylactic treatment by bathing them twice in 0.05% potassium permanganate ($KMnO_4$) solution for 2 min to avoid any dermal infections. The fishes were then acclimatized for one month under laboratory condition before CPF exposure. The fishes were fed boiled eggs; goat liver and poultry waste material. The faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Every effort as suggested by Bennett and Dooley (1982) was made to maintain optimal conditions during acclimatization.

For the present study, technical-grade CPF (20% EC) with trade name Tricel (manufactured by Excel crop care Ltd. Mumbai) was purchased from market.

2.2. Determination of sub lethal concentrations

The acute toxicity bioassays to determine the LC_{50} – 96 h value of CPF were conducted in the semi-static system. A facility for oxygenation of the test solution was provided with the help of showers fixed above the test chambers. The acute bioassay procedure was based on standard methods (APHA et al., 2005). The stock solution of CPF was prepared by dissolving it in acetone.

A set of 10 acclimatized fish specimens was randomly exposed to each of the six CPF target concentrations (0.3, 0.6, 0.8, 1.0, 1.2 and 1.5 mg/l) and the experiment was repeated twice to obtain the LC_{50} – 96 h value of the test chemical for the species.

The LC_{50} – 96 h value of CPF was determined as 811.98 μg/l for *C. punctauts* following the probit analysis method as described by Finney (1971). Based on the LC_{50} – 96 h value, the three test concentrations of CPF viz., sublethal 1 (1/4th of LC_{50} = ~203.0 μg/l), sublethal 2 (1/8th of LC_{50} = ~102.0 μg/l) and nonlethal (1/12th of LC_{50} = ~68.0 μg/l) were estimated.

2.3. In vivo exposure experiment

The fish specimens were exposed to the three aforementioned test concentrations of CPF in a semi-static system with the change of test water on every 96 h. The exposure was continued up to 35 days and tissue sampling was done at intervals of 1, 3, 5, 7, 14, 21, 28 and 35 days at the rate of five fishes per duration. The fishes maintained in tap water were considered as negative control. The concentration of acetone was 0.1% in all test solutions and solvent control. An additional set for positive control (cyclophosphamide 20 mg/kg of body weight) was separately maintained.

On each sampling day, the blood and gills were collected and immediately processed for MN and comet assays. The blood samples were collected from the fish by caudal vein puncture technique using heparinized syringe. The physicochemical properties of test water, namely temperature, pH, conductivity, dissolved oxygen, chloride, total hardness and total alkalinity were analyzed by standard methods (APHA et al., 2005).

2.4. Micronucleus assay

Immediately after collection a drop of blood was smeared on a clean microscopic slide. After drying, the smears were fixed in methanol for 10 min and left to air-dry at room temperature and finally stained with 6% Giemsa in Sorenson buffer (pH 6.9) for 20 min. A total of 2000 erythrocytes were examined for each specimen under the light microscope (Leitz Wetzlar Germany, Type 307–083.103; oil immersion lens, 100/1.25). The following criteria for the identification of micronuclei (MNi) were used no connection with the main nucleus, same color and intensity as the main nucleus and an area smaller than one-third of the main nucleus (Schmid, 1975; Das and Nanda,1986; Fenech, 2000). MN frequency was calculated using the formula:

$$\mathrm{MN}\% = \frac{\text{Number of cells containing micronucleus}}{\text{Total number of cells counted}} \times 100.$$

2.5. Alkaline single-cell gel electrophoresis

The alkaline single-cell gel electrophoresis was performed as a three-layer procedure (Singh et al., 1988) with slight modifications (Klaude et al., 1996). The gill tissue (~50 mg) was homogenized in ice-cold homogenization buffer (1X Hanks' balanced salt solution, 20 mM EDTA, 10% di methyl sulphoxide (DMSO), pH 7.0–7.5) followed by centrifugation at 3000 rpm at 4 °C for 5 min. The cell pellet was then suspended in chilled phosphate buffered saline for comet assay.

The lymphocytes were separated from blood using histopaque density gradient centrifugation and the cells were diluted 20-fold for the comet assay. Viability of both the lymphocytes and gill cells was evaluated by the trypan blue exclusion test method (Anderson et al., 1994). The tissue samples showing cell viability higher than 84% were further processed for comet assay. In brief, about 15 μl of cell suspension (approx. 20,000 cells) was mixed with 85 μl of 0.5% low melting-point agarose and layered on one end of a frosted glass slide, coated with a layer of 200 μl of 1% normal agarose. It was covered with a third layer of 100 μl low melting-point agarose. After solidification of the gel, the slides were immersed in lysing solution (2.5 M NaCl, 100 mM Na_2 EDTA, 10 mM Tris, pH 10 with 10% DMSO and 1% Triton X-100 added fresh) overnight at 4 °C. The slides were then placed in a horizontal gel electrophoresis unit, immersed in fresh cold alkaline electrophoresis buffer (300 mM NaOH, 1 mM Na_2 EDTA and 0.2% DMSO, pH > 13.5), and left in solution for 20 min at 4 °C for the DNA unwinding and conversion of alkali–labile sites to single strand breaks. Electrophoresis was carried out using the same solution at 4 °C for 20 min, using 15 V (0.8 V/cm) and 300 mA. The slides were neutralized gently with 0.4 M Tris buffer at pH 7.5 and stained with 75 μl ethidium bromide (20 μg/ml). For positive control, the lymphocytes and gill cells were treated with 100 μM H_2O_2 for 10 min at 4 °C. Two slides per specimen were prepared and 25 cells per slide (250 cells per concentration) were scored randomly and analyzed using an image analysis system (Komet-5.5, Kinetic Imaging) attached to a fluorescent microscope (Leica) equipped with appropriate filters. The parameter selected for quantification of DNA damage was percent tail DNA as determined by the software.

2.6. Statistical analysis

The one-way analysis of variance (ANOVA) was applied to compare the mean differences in the% tail DNA between tissues within concentration, between concentrations within tissue and between durations within concentration and tissue. The% MN frequencies were compared between durations within concentration and between concentrations within duration using Mann–Whitney test. The *P* values less than 0.01 were considered statistically significant.

3. Results

3.1. Physicochemical properties of the test water

The test water temperature varied from 26.7 to 28.4 °C and the pH ranged from 7.2 to 8.1. The dissolved oxygen concentration was normal, varied from 6.0 to 8.05 mg/l, during experimental period. The conductivity of the water ranged from 248 to 296 μM/cm and the chloride, total hardness and total alkalinity ranged from 45–54 mg/l, 160–180 mg/l and 260–290 mg/l, as $CaCO_3$, respectively.

3.2. DNA damage

The fish specimens exposed to different concentrations of CPF exhibited significantly higher DNA damage in their tissues than the control sample and solvent control (Table 1, Figs. 1a and b, 2a and b, 3a–c). A significant effect of the duration of exposure on DNA damage was observed in specimens exposed to CPF. The highest DNA damage in the tissues was observed on day 5 for all treatment groups. Afterwards, there was a gradual non-linear decrease as the experiment the advanced. The decline in DNA damage

Table 1
Mean percentage tail DNA in gill cells and lymphocytes of *C. punctatus* exposed to chlorpyrifos.

Exposure time	Gill (μg/1)			Lymphocytes (μg/1)		
	203.0	102.0	68.0	203.0	102.0	68.0
Control	4.34 ± 0.28^{A}	4.34 ± 0.28^{A}	4.34 ± 0.28^{A}	3.92 ± 0.19^{A}	3.92 ± 0.19^{A}	3.92 ± 0.19^{A}
Solvent control	6.32 ± 0.40^{A}	6.32 ± 0.40^{A}	6.32 ± 0.40^{A}	6.28 ± 0.41^{A}	6.28 ± 0.41^{A}	6.28 ± 0.41^{A}
1 day	10.70 ± 0.45^{a1B}	9.78 ± 0.36^{a12B}	9.61 ± 0.20^{a2B}	9.10 ± 0.59^{b1B}	11.25 ± 0.53^{b2B}	7.04 ± 0.37^{b3B}
3 days	17.91 ± 0.64^{a1C}	12.39 ± 0.48^{a2C}	11.84 ± 0.45^{a2C}	13.72 ± 0.61^{b1C}	11.63 ± 0.57^{a2B}	9.38 ± 0.59^{a3C}
5 days	24.10 ± 0.89^{a1D}	18.44 ± 0.48^{a2D}	15.35 ± 0.40^{a3D}	22.67 ± 0.51^{b1D}	16.39 ± 0.94^{b2C}	14.17 ± 0.65^{b3D}
7 days	20.34 ± 0.50^{a1E}	15.27 ± 0.41^{a2E}	13.52 ± 0.47^{a3E}	17.64 ± 0.42^{b1E}	11.97 ± 0.48^{b2B}	9.35 ± 0.61^{b3C}
14 days	19.03 ± 0.50^{a1E}	13.80 ± 0.39^{a2E}	10.54 ± 0.53^{a3F}	15.92 ± 0.50^{b1F}	11.08 ± 0.80^{b2B}	8.87 ± 0.61^{b3C}
21 days	13.17 ± 0.40^{a1F}	11.68 ± 0.57^{a2F}	10.18 ± 0.47^{a2B}	12.10 ± 0.41^{a1C}	10.30 ± 0.52^{a1BD}	8.22 ± 0.60^{b2C}
28 days	10.12 ± 0.27^{a1B}	10.46 ± 0.27^{a1FG}	9.87 ± 0.42^{a2B}	10.67 ± 0.39^{a1B}	9.47 ± 0.63^{a1DE}	7.35 ± 0.36^{b2B}
35 days	9.95 ± 0.25^{a1B}	9.52 ± 0.58^{a1G}	9.05 ± 0.46^{a1B}	10.04 ± 0.37^{a1B}	8.12 ± 0.46^{b2E}	7.16 ± 0.37^{b2B}

Values with different alphabet superscripts differ significantly ($P < 0.01$) between tissues within concentration. Values with different numeric superscripts differ significantly ($P < 0.01$) between concentrations within tissue. Values with different capital alphabet superscripts differ significantly ($P < 0.01$) between durations within concentration and tissue.

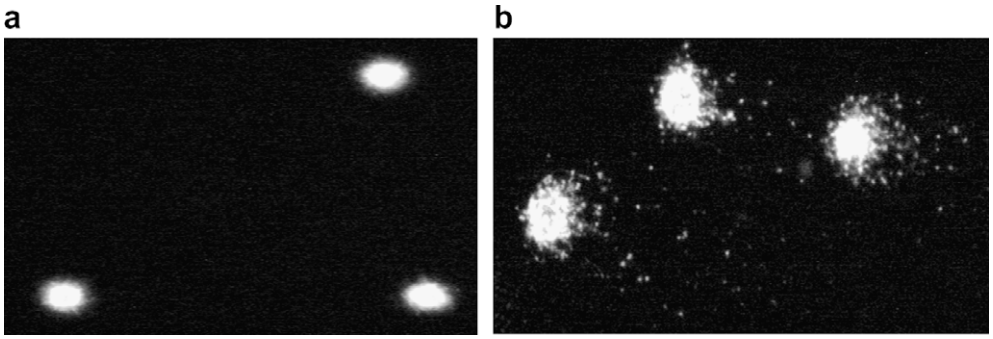


Fig. 1. (a) Control gill cells and (b) gill cells after exposure to chlorpyrifos.

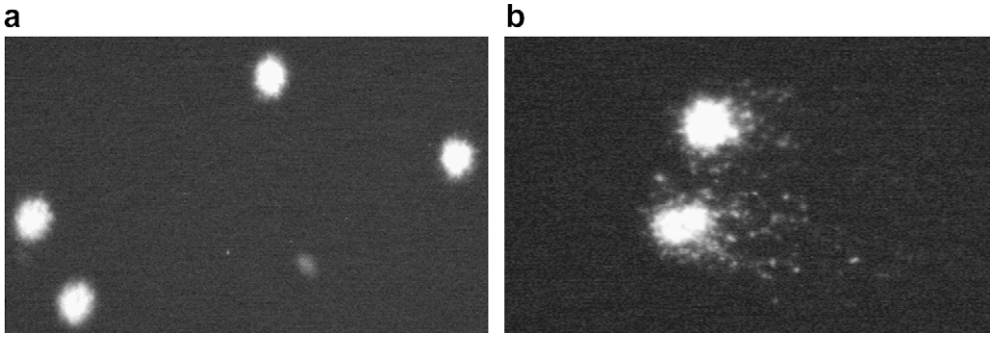


Fig. 2. (a) Control lymphocytes and (b) lymphocytes after exposure to chlorpyrifos.

after day 5 to day 21 was significantly evident for sublethal 1 concentration of CPF in all tissues except between day 7 and day 14 in gill cells. With sublethal 2 concentration, the decline of DNA damage was significant up to day 21 in gill cells (Table 1, Fig. 3a and b). At nonlethal concentrations the decrease in DNA damage from day 5 to day 7 was significantly evident for lymphocytes and from day 5 to day 14 for gill cells (Fig. 3c).

The study also showed a significant effect of CPF on the induction of DNA damage especially in the gill cells (Table 1, Fig. 3a–c). In general, the DNA damage was found to be concentrations dependent in both the tissues, with the highest DNA damage at the sublethal 1, followed by sublethal 2 and nonlethal concentrations.

With regard to the variation in DNA damage between the tissues, the gill cells exhibited comparatively higher DNA damage than lymphocytes at most of the concentrations and durations (Fig. 3a–c). At day 5, the highest DNA damage was (24.10%) in gill cells followed by lymphocytes (22.67%) at the sublethal 1 (Table 1).

3.3. Induction of MNi

The erythrocytes of *C. punctatus* were elliptical with a centrally located oblong nucleus. The frequency of MNi induced in the peripheral blood erythrocytes was determined at different concentrations of CPF and the results are summarized in Table 2. The observed MNi (Fig. 4a and b) showed similar features as described by Schmid (1975). Time response graphs at each concentration level are presented in Fig. 5. The induction of MNi at sublethal 1 concentration of CPF, all exposure times, was significantly higher than nonlethal and control groups, whereas they were not induced significantly on days 1, 21, 28 and 35 as compared to sublethal 2.

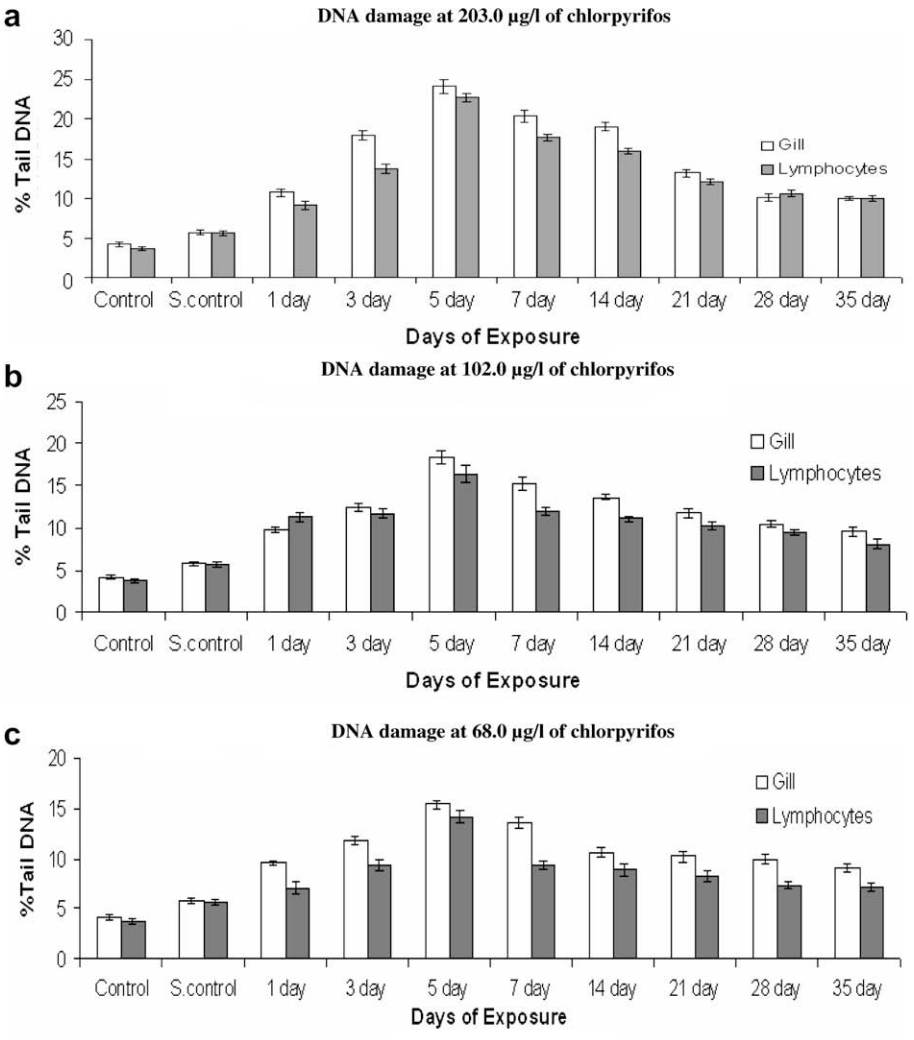


Fig. 3. DNA damage in lymphocytes and gill cells by (a) 203.0 µg/l, (b) 102.0 µg/l and (c) 68.0 µg/l of chlorpyrifos.

Further, a significant effect of durations on induction of MNi has been observed for all the concentrations of CPF. We have observed the highest MNi frequency of 1.62% on day 14 at sublethal 1 concentration and 1.53% on day 21 for sublethal 2 and 1.16% for non-lethal concentrations. The solvent control (acetone) was unable to induce any significant MNi frequency in comparison to negative control.

4. Discussion

The comet assay under alkaline conditions (pH > 13) is able to detect DNA damage, i.e. single strand breakage or other lesions, such as alkali–labile sites, DNA cross-links (Tice, 1995) and incomplete excision repair events (Gedic et al., 1992). It offers considerable advantages over other cytogenetic methods like chromosome aberrations, sister chromatid exchanges and micronucleus test used to detect DNA damage, because for comet assay the cells need not to be mitotically active (Buschini et al., 2003). Therefore, it has been widely used in the fields of genetic toxicology and environmental biomonitoring (Tice et al., 2000), including aquatic organisms (Mitchelmore and Chipman, 1998; Lee and Steinert, 2003), as powerful tool for measuring the relationship between DNA damage and the exposure of aquatic organisms to genotoxic pollutants.

Long-term genotoxicity studies can be important approach for achieving greater insight into the organism's DNA repair ability and other protective mechanisms for excreting the toxic chemicals. The significant induction of MNi in erythrocytes and DNA damage in gill cells and lymphocytes of *C. punctatus* due to CPF exposure at different concentrations as well as exposure durations has indicated its potential genotoxic and mutagenic properties. Further, a differential tissue response for DNA damage induced by CPF exposure was evident. The variation in DNA damage between tissues could be explained by the number of the alkali–labile sites, being variable in DNA from different tissues and by the different cell types having different background levels of DNA single strand breaks due to variation in excision repair activity, metabolic activity, antioxidant concentrations and other factors (Lee and Steinert, 2003). The results obtained in the present study are in agreement with the findings of Blasiak et al. (1999) for the human lymphocytes exposed to malathion and its two analogues and Masauda et al., 2004 in the gill cells of gold fish (*Carassius auratus*) for PBTA-6 and ADDB. The DNA damage detected in this study could have originated from DNA single strand breaks, DNA double strands break, DNA adducts formations, DNA–DNA and DNA–protein cross-links (Mitchelmore and Chipman, 1998) resulting from the interaction of pesticides or their metabolites with DNA (Fairbairn et al., 1995). Anitha et al. (2000) carried out genotoxicity eval-

Table 2
Incidence of MNi in peripheral erythrocytes of *C. punctatus* exposed to different concentrations of chlorpyrifos.

Exposure time	Dosage	No. of fishes observed	No. of cells observed	MN frequencies (%)±SE
1day	Control	5	10,057	0.028 ± 0.09^{a}
	Solvent control	5	10,140	0.040 ± 0.10^{a}
	Positive control	5	10,316	0.058 ± 0.10^{1a}
	203.0 μg/l	5	10,127	0.070 ± 0.15^{1b}
	102.0 μg/l	5	10,120	0.064 ± 0.10^{1b}
	68.0 μg/l	5	10,155	0.050 ± 0.07^{1a}
3 days	Control	5	10,015	0.029 ± 0.09^{a}
	Solvent control	5	10,160	0.04 ± 0.10^{a}
	Positive control	5	10,363	0.16 ± 0.31^{2b}
	203.0 μg/l	5	10,182	0.24 ± 0.30^{2c}
	102.0 μg/l	5	10,273	0.13 ± 0.10^{2d}
	68.0 μg/l	5	10,241	0.11 ± 0.08^{2d}
5 days	Control	5	10,035	0.044 ± 0.10^{a}
	Solvent control	5	10,210	0.047 ± 0.11^{a}
	Positive control	5	10,338	0.19 ± 0.09^{3b}
	203.0 μg/l	5	10,007	0.91 ± 0.18^{3c}
	102.0 μg/l	5	10,046	0.61 ± 0.08^{3d}
	68.0 μg/l	5	10,305	0.39 ± 0.06^{3e}
7 days	Control	5	10,014	0.042 ± 0.10^{a}
	Solvent control	5	10,210	0.048 ± 0.11^{a}
	Positive control	5	10,287	1.17 ± 0.12^{4b}
	203.0 μg/l	5	10,273	1.20 ± 0.14^{4b}
	102.0 μg/l	5	10,246	0.86 ± 0.10^{4c}
	68.0 μg/l	5	10,179	0.64 ± 0.08^{4d}
14 days	Control	5	10,000	0.039 ± 0.09^{a}
	Solvent control	5	10,210	0.042 ± 0.10^{a}
	Positive control	5	10,150	1.09 ± 0.11^{5b}
	203.0 μg/l	5	10,182	1.62 ± 0.26^{5c}
	102.0 μg/l	5	10,529	1.38 ± 0.20^{5d}
	68.0 μg/l	5	10,291	1.12 ± 0.11^{5b}
21days	Control	5	10,030	0.040 ± 0.10^{a}
	Solvent control	5	10,210	0.042 ± 0.10^{a}
	Positive control	5	10,268	1.10 ± 0.11^{5b}
	203.0 μg/l	5	10,250	1.56 ± 0.24^{5c}
	102.0 μg/l	5	10,146	1.53 ± 0.23^{6c}
	68.0 μg/l	5	10,318	1.16 ± 0.13^{5b}
28days	Control	5	10,010	0.039 ± 0.09^{a}
	Solvent control	5	10,210	0.040 ± 0.10^{a}
	Positive control	5	10,276	$0.97 \pm 0.10^{6\ b}$
	203.0 μg/l	5	10,089	1.50 ± 0.24^{6c}
	102.0 μg/l	5	10,176	1.44 ± 0.23^{5c}
	68.0 μg/l	5	10,015	1.04 ± 0.11^{6b}
35days	Control	5	10,000	0.037 ± 0.07^{a}
	Solvent control	5	10,210	0.041 ± 0.09^{a}
	Positive control	5	10,210	0.81 ± 0.16^{7b}
	203.0 μg/l	5	10,306	1.31 ± 0.21^{7c}
	102.0 μg/l	5	10,186	1.26 ± 0.17^{7c}
	68.0 μg/l	5	10,272	0.89 ± 0.09^{7d}

Values with different numeric superscripts differ significantly ($P < 0.01$) between durations within concentration. Values with different alphabet superscripts differ significantly ($P < 0.01$) between concentrations within duration.

uation of heat shock in the gold fish *C. auratus* using both micronucleus and comet assays. However, the clastogenic effects of pollutants can be measured in different target tissues such as erythrocytes, gills, kidney, liver etc. (Hayashi et al., 1998) but the erythrocyte MNi test has been used with different fish species to monitor aquatic pollution displaying mutagenic features.

The higher DNA damage in gill cells could be explained by the gill being appropriate organ that is directly and constantly exposed to the DNA damaging chemicals dissolved in the water (Dzwonkowska and Hubner, 1986), where as lymphocytes receive chemicals when they enter the circulatory system. In the present study, in general, gill cells showed significantly higher DNA damage than the lymphocytes when over all comparisons are made. The suitability of gill tissues for genotoxicity studies has also been demonstrated earlier using shellfish (Sasaki et al., 1997). The observed tissue-specific response may also be due to physiochemical activities distinctive to these organs, with respect to either the activation or detoxification of pollutants or the repair of different types of strand breaks.

In the present study, CPF induced significantly higher number of MNi formation that increased with concentrations and durations. Cyclophosphamide (20 mg/kg body weight) has widely been used as a positive control because of its alkylating activity. Cyclophosphamide itself is not genotoxic but undergoes complex metabolic activation by mixed function oxidases, which result in genotoxic metabolites (Colvin and Chabner, 1990). Al-Sabti and Metcalfe (1995) demonstrated that maximal micronucleus induction normally occurred one to five days post-exposure, which agrees with our results. Based on two samplings time, Hooftman and de Raat (1982) and Das and Nanda (1986) reported a concentration and time-dependent increase in MNi induction in the fish erythrocytes, an effect corroborated by the present work. Time-dependent responses have also been observed in amphibians exposed to radiation (Siboulet et al., 1984; Fernanadez et al., 1993). The results of this study emphasize the importance of the MN assay as an early biological marker of exposure of fish to clastogenic pollutants in the aquatic environment. In present study, the general comparison between comet and MN assays showed that both concentrations and durations are effective in induction of MNi and DNA damage due to CPF exposure.

The mechanism of DNA strand breaks due to CPF exposure is poorly understood and little is known about CPF or its metabolites that are responsible for production of DNA strand breaks. Wild (1975) observed that the phosphorus moiety in organophosphates appears to be a good substrate for nucleophilic attack. This may cause phosphorylation of the DNA, which is an instance of DNA damage. Thus, it is probable that CPF could cause alterations in DNA of *C. punctatus* resulting in formation of comets. The toxic action of xenobiotics like CPF is counteracted by glutathione (GSH) and glutathione dependent enzymes systems. Depletion of cellular

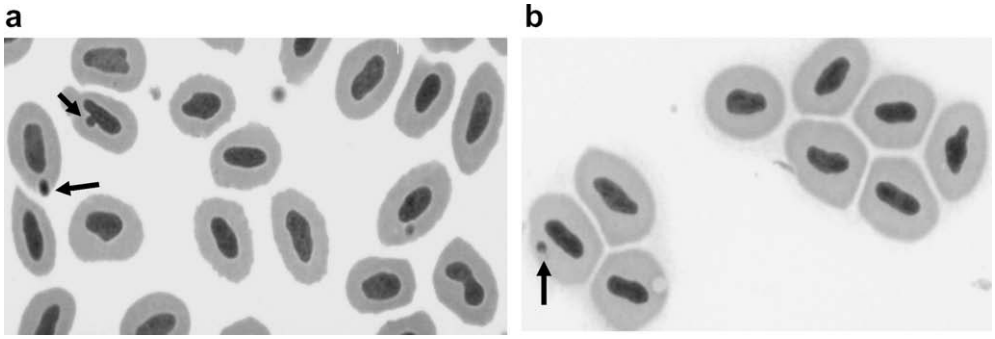


Fig. 4. (a) Micronuclei formation in the erythrocytes after exposure to cyclophasphamide and (b) micronuclei formation in the erythrocytes after exposure to chlorpyrifos.

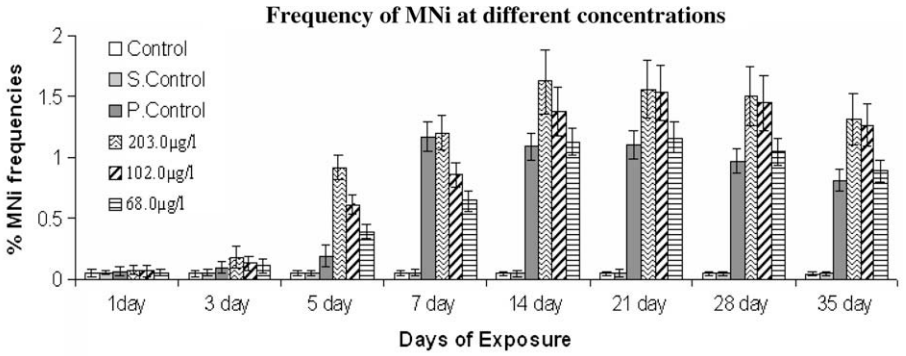


Fig. 5. Variation in MN frequency for each concentration of chlorpyrifos in relation to different exposure time.

GSH content below the critical level prevents the conjugation of xenobiotics to GSH and enables them to freely combine covalently with DNA, RNA or cell proteins and thus lead to cell damage (Meister and Andersen, 1983). DNA damage could also be due to formation of GSH conjugates, which might deplete the cellular GSH, content and induce oxidative stress (Yamano and Morita, 1995).

Reduction of GSH by xenobiotics below a certain level allows the enhancement of lipid peroxidation evoked by endogenous substances. This can lead to induction of reactive oxygen species, which could also contribute to formation of DNA single, and/or double strand breaks (Banu et al., 2001). The observed the DNA damage in our study also may have been caused for the same reason. The genotoxic properties of pesticide also depend on chemical structures. Since there is no direct evidence, it is difficult to determine the actual cause for the observed DNA strand breaks in the present study and this will be addressed in further studies.

In our experiments, it was observed that CPF produced a concentration-dependent increase in DNA single-strand breaks in the form of comet induction and a time-dependent decrease in the damage, due to the DNA repair. The genotoxic properties of CPF have been studied in a variety of assays in the past, but the results were contradictory (Isidate, 1987; Patnaik and Tripathy, 1992; Gollapudi et al., 1995). Hence, hazardous effects of this pesticide are a matter of concern due to human exposure consequent to the heavy use of CPF in India. Therefore, in an attempt to resolve the ambiguities, we investigated the *in vivo* genotoxic and mutagenic effects of CPF in *C. punctatus* erythrocytes lymphocytes and gill cells.

The decrease in DNA damage has been observed in the tissues of fishes exposed to different concentrations of CPF, although the decrease was non-linear, which may indicate repair of damaged DNA, loss of heavily damaged cells, or both (Miyamae et al., 1997; Banu et al., 2001). This inverse relationship between time of exposure and DNA damage may be due to toxicity of contaminants that could disturb the enzymatic processes in the formation of DNA damage (Rank and Jensen, 2003). Another possible explanation could be the gene activation of metabolizing enzymes such as cytochrome P450 in various tissues that provides a defensive mechanism against the persistent organic pollutants (Wong et al., 2001). Similar repair mechanism was observed with malathion analogues in isolated human lymphocytes (Blasiak et al., 1999) and with monocrotophos in fish (Banu et al., 2001), using comet assay.

One of the advantages of comet and MN assays are that both can be used for the simultaneous assessment of DNA damage in many tissues from the same animal, the comparison of their responses under identical treatment conditions. Thus these techniques can be used in combination for screening genotoxic effect of chemicals and for investigating the implications of DNA damage and its recovery in the sentinel fish species. These biomarkers have also opened a broad perspective in aquatic toxicology, as fish erythrocytes and gills are constantly being exposed to environmental pollutants.

The results of this investigation may help in guarding against the genetic hazard to human population and the environment through judicious and careful use of this pesticide in agricultural and non-agricultural areas.

5. Conclusion

The technical-grade CPF was found to be mutagenic and genotoxic to fishes even at nonlethal concentration (i.e. 1/12th of LC_{50} = ~68.0 µg/l), which indicates apprehension about the potential hazards of CPF to aquatic organisms. The present investigations indicated that the comet and MN assays are sensitive tools for demonstration of genotoxic effects of CPF in different fish tissues. Further, the comparison of DNA damage between the tissues showed that gill cells were more sensitive than lymphocytes. Thus, the combined approach using both the assays opened a broad perspective in aquatic toxicology, as fish erythrocytes and gills are constantly being exposed to environmental pollutants.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors are grateful to the Director, National Bureau of Fish Genetic Resources, Lucknow, India for providing necessary facilities for carrying out this work.

References

Al-Sabti, K., Metcalfe, C.D., 1995. Fish micronuclei for assessing genotoxicity in water. Mutat. Res. 343, 121–135.
Amaraneni, S.R., 2006. Distribution of pesticides, PAHs and heavy metals in prawn ponds near Kolleru Lake wetland, India. Environ. Int. 32, 294–302.
Amaraneni, S.R., Pillai, R.R., 2001. Concentrations of pesticide residues in tissues of fish from Kolleru Lake in India. Environ. Toxicol. 16, 550–556.
Amitai, G., Moorad, D., Adani, A., Doctor, B.P., 1998. Inhibition of acetylcholinesterase and butyrylcholinesterase by chlorpyrifos-oxon. Biochem. Pharmacol. 56, 293–299.
Anderson, D., Yu, T.W., Philips, B.J., Schmerzer, P., 1994. The effect of various antioxidant and other modifying agents on oxygen radical-generated DNA damage in human lymphocytes in the COMET assay. Mutat. Res. 307, 261–271.
Anitha, B., Chandra, N., Gopinath, P.M., Durairaj, D., 2000. Genotoxicity evaluation of heat shock in gold fish (*Carassius auratus*). Mutat. Res. 469, 1–8.
APHA, AWWA, WPCF, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Publication of Health Association, Washington, DC.

Bagchi, D., Bagchi, M., Hassoun, E.A., Stohs, S.J., 1995. *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology 104, 129–140.

Banu, B.S., Danadevi, K., Rahman, M.F., Ahuja, Y.R., Kaiser, J., 2001. Genotoxic effect of monocrotophos to sentinel species using comet assay. Food Chem. Toxicol. 39, 361–366.

Bennett, R.O., Dooley, J.K., 1982. Copper uptake by two sympatric species of killifish *Fundulus heteroclitus* (L.) and *L. majalis* (Walkbaum). J. Fish Biol. 21, 381–398.

Blasiak, J., Jaloszynski, P., Trzeciak, A., Szyfter, K., 1999. *In vitro* studies on the genotoxicity of the organophosphorus insecticide malathion and its two analogues. Mutat. Res. 445, 275–283.

Buschini, A., Carboni, P., Martino, A., Poli, P., Rossi, C., 2003. Effects of temperature on baseline and genotoxicant-induced DNA damage in haemocytes of *Dreissena polymorpha*. Mutat. Res. 537, 81–92.

Colvin, M., Chabner, B.A., 1990. Alkylating agents. In: Chabner, B.A., Collins, J.M. (Eds.), Cancer Chemotherapy: Principles and Practices. J.B. Lippencott, Philadelphia, pp. 276–313.

Centre for Science and Environment, Issue in Depth, 2006. <http://www.cseindia.org/misc/cola-indepth/cola2006/cola_press2006.htm>.

Dam, K., Garcia, S.J., Seidler, F.J., Slotkin, T.A., 1999. Neonatal chlorpyrifos exposure alters synaptic development and neuronal activity in cholinergic and catecholaminergic pathways. Brain Res. Dev. Brain Res. 116, 9–20.

Das, R.K., Nanda, N.K., 1986. Induction of micronuclei in peripheral erythrocytes of fish *Heteropneustes fossilis* by mitomycin C and paper mill effluent. Mutat. Res. 175, 67–71.

Dimitrov, B., Gadeva, P., 1997. studies on the insecticide dursban in root meristem cells of *Crepis capillaris* L.. Environ. Exp. Bot. 37, 199–209.

Dzwonkowska, A., Hubner, H., 1986. Induction of chromosomal aberrations in the Syrian hamster by insecticides tested *in vivo*. Arch. Toxicol. 58, 152–156.

Ecobichon, D.J., 1991. Toxic effect of pesticides. In: Amdur, M.O., Doull, J., Klaassen, C.D. (Eds.), Cassarett and Doull's Toxicology, fourth ed. Pergamon, New York, pp. 565–622.

EPA, 1985. Disciplinary review ecological effects profile. Available from US Environ. Protection Agency, Office of Pesticide Programs, 401 M St. S.W., Washington DC 20460, 10p.

EPA, 1986. Ambient water quality criteria for chlorpyrifos –1986 US Environ. Protection Agency Report 440/5-86-005, 64p.

Fairbairn, D.W., Olive, P.L., O'Neill, K.L., 1995. The comet assay: a comprehensive review. Mutat. Res. 399, 37–59.

Fenech, M., 2000. The *in vitro* micronucleus technique. Mutat. Res. 455, 81–95.

Fernanadez, M., L'Haridon, J., Gauthier, L., Zoll-Moreux, C., 1993. Amphibian micronucleus test(s): a simple and reliable method for evaluating *in vivo* genotoxic effects of freshwater pollutants and radiation. Initial assessment. Mutat. Res. 292, 83–99.

Finney, D.J., 1971. Probit Analysis. University Press, Cambridge. p. 333.

Gedic, C.M., Ewen, S.W.B., Collins, A.R., 1992. Single cell gel electrophoresis applied to analysis of UV-C damage and its repair in human cells. Int. J. Radiat. Biol. 62, 313–320.

Gollapudi, B.B., Mendrala, A.L., Linscombe, V.A., 1995. Evaluation of the genetic toxicity of the organophosphate insecticide chloropyrifos. Mutat. Res. 342, 25–36.

Gomes, J., Dawodu, A.H., Llyod, O., Revitt, D.M., Anilal, S.V., 1999. Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorus pesticides. Hum. Exp. Toxicol. 18, 33–37.

Hayashi, M., Ueda, T., Uyeno, L., Wada, K., Kinae, N., Saotome, K., Tanaka, N., Takai, A., Sasaki, Y.F., Asano, N., Sifuni, Y., Ojimma, T., 1998. Development of genotoxicity assay systems that use aquatic organisms. Mutat. Res. 399, 125–133.

Hooftman, R.N., de Raat, W.K., 1982. Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocyte of the eastern mudminnow *Umbra pygmaea* by ethyl methane sulphonate. Mutat. Res. 104, 147–152.

Isidate Jr., M., 1987. Chromosomal Aberrations Test *In vitro*. L.I.C. Inc., Tokyo.

Industrial Toxicology Research Centre (ITRC), 1989. Toxicity Data Handbook: Pesticide-B TDS, 192, 4, pp. 458–463.

Johnson, W.W., Finley, M.T., 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. US Fish Wild. Serv. Resour. Publ.. pp. 98–137.

Kassie, F., Parzefall, W., Knasmuller, S., 2000. Single cell gel electrophoresis assay: a new technique for human biomonitoring studies. Mutat. Res. 463, 13–31.

Klaude, M., Eriksson, S., Nygren, J., Ahnstrom, G., 1996. The comet assay: mechanisms and technical considerations. Mutat. Res. 363, 89–96.

Kumar, A., Nadda, G., Shanker, A., 2004. Determination of chlorpyrifos 20% EC (Dursban 20 EC) in scented rose and its products. J. Chromatogr. A1050, 193–199.

Lee, R.F., Steinert, S., 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. Mutat. Res. 544, 43–64.

Marrs, T.C., 1993. Organophosphate poisoning. Pharmacol. Ther. 58, 51–66.

Masauda, S., Deguchi, Y., Masauda, Y., Watanabe, T., Nukya, H., Terao, Y., Takamura, T., Wakabyashi, K., Kinae, N., 2004. Genotoxicity of 2-[2-(acetyl amino)-4-[2-bis (hydroxyethyl)]-5methoxyphenyl]-5-amino-7-bromo-4-chloro-2H benzoate (PBTA-6) and 4-amino-3,3,dichloro-5,4,dinitro-biphenyl (ADDB) in goldfish (*Carassius auratus*) using the micronucleus test and comet assay. Mutat. Res. 560, 33–40.

Meister, A., Andersen, M.E., 1983. Glutathione. Ann. Rev. Biochem. 52, 711–760.

Mitchelmore, C.L., Chipman, J.K., 1998. DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. Mutat. Res. 399, 135–147.

Miyamae, Y., Zaizen, K., Obara, K., Mine, Y., Sasaki, Y.F., 1997. Detection of DNA lesions induced by chemical mutagens by the single cell gel electrophoresis (comet assay) and relationship of onset of DNA damage and the characteristics mutagens. Mutat. Res. 393, 99–106.

Muscarella, D.E., Keown, J.F., Bloom, S.E., 1984. Evaluation of the genotoxic and embryotoxic potential of chlorpyrifos and its metabolites *in vivo* and *in vitro*. Environ. Mutagen. 6, 13–23.

Muto, M.A., Lobelle Jr., F., Bidanset, J.H., Wurpel, J.N., 1992. Embryotoxicity and neurotoxicity in rats associated with prenatal exposure to DURSBAN. Vet. Hum. Toxicol. 34, 498–501.

Norppa, H., Falck, G.C.-M., 2003. What do human micronuclei contain? Mutagenesis 18, 221–233.

Patnaik, K., Tripathy, N.K., 1992. Farm grade chloropyrifos (Dermot) is genotoxic in somatic and germ-line cells of *Drosophila*. Mutat. Res. 279, 15–20.

Porichha, S.K., Sarangi, P.K., Prasad, R., 1998. Genotoxic effect of chlorpyrifos in *Channa punctatus*. Pres. Cytol. Genet. 9, 631–638.

Rahman, M.F., Mahboob, M., Danadevi, K., Banu, B.S., Grover, P., 2002. Assessment of genotoxic effect of chlorpyrifos and acephate by the comet assay in mice leukocyte. Mutat. Res. 516, 139–147.

Rank, J., Jensen, K., 2003. Comet assay on gill and hemocytes from the blue mussel *Mytilus edulis*. Ecotoxicol. Environ. Saf. 54, 323–329.

Sasaki, Y.F., Izumiyama, F., Nishidate, E., Ishibashi, S., Tsuda, S., Matsusaka, N., Asano, N., Saotome, K., Sofuni, T., Hayashi, M., 1997. Detection of genotoxicity of polluted seawater using shellfish and alkaline single-cell gel electrophoresis (SCGE) assay: a preliminary study. Mutat. Res. 393, 133–139.

Schimmel, S.C., Garnas, R.L., Patrick, J.M., Moore, J.C., 1983. Acute toxicity, bioconcentration, and persistence of AC 222,705, benthiocarb, chlorpyrifos, fenvalerate, methyl parathion, and permethrin in the estuarine environment. J. Agric. Food Chem. 31, 104–113.

Schmid, W., 1975. The micronucleus test. Mutat. Res. 31, 9–15.

Siboulet, R., Grinfeld, P., Deparis, P., Jaylet, A., 1984. Micronuclei in red blood cells of the new *Pleurodeles waltl* Michah: induction with X-rays and chemicals. Mutat. Res. 125, 275–281.

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantification of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 184–191.

Song, X., Violin, J.D., Seidler, F.J., Slotkin, T.A., 1998. Modelling the developmental neurotoxicity of chlorpyrifos *in vitro*: macromolecule synthesis in PC12 cells. Toxicol. Appl. Pharmacol. 151, 182–191.

Thrasher, J.D., Madison, R., Broughton, A., 1993. Immunologic abnormalities in humans exposed to chlorpyrifos: preliminary observations. Arch. Environ. Health 48, 89–93.

Tice, R.R., 1995. The Single cell gel/comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cells. In: Phillips, D.H., Venitt, S. (Eds.), Environmental Mutagenesis. Bios Scientific Publishers, Oxford, UK, pp. 315–339.

Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H.Y., Miyamae, R.E., Ryu, J.C., Sasaki, Y.F., 2000. Single cell gel/comet assay: guidelines for *in vitro* genetic toxicology testing. Environ. Mol. Mutagen. 35, 206–221.

Tucker, J.D., Preston, R.J., 1996. Chromosome aberrations, micronuclei, aneuploidy, sister chromatid exchanges and cancer risk assessment. Mutat. Res. 365, 147–159.

USDA, 1994. Agriculture Chemical Usage Field Crop Study. US Department of Agriculture, Washington, DC, pp. 396–432.

Whitney, K.D., Seidler, F.J., Slotkin, T.A., 1995. Developmental neurotoxicity of chlorpyrifos: cellular mechanisms. Toxicol. Appl. Pharmacol. 134, 53–62.

Wild, D., 1975. Mutagenicity studies on organophosphorus insecticides. Mutat. Res. 32, 133–150.

Wong, C.K.C., Yeung, H.Y., Woo, P.S., Wong, M.H., 2001. Specific expression of cytochrome P4501A1 gene in gill, intestine and liver of Tilapia exposed to coastal sediments. Aquatic Toxicol. 54, 69–80.

Yamano, T., Morita, S., 1995. Effects of pesticides on isolated hepatocytes, mitochondria and microsomes II. Arch. Environ. Contam. Toxicol. 28, 1–7.

Yasmashita, M., Tanaka, J., Ando, Y., 1997. Human mortality in organophosphate poisonings. Vet. Hum. Toxicol. 39, 84–85.

March 5, 2021

OPP Docket # EPA-HQ-OPP-2008-0850-0750
Environmental Protection Agency Docket Center (EPA/DC)
1200 Pennsylvania Ave. NW
Washington, DC 20460-0001

Subject: Letter Urging Expeditious Action to Ban Chlorpyrifos

The undersigned 101 farmworker, public health, environmental, labor, and faith organizations urge the EPA to immediately revoke all food tolerances for chlorpyrifos and initiate the cancellation process to end all uses of this neurotoxic pesticide.

Chlorpyrifos, which belongs to a nerve-agent class of pesticides called organophosphates (OPs), is used on an extensive variety of crops and is acutely toxic and associated with neurodevelopmental harms in children. Yet, in its proposed interim registration review decision, the EPA is proposing to allow 11 food uses of chlorpyrifos to continue at the urging of industry.

Peer-reviewed studies and EPA's own Scientific Advisory Panel have demonstrated that chlorpyrifos damages children's brains; prenatal exposure to very low levels of chlorpyrifos — levels far lower than what EPA used to set regulatory limits — harms babies permanently. Studies show that exposure to chlorpyrifos, and other OP pesticides during pregnancy, is associated with lower birth weight, attention deficit disorders, autism spectrum disorder, reduced IQ, and loss of working memory.[1] It is also unsafe for workers even with the most protective equipment.

In 2014, EPA released a risk assessment finding unsafe drinking water contamination from chlorpyrifos and it proposed to ban chlorpyrifos from food in 2015. In 2016, EPA released a revised human health risk assessment, which confirmed that exposures to chlorpyrifos are unsafe whether in food, pesticide drift, or drinking water; toddlers were being exposed to levels 140 times what is considered safe in food and all drinking water exposures were found to be unsafe. But in 2020, EPA released a new risk assessment, which abandoned attempts to protect children from the low-level exposures that damage their brains.

Under the law, EPA must find reasonable certainty of no harm to children from pesticides. It cannot make this finding for any use of chlorpyrifos on food. The only outcome that protects our children and complies with the law is to revoke all food tolerances and end all food uses as soon as possible. The 2015 proposed tolerance revocation would have prohibited chlorpyrifos on food six months after the rule became final. EPA should adhere to that timetable.

---

[1] See: "Brain anomalies in children exposed prenatally to a common organophosphate pesticide," Rauh et. al., *Proceedings of the National Academy of Sciences of the United States of America*, reviewed for review February 27, 2012. Viewable: https://www.pnas.org/content/pnas/109/20/7871.full.pdf (last visited 1/29/21)
"Prenatal exposure to organophosphate pesticides and functional neuroimaging in adolescents living in proximity to pesticide application," Sagiv et. al., *Proceedings of the National Academy of Sciences of the United States of America,* received for review March 6, 2019. Viewable: https://www.pnas.org/content/116/37/18347 (last visited 1/29/21)

Ending use of chlorpyrifos on food will protect the farmworkers who grow that food. However, chlorpyrifos is also used in other ways that expose workers to extremely dangerous amounts of the pesticide. For example, chlorpyrifos is used in greenhouses on ornamental plants. The greenhouse workers face unconscionable risks. And under EPA's 2020 risk assessment and proposed decision, the agency finds that the workers who mix and apply chlorpyrifos will face unsafe exposures from more than 100 tasks; workers who re-enter fields sprayed with chlorpyrifos will be at risk as well.

The EPA is proposing to allow these risks to continue because of the economic benefits of using chlorpyrifos compared to other currently available chemical pesticide alternatives. In making this proposal, EPA is ignoring non-chemical methods of pest control as well as the economic costs and hardships caused by pesticide poisonings, learning disabilities, reduced IQ in children, and environmental harm from chlorpyrifos use; this pesticide also contaminates surface water and harms threatened and endangered species, including birds, Pacific salmon, Southern Resident Killer Whales, and other mammals.

For the workers, the EPA is considering requiring more protective clothing and gear, but those measures can cause heat stress in many regions where workers toil in hot temperatures. And the agency is proposing to afford farmworkers less protection than industrial workers where personal protective equipment is the last resort, employed only if the exposures cannot be prevented.

When the Trump EPA derailed the proposed tolerance revocation, states like Hawaii, California, Oregon, and New York stepped in. In California, 99% of chlorpyrifos uses are now banned. While states can end use of chlorpyrifos, they cannot prevent residues of chlorpyrifos on their food. Only the EPA can do that. All Americans need the EPA to do its job and ensure our food is safe for children.

We, therefore, urge the EPA to immediately:

1. **Revoke the 2019 final order[2] that denied the 2007 petition to ban food uses of chlorpyrifos;[3]**
2. **Grant the 2007 petition;**
3. **Finalize the 2015 proposed order revoking all chlorpyrifos food tolerances;[4] and**
4. **Initiate the cancellation process for all uses of chlorpyrifos.**

The proposed interim decision on chlorpyrifos fails to protect the health of workers and children from a pesticide that is widely recognized as unsafe. Only banning the pesticide can truly protect children, workers, and the environment.

Signed,

ActionAid USA
American Bird Conservancy
American Public Health Association
Association of Farmworker Opportunity Programs
Beyond Pesticides

[2] 84 Fed. Reg. 35555 (Jul. 24, 2019)
[3] 72 Fed. Reg. 58845 (Oct. 17, 2007)
[4] 80 Fed. Reg. 69079 (Nov. 6, 2015)

Center for an Ecology-Based Economy
Center for Biological Diversity
Center for Energy & Environmental Education
Center for Food Safety
Child Labor Coalition
Citizens Campaign for the Environment
Clean Water Action/Clean Water Fund
Community Action Works
Conservation Law Foundation
CREA: Center for Reflection, Education and Action
CRLA Foundation
Defend Our Health
Earth Action, Inc.
Earth Ethics, Inc.
Earthjustice
East Yard Communities for Environmental Justice
Endangered Species Coalition
Environment Maine
Environmental Advocates NY
Experimental Farm Network
Farmworker and Landscaper Advocacy Project
Farmworker Association of Florida
Farmworker Justice
Fayetteville PACT
Fertile Acres
First Focus on Children
Food & Water Watch
Food Empowerment Project
Four Harbors Audubon Society
FreshWater Accountability Project
Friends of the Earth
Genesee Valley Audubon Society
Georgia STAND-UP
Global Labor Justice - International Labor Rights Forum
Green America
Green Inside and Out
Human Rights Watch
Huntington Breast Cancer Action Coalition
International Corporate Accountability Roundtable (ICAR)
International Initiative to End Child Labor
Justice for Migrant Women
Labor Council for Latin American Advancement
LEAD for Pollinators., Inc.
League of Conservation Voters
League of United Latin American Citizens (LULAC)
Learning Disabilities Association of America
Learning Disabilities Association of Arkansas
Learning Disabilities Association of Connecticut

Learning Disabilities Association of Georgia
Learning Disabilities Association of Illinois
Learning Disabilities Association of Maine
Learning Disabilities Association of Minnesota
Learning Disabilities Association of New Jersey
Learning Disabilities Association of Pennsylvania
Learning Disabilities Association of South Carolina
Learning Disabilities Association of Tennessee
Learning Disabilities Association of Texas
Learning disabilities association of Utah
Learning Disabilities Association of Wisconsin
Maine Audubon
Maine Conservation Voters
Maine Organic Farmers and Gardeners Association
Maine Unitarian Universalist State Advocacy Network
maine*taine
Maryland Pesticide Education Network
Media Voices for Children
Mighty Earth
Migrant Clinicians Network
MOM's Organic Market
National Consumers League
National Nurses United
Natural Resources Defense Council
NC Farmworkers' Project
New Mexico Center on Law and Poverty
Northeast Organic Farming Association -- New York (NOFA-NY)
Northwest Center for Alternatives to Pesticides
Northwest Workers' Justice Project
NYC Audubon
Operation Splash
Oregon Physicians for Social Responsibility
Pesticide Action Network
Physicians for Social Responsibility
Physicians for Social Responsibility Maine Chapter
Pineros y Campesinos Unidos del Noroeste (PCUN)
Public Citizen
Rachel Carson Council
RESTORE: The North Woods
Santa Cruz Climate Action Network
Sierra Club
Student Action with Farmworkers
The Oakland Institute
The Xerces Society for Invertebrate Conservation
Toxic Free North Carolina
Union of Concerned Scientists
UFW Foundation
Virginia Association for Biological Farming

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™

FROM THE AMERICAN ACADEMY OF PEDIATRICS

Organizational Principles to Guide and Define the Child Health Care System and/or Improve the Health of all Children

POLICY STATEMENT

Pesticide Exposure in Children

COUNCIL ON ENVIRONMENTAL HEALTH

KEY WORDS
pesticides, toxicity, children, pest control, integrated pest management

ABBREVIATIONS
EPA—Environmental Protection Agency
IPM—integrated pest management

www.pediatrics.org/cgi/doi/10.1542/peds.2012-2757

doi:10.1542/peds.2012-2757

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

abstract

FREE

This statement presents the position of the American Academy of Pediatrics on pesticides. Pesticides are a collective term for chemicals intended to kill unwanted insects, plants, molds, and rodents. Children encounter pesticides daily and have unique susceptibilities to their potential toxicity. Acute poisoning risks are clear, and understanding of chronic health implications from both acute and chronic exposure are emerging. Epidemiologic evidence demonstrates associations between early life exposure to pesticides and pediatric cancers, decreased cognitive function, and behavioral problems. Related animal toxicology studies provide supportive biological plausibility for these findings. Recognizing and reducing problematic exposures will require attention to current inadequacies in medical training, public health tracking, and regulatory action on pesticides. Ongoing research describing toxicologic vulnerabilities and exposure factors across the life span are needed to inform regulatory needs and appropriate interventions. Policies that promote integrated pest management, comprehensive pesticide labeling, and marketing practices that incorporate child health considerations will enhance safe use. *Pediatrics* 2012;130:e1757–e1763

INTRODUCTION

Pesticides represent a large group of products designed to kill or harm living organisms from insects to rodents to unwanted plants or animals (eg, rodents), making them inherently toxic (Table 1). Beyond acute poisoning, the influences of low-level exposures on child health are of increasing concern. This policy statement presents the position of the American Academy of Pediatrics on exposure to these products. It was developed in conjunction with a technical report that provides a thorough review of topics presented here: steps that pediatricians should take to identify pesticide poisoning, evaluate patients for pesticide-related illness, provide appropriate treatment, and prevent unnecessary exposure and poisoning.[1] Recommendations for a regulatory agenda are provided as well, recognizing the role of federal agencies in ensuring the safety of children while balancing the positive attributes of pesticides. Repellents reviewed previously (eg, N,N-diethyl-meta-toluamide, commonly known as DEET; picaridin) are not discussed.[2]

SOURCES AND MECHANISMS OF EXPOSURE

Children encounter pesticides daily in air, food, dust, and soil and on surfaces through home and public lawn or garden application, household insecticide use, application to pets, and agricultural product

TABLE 1 Categories of Pesticides and Major Classes

Pesticide category	Major Classes	Examples
Insecticides	Organophosphates	Malathion, methyl parathion, acephate
	Carbamates	Aldicarb, carbaryl, methomyl, propoxur
	Pyrethroids/pyrethrins	Cypermethrin, fenvalerate, permethrin
	Organochlorines	Lindane
	Neonicotinoids	Imidacloprid
	N-phenylpyrazoles	Fipronil
Herbicides	Phosphonates	Glyphosate
	Chlorophenoxy herbicides	2,4-D, mecoprop
	Dipyridyl herbicides	Diquat, paraquat
	Nonselective	Sodium chlorate
Rodenticides	Anticoagulants	Warfarin, brodifacoum
	Convulsants	Strychnine
	Metabolic poison	Sodium fluoroacetate
	Inorganic compounds	Aluminum phosphide
Fungicides	Thiocarbamates	Metam-sodium
	Triazoles	Fluconazole, myclobutanil, triadimefon
	Strobilurins	Pyraclostrobin, picoxystrobin
Fumigants	Halogenated organic	Methyl bromide, Chloropicrin
	Organic	Carbon disulfide, Hydrogen cyanide, Naphthalene
	Inorganic	Phosphine
Miscellaneous	Arsenicals	Lead arsenate, chromated copper arsenate, arsenic trioxide
	Pyridine	4-aminopyridine

residues.[3–9] For many children, diet may be the most influential source, as illustrated by an intervention study that placed children on an organic diet (produced without pesticide) and observed drastic and immediate decrease in urinary excretion of pesticide metabolites.[10] In agricultural settings, pesticide spray drift is important for residences near treated crops or by take-home exposure on clothing and footwear of agricultural workers.[9,11,12] Teen workers may have occupational exposures on the farm or in lawn care.[13–15] Heavy use of pesticides may also occur in urban pest control.[16]

Most serious acute poisoning occurs after unintentional ingestion, although poisoning may also follow inhalational exposure (particularly from fumigants) or significant dermal exposure.[17]

ACUTE PESTICIDE TOXICITY

Clinical Signs and Symptoms

High-dose pesticide exposure may result in immediate, devastating, even lethal consequences. Table 2 summarizes features of clinical toxicity for the major pesticides classes. It highlights the similarities of common classes of pesticides (eg, organophosphates, carbamates, and pyrethroids) and underscores the importance of discriminating among them because treatment modalities differ. Having an index of suspicion based on familiarity with toxic mechanisms and taking an environmental history provides the opportunity for discerning a pesticide's role in clinical decision-making.[18] Pediatric care providers have a poor track record for recognition of acute pesticide poisoning.[19–21] This reflects their self-reported lack of medical education and self-efficacy on the topic.[22–26] More in-depth review of acute toxicity and management can be found in the accompanying technical report or recommended resources in Table 3.

The local or regional poison control center plays an important role as a resource for any suspected pesticide poisoning.

There is no current reliable way to determine the incidence of pesticide exposure and illness in US children. Existing data systems, such as the American Association of Poison Control Centers' National Poison Data System or the National Institute for Occupational Safety and Health's Sentinel Event Notification System for Occupational Risks,[27,28] capture limited information about acute poisoning and trends over time.

There is also no national systematic reporting on the use of pesticides by consumers or licensed professionals. The last national survey of consumer pesticide use in homes and gardens was in 1993 (Research Triangle Institute study).[29]

Improved physician education, accessible and reliable biomarkers, and better diagnostic testing methods to readily identify suspected pesticide illness would significantly improve reporting and surveillance. Such tools would be equally important in improving clinical decision-making and reassuring families if pesticides can be eliminated from the differential diagnosis.

The Pesticide Label

The pesticide label contains information for understanding and preventing acute health consequences: the active ingredient; signal words identifying acute toxicity potential; US Environmental Protection Agency (EPA) registration number; directions for use, including protective equipment recommendations, storage, and disposal; and manufacturer's contact information.[30] Basic first aid advice is provided, and some labels contain a "note for physicians" with specific relevant medical information. The label does not specify the pesticide class or "other"/"inert" ingredients that may have significant toxicity and can account for up to 99% of the product.

Chronic toxicity information is not included, and labels are predominantly available in English. There is significant use of illegal pesticides (especially in immigrant communities), off-label use, and overuse, underscoring the importance of education, monitoring, and enforcement.[31]

TABLE 2 Common Pesticides: Signs, Symptoms, and Management Considerations[a]

Class	Acute Signs and Symptoms	Clinical Considerations
Organophosphate and N-methyl carbamate insecticides	• Headache, nausea, vomiting, abdominal pain, and dizziness • Hypersecretion: sweating, salivation, lacrimation, rhinorrhea, diarrhea, and bronchorrhea • Muscle fasciculation and weakness, and respiratory symptoms (bronchospasm, cough, wheezing, and respiratory depression) • Bradycardia, although early on, tachycardia may be present • Miosis • Central nervous system: respiratory depression, lethargy, coma, and seizures	• Obtain red blood cell and plasma cholinesterase levels • Atropine is primary antidote • Pralidoxime is also an antidote for organophosphate and acts as a cholinesterase reactivator • Because carbamates generally produce a reversible cholinesterase inhibition, pralidoxime is not indicated in these poisonings
Pyrethroid insecticides	• Similar findings found in organophosphates including the hypersecretion, muscle fasciculation, respiratory symptoms, and seizures • Headache, fatigue, vomiting, diarrhea, and irritability • Dermal: skin irritation and paresthesia	• At times have been mistaken for acute organophosphate or carbamate poisoning • Symptomatic treatment • Treatment with high doses of atropine may yield significant adverse results • Vitamin E oil for dermal symptoms
Neonicotinoid insecticides	• Disorientation, severe agitation, drowsiness, dizziness, weakness, and in some situations, loss of consciousness • Vomiting, sore throat, abdominal pain • Ulcerations in upper gastrointestinal tract	• Supportive care • Consider sedation for severe agitation • No available antidote • No available diagnostic test
Fipronil (N-phenylpyrazole insecticides)	• Nausea and vomiting • Aphthous ulcers • Altered mental status and coma • Seizures	• Supportive care • No available antidote • No available diagnostic test
Lindane (organochlorine insecticide)	• Central nervous system: mental status changes and seizures • Paresthesia, tremor, ataxia and hyperreflexia	• Control acute seizures with lorazepam • Lindane blood level available as send out
Glyphosate (phosphonate herbicides)	• Nausea and vomiting • Aspiration pneumonia type syndrome • Hypotension, altered mental status, and oliguria in severe cases • Pulmonary effects may in fact be secondary to organic solvent	• Supportive care • Pulmonary effects may be secondary to organic solvent
Chlorophenoxy herbicides	• Skin and mucous membrane irritation • Vomiting, diarrhea, headache, confusion • Metabolic acidosis is the hallmark • Renal failure, hyperkalemia, and hypocalcemia • Probable carcinogen	• Consider urine alkalinization with sodium bicarbonate in IV fluids
Rodenticides (long-acting anticoagulants)	• Bleeding: gums, nose, and other mucous membrane sites • Bruising	• Consider PT (international normalized ratio) • Observation may be appropriate for some clinical scenarios in which it is not clear a child even ingested the agent • Vitamin K indicated for active bleeding (IV vitamin K) or for elevated PT (oral vitamin K)

IV, intravenous; PT, prothrombin time.

[a] Expanded version of this table is available in the accompanying technical report.[1]

CHRONIC EFFECTS

Dosing experiments in animals clearly demonstrate the acute and chronic toxicity potential of multiple pesticides. Many pesticide chemicals are classified by the US EPA as carcinogens. The past decade has seen an expansion of the epidemiologic evidence base supporting adverse effects after acute and chronic pesticide exposure in children. This includes increasingly sophisticated studies addressing combined exposures and genetic susceptibility.[1]

Chronic toxicity end points identified in epidemiologic studies include adverse birth outcomes including preterm birth, low birth weight, and congenital

TABLE 3 Pesticide and Child Health Resources for the Pediatrician

Topic/Resource	Additional Information	Contact Information
Management of acute pesticide poisoning		
Recognition and Management of Pesticide Poisonings	Print: fifth (1999) is available in Spanish, English; 6th edition available 2013	http://www.epa.gov/pesticides/safety/healthcare/handbook/handbook.htm
Regional Poison Control Centers		1 (800) 222-1222
Chronic exposure information and specialty consultation		
The National Pesticide Medical Monitoring Program (NPMMP)	Cooperative agreement between Oregon State University and the US EPA. NPMMP provides informational assistance by E-mail in the assessment of human exposure to pesticides	npmmp@oregonstate.edu or by fax at (541) 737-9047
Pediatric Environmental Health Specialty Units (PEHSUs)	Coordinated by the Association of Occupational and Environmental Clinics to provide regional academically based free consultation for health care providers	www.aoec.org/PEHSU.htm; toll-free telephone number (888) 347-AOEC (extension 2632)
Resources for safer approaches to pest control		
US EPA	Consumer information documents	www.epa.gov/oppfead1/Publications/Cit_Guide/citguide.pdf
Citizens Guide to Pest Control and Pesticide Safety	• Household pest control • Alternatives to chemical pesticides • How to choose pesticides • How to use, store, and dispose of them safely • How to prevent pesticide poisoning • How to choose a pest-control company	
Controlling pests	Recommended safest approaches and examples of programs	www.epa.gov/pesticides/controlling/index.htm
The University of California Integrative Pest Management Program	Information on IPM approaches for common home and garden pests	www.ipm.ucdavis.edu
Other resources		
National research programs addressing children's health and pesticides	• NIEHS/EPA Centers for Children's Environmental Health & Disease Prevention Research • The National Children's Study	www.niehs.nih.gov/research/supported/centers/prevention www.nationalchildrensstudy.gov/Pages/default.aspx
US EPA	Pesticide product labels	www.epa.gov/pesticides/regulating/labels/product-labels.htm#projects
The National Library of Medicine "Tox Town"	Section on pesticides that includes a comprehensive and well-organized list of web link resources on pesticides	http://toxtown.nlm.nih.gov/text_version/chemicals.php?id=23

anomalies, pediatric cancers, neurobehavioral and cognitive deficits, and asthma. These are reviewed in the accompanying technical report. The evidence base is most robust for associations to pediatric cancer and adverse neurodevelopment. Multiple case-control studies and evidence reviews support a role for insecticides in risk of brain tumors and acute lymphocytic leukemia. Prospective contemporary birth cohort studies in the United States link early-life exposure to organophosphate insecticides with reductions in IQ and abnormal behaviors associated with attention-deficit/hyperactivity disorder and autism. The need to better understand the health implications of ongoing pesticide use practices on child health has benefited from these observational epidemiologic data.[32]

EXPOSURE PREVENTION APPROACHES

The concerning and expanding evidence base of chronic health consequences of pesticide exposure underscores the importance of efforts aimed at decreasing exposure.

Integrated pest management (IPM) is an established but undersupported approach to pest control designed to minimize and, in some cases, replace the use of pesticide chemicals while achieving acceptable control of pest populations.[33] IPM programs and knowledge have been implemented in agriculture and to address weeds and pest control in residential settings and schools, commercial structures, lawn and turf, and community gardens. Reliable resources are available from the US EPA and University of California—Davis (Table 3). Other local policy approaches in use are posting warning signs of pesticide use, restricting spray zone buffers at schools, or restricting specific types of pesticide products in schools. Pediatricians can

play a role in promotion of development of model programs and practices in the communities and schools of their patients.

RECOMMENDATIONS

Three overarching principles can be identified: (1) pesticide exposures are common and cause both acute and chronic effects; (2) pediatricians need to be knowledgeable in pesticide identification, counseling, and management; and (3) governmental actions to improve pesticide safety are needed. Whenever new public policy is developed or existing policy is revised, the wide range of consequences of pesticide use on children and their families should be considered. The American Academy of Pediatrics, through its chapters, committees, councils, sections, and staff, can provide information and support for public policy advocacy efforts. See http://www.aap.org/advocacy.html for additional information or contact chapter leadership.

Recommendations to Pediatricians

1. Acute exposures: become familiar with the clinical signs and symptoms of acute intoxication from the major types of pesticides. Be able to translate clinical knowledge about pesticide hazards into an appropriate exposure history for pesticide poisoning.
2. Chronic exposures: become familiar with the subclinical effects of chronic exposures and routes of exposures from the major types of pesticides.
3. Resource identification: know locally available resources for acute toxicity management and chronic low-dose exposure (see Table 3).
4. Pesticide labeling knowledge: Understand the usefulness and limitations of pesticide chemical information on pesticide product labels.
5. Counseling: Ask parents about pesticide use in or around the home to help determine the need for providing targeted anticipatory guidance. Recommend use of minimal-risk products, safe storage practices, and application of IPM (least toxic methods), whenever possible.
6. Advocacy: work with schools and governmental agencies to advocate for application of least toxic pesticides by using IPM principles. Promote community right-to-know procedures when pesticide spraying occurs in public areas.

Recommendations to Government

1. Marketing: ensure that pesticide products as marketed are not attractive to children.
2. Labeling: include chemical ingredient identity on the label and/or the manufacturer's Web site for all product constituents, including inert ingredients, carriers, and solvents. Include a label section specific to "Risks to children," which informs users whether there is evidence that the active or inert ingredients have any known chronic or developmental health concerns for children. Enforce labeling practices that ensure users have adequate information on product contents, acute and chronic toxicity potential, and emergency information. Consider printing or making available labels in Spanish in addition to English.
3. Exposure reduction: set goal to reduce exposure overall. Promote application methods and practices that minimize children's exposure, such as using bait stations and gels, advising against overuse of pediculicides. Promote education regarding proper storage of product.
4. Reporting: make pesticide-related suspected poisoning universally reportable and support a systematic central repository of such incidents to optimize national surveillance.
5. Exportation: aid in identification of least toxic alternatives to pesticide use internationally, and unless safer alternatives are not available or are impossible to implement, ban export of products that are banned or restricted for toxicity concerns in the United States.
6. Safety: continue to evaluate pesticide safety. Enforce community right-to-know procedures when pesticide spraying occurs in public areas. Develop, strengthen, and enforce standards of removal of concerning products for home or child product use. Require development of a human biomarker, such as a urinary or blood measure, that can be used to identify exposure and/or early health implications with new pesticide chemical registration or reregistration of existing products. Developmental toxicity, including endocrine disruption, should be a priority when evaluating new chemicals for licensing or reregistration of existing products.
7. Advance less toxic pesticide alternatives: increase economic incentives for growers who adopt IPM, including less toxic pesticides. Support research to expand and improve IPM in agriculture and nonagricultural pest control.
8. Research: support toxicologic and epidemiologic research to better identify and understand health risks associated with children's exposure to pesticides. Consider supporting another national study of pesticide use in the home and garden setting of US households as a targeted initiative or through cooperation with existing research opportunities (eg, National Children's Study, NHANES).
9. Health provider education and support: support educational efforts to increase the capacity of pediatric health care providers to diagnose and manage acute pesticide

poisoning and reduce pesticide exposure and potential chronic pesticide effects in children. Provide support to systems such as Poison Control Centers to provide timely, expert advice on exposures. Require the development of diagnostic tests to assist providers with diagnosing (and ruling out) pesticide poisoning.

LEAD AUTHORS

James R. Roberts, MD, MPH
Catherine J. Karr, MD, PhD

COUNCIL ON ENVIRONMENTAL HEALTH EXECUTIVE COMMITTEE, 2012–2013

Jerome A. Paulson, MD, Chairperson
Alice C. Brock-Utne, MD
Heather L. Brumberg, MD, MPH
Carla C. Campbell, MD
Bruce P. Lanphear, MD, MPH
Kevin C. Osterhoudt, MD, MSCE
Megan T. Sandel, MD
Leonardo Trasande, MD, MPP
Robert O. Wright, MD, MPH

FORMER EXECUTIVE COMMITTEE MEMBERS

Helen J. Binns, MD, MPH
James R. Roberts, MD, MPH
Catherine J. Karr, MD, PhD
Joel A. Forman, MD
James M. Seltzer, MD

LIAISONS

Mary Mortensen, MD – *Centers for Disease Control and Prevention/National Center for Environmental Health*
Walter J. Rogan, MD – *National Institute of Environmental Health Sciences*
Sharon Savage, MD – *National Cancer Institute*

STAFF

Paul Spire

REFERENCES

1. Roberts JR, Karr CK; American Academy of Pediatrics, Council on Environmental Health. Technical report—pesticide exposure in children. *Pediatrics*. 2012:130(6)
2. Katz TM, Miller JH, Hebert AA. Insect repellents: historical perspectives and new developments. *J Am Acad Dermatol*. 2008; 58(5):865–871
3. Lewis RG, Fortune CR, Blanchard FT, Camann DE. Movement and deposition of two organophosphorus pesticides within a residence after interior and exterior applications. *J Air Waste Manag Assoc*. 2001;51(3):339–351
4. Hore P, Robson M, Freeman N, et al. Chlorpyrifos accumulation patterns for child-accessible surfaces and objects and urinary metabolite excretion by children for 2 weeks after crack-and-crevice application. *Environ Health Perspect*. 2005;113 (2):211–219
5. Gurunathan S, Robson M, Freeman N, et al. Accumulation of chlorpyrifos on residential surfaces and toys accessible to children. *Environ Health Perspect*. 1998;106(1):9–16
6. Fenske RA, Black KG, Elkner KP, Lee CL, Methner MM, Soto R. Potential exposure and health risks of infants following indoor residential pesticide applications. *Am J Public Health*. 1990;80(6):689–693
7. Nishioka MG, Lewis RG, Brinkman MC, Burkholder HM, Hines CE, Menkedick JR. Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: comparing exposure estimates from various media for young children. *Environ Health Perspect*. 2001;109(11):1185–1191
8. Coronado GD, Vigoren EM, Thompson B, Griffith WC, Faustman EM. Organophosphate pesticide exposure and work in pome fruit: evidence for the take-home pesticide pathway. *Environ Health Perspect*. 2006;114(7):999–1006
9. Lu C, Fenske RA, Simcox NJ, Kalman D. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. *Environ Res*. 2000;84(3): 290–302
10. Lu C, Toepel K, Irish R, Fenske RA, Barr DB, Bravo R. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. *Environ Health Perspect*. 2006;114(2):260–263
11. Curl CL, Fenske RA, Kissel JC, et al. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. *Environ Health Perspect*. 2002;110(12):A787–A792
12. Curwin BD, Hein MJ, Sanderson WT, et al. Pesticide contamination inside farm and nonfarm homes. *J Occup Environ Hyg*. 2005; 2(7):357–367
13. Shipp EM, Cooper SP, del Junco DJ, Bolin JN, Whitworth RE, Cooper CJ. Pesticide safety training among adolescent farmworkers from Starr County, Texas. *J Agric Saf Health*. 2007;13(3):311–321
14. Gamlin J, Diaz Romo P, Hesketh T. Exposure of young children working on Mexican tobacco plantations to organophosphorous and carbamic pesticides, indicated by cholinesterase depression. *Child Care Health Dev*. 2007;33(3):246–248
15. Eckerman DA, Gimenes LA, de Souza RC, Lopes Galvão PR, Sarcinelli PN, Chrisman JR. Age related effects of pesticide exposure on neurobehavioral performance of adolescent farm workers in Brazil. *Neurotoxicol Teratol*. 2007;29(1):164–175
16. Landrigan PJ, Claudio L, Markowitz SB, et al. Pesticides and inner-city children: exposures, risks, and prevention. *Environ Health Perspect*. 1999;107(suppl 3):431–437
17. Reigart JR, Roberts JR. *Recognition and Management of Pesticide Poisoning*, 5th ed. Washington, DC: US Environmental Protection Agency; 1999
18. American Academy of Pediatrics, Committee on Environmental Health. Taking an environmental history and giving anticipatory guidance. In: Etzel RA, Balk SJ, eds. *Pediatric Environmental Health*. 2nd ed. Elk Grove Village, IL: American Academy of Pediatrics; 2003:39–56
19. Sofer S, Tal A, Shahak E. Carbamate and organophosphate poisoning in early childhood. *Pediatr Emerg Care*. 1989;5(4):222–225
20. Zwiener RJ, Ginsburg CM. Organophosphate and carbamate poisoning in infants and children. *Pediatrics*. 1988;81(1):121–126
21. Lifshitz M, Shahak E, Sofer S. Carbamate and organophosphate poisoning in young children. *Pediatr Emerg Care*. 1999;15(2): 102–103
22. Balbus JM, Harvey CE, McCurdy LE. Educational needs assessment for pediatric health care providers on pesticide toxicity. *J Agromed*. 2006;11(1):27–38
23. Kilpatrick N, Frumkin H, Trowbridge J, et al. The environmental history in pediatric practice: a study of pediatricians' attitudes, beliefs, and practices. *Environ Health Perspect*. 2002;110(8):823–871
24. Trasande L, Schapiro ML, Falk R, et al. Pediatrician attitudes, clinical activities, and knowledge of environmental health in Wisconsin. *WMJ*. 2006;105(2):45–49
25. Karr C, Murphy H, Glew G, Keifer MC, Fenske RA. Pacific Northwest health professionals survey on pesticides and children. *J Agromed*. 2006;11(3-4):113–120

26. Roberts JR, Balk SJ, Forman J, Shannon M. Teaching about pediatric environmental health. *Acad Pediatr*. 2009;9(2):129–130
27. Bronstein AC, Spyker DA, Cantilena LR Jr, Green JL, Rumack BH, Dart RC. 2010 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 26th Annual Report. *Clin Toxicol*. 2011;49(10):910–941
28. Baker EL. Sentinel Event Notification System for Occupational Risks (SENSOR): the concept. *Am J Public Health*. 1989;79(suppl):18–20
29. Whitmore RW, Kelly JE, Reading PL, et al. Pesticides in urban environments. *ACS Symp Ser*. 1993;522(3):18–36
30. US Environmental Protection Agency. Pesticide product labels. Available at: www.epa.gov/pesticides/regulating/labels/product-labels.htm#projects. Accessed October 15, 2012
31. US Environmental Protection Agency. Illegal pesticide products. Available at: www.epa.gov/opp00001/health/illegalproducts. Accessed October 15, 2012
32. Kimmel CA, Collman GW, Fields N, Eskenazi B. Lessons learned for the National Children's Study from the National Institute of Environmental Health Sciences/U.S. Environmental Protection Agency Centers for Children's Environmental Health and Disease Prevention Research. *Environ Health Perspect*. 2005;113(10):1414–1418
33. US General Accounting Office. Agricultural pesticides: Management improvements needed to further promote integrated pest management. Available at: www.gao.gov/new.items/d01815.pdf. Accessed October 15, 2012

Pesticide Exposure in Children

COUNCIL ON ENVIRONMENTAL HEALTH

Pediatrics 2012;130;e1757
DOI: 10.1542/peds.2012-2757 originally published online November 26, 2012;

PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Pesticide Exposure in Children

COUNCIL ON ENVIRONMENTAL HEALTH

Pediatrics 2012;130;e1757
DOI: 10.1542/peds.2012-2757 originally published online November 26, 2012;

stamford advocate

OPINION: Grass doesn't need to be any greener

Priscilla Feral

June 3, 2020Updated: June 3, 2020 8:24 a.m.

A pesticide warning sign in Norwalk.

Erik Trautmann / Hearst Connecticut Media

As communities try to protect themselves from COVID-19, a respiratory disease that has brought the world to its knees, claiming 106,241 lives in the United States so far, we at Pesticide Free Rowayton were alarmed this weekend to see a bare foot child playing on a lawn doused in pesticides — the yellow sign still posted revealing it was sprayed just three days earlier.

On the same Rowayton Street, a father of two young children was seen spraying Roundup on some weeds that dared to poke through his gravel driveway.

Everyone wants the perfect yard, but at what cost, especially during a pandemic? A recent study revealed workers exposed to pesticides on the job may be more likely than other people to develop chronic obstructive pulmonary disease, bronchitis and other breathing problems, and all of these things make people more susceptible to COVID-19.

Toxic pesticides and herbicides put people, pets, pollinators and wildlife in harm's way. Without bees, butterflies, insects and birds there would be no ecosystems; there would be no us.

Roundup, the most popular weed killer in the world, has as its most active ingredients glyphosate and 2,4-D, which are particularly dangerous. Last year, a jury awarded a couple $2 billion in damages after concluding that sustained exposure to Roundup led to their cancer diagnoses.

The truth is there is no official scientific standard for how long people should stay off a lawn after it is treated. One size does not fit all because different populations — young children, pregnant mothers, the elderly and those who may be immuno-compromised — are more sensitive to pesticide exposure.

A 2013 study that tested dogs found that they had lawn pesticides in their urine for at least 48 hours after spraying. A 2001 study also found that a week

after lawn treatments, 2, 4-D was found on all indoor air surfaces after wafting in through various openings in 13 different homes.

Even if the active ingredient in a pesticide is gone, it may still leave behind breakdown products that can be even more toxic than the active ingredient, according to Beyond Pesticides, a D.C.-based nonprofit. One notable example is the neonicotinoid thiamethoxam, which is registered for home lawn use to treat grubs, and is found in the product Meridian 25WG.

All of this data is inconvenient to the greedy pesticide industry whose “keep off the grass for 24 hours” yellow warning signs mislead the public into thinking pesticides aren’t as deadly as they are.

Of the 30 most commonly used lawn pesticides, 17 are possible or known carcinogens, 18 have the potential to disrupt the hormonal system, 19 are linked to reproductive effects and sexual dysfunction, 11 have been linked to birth defects, 14 are neurotoxic, 24 can cause kidney or liver damage, 25 are irritants, 19 are detected in groundwater and 20 have the ability to leach into drinking water sources.

Likewise, 30 are toxic to fish and other aquatic organisms vital to our ecosystem, 29 are toxic to bees, 14 are toxic to mammals and 22 are toxic to birds.

The good news is it has never been easier to go pesticide free as organic lawn care has become mainstream.

The first step is testing your soil to determine what organic supplements you should add to make it healthy. If your soil is hard, compacted and full of weeds or bare spots, aerate it. Use grass seed on bare spots to crowd out weeds. Apply corn gluten meal as a pre-emergent for weed prone areas. And instead of using toxic herbicides, weeds can be pulled by hand or sprayed with horticultural vinegar.

Rowayton can be a role model for other communities by going pesticide-free one lawn at a time. These unprecedented times have taught us our lives have more meaning when we rescue ourselves and model the leadership and initiatives we hope to see across the state, nation and globe.

Priscilla Feral is president of Friends of Animals, which is based in Darien (friendsofanimals.org/). Pesticide Free Rowayton, a project of Friends of animals, is promoting nontoxic lawns.