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Intersections between neonicotinoid seed treatments and honey bees

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A growing understanding of the often subtle unintended impacts of neonicotinoid seed treatments on both non-target organisms and their environment have led to concerns about the suitability of current pest management approaches in large scale agriculture. Several neonicotinoid compounds are used in seed treatments of the most widely grown grain and oilseed crops worldwide. Most applications are made prophylactically and without prior knowledge of pest populations. A growing body of evidence suggests that these compounds become contaminants of soil, water, and plant products, including pollen and nectar. These unforeseen routes of exposure are documented to have negative impacts on honey bee health and also have potential to exert effects on a broader environmental scale.

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Seed treatments as crop protectants in agriculture

Concerns regarding the unintended consequences of pesticide use have recently received increased attention from researchers and regulatory bodies alike, particularly in the case of the neonicotinoid class of insecticides and their impacts on insect pollinators and ecosystems [1,2••,3,4••]. In the case of many of the principal agronomic crops grown worldwide (including maize, soybeans, wheat, canola, as well as cotton), neonicotinoids are routinely applied to seeds to guard against early season insect pests. In North America alone, these crops represent approximately 115 million hectares of production annually (94.5 million hectares in the United States and 21.5 million in Canada) [5,6]. Notably, this rapid adoption has occurred in the absence of any documented increase in pest threat [7]. The use of neonicotinoids as seed treatments began with the registration of imidacloprid in 1994, and it is now estimated that 60% of applications of neonicotinoid insecticides are delivered via soil or seed treatments [8], often in combination with protectant fungicides. The predominant neonicotinoids used in seed treatment formulations for grain and oilseed crops are thiamethoxam, its metabolite clothianidin, and imidacloprid. Although these formulations can provide crop protection, particularly from aphids and other sucking insects [9], the economic benefits associated with their use have been difficult to quantify in the major cropping systems where they are used, including maize [10–13] and soybeans [14,15]. These compounds also carry risks to beneficial insects and non-target areas surrounding fields both during and after planting. Chemical characteristics of these compounds that are frequently cited as beneficial for pest management include high water solubility that facilitates systemic movement through plant tissues and high persistence in soils. However, these same characteristics can enhance the potential for neonicotinoid active ingredients used in seed treatments to exert impacts on non-target areas and organisms within and beyond both the planted field and cropping season. In the sections below and the attached table, we outline the principal routes through which honey bees and other pollinators may encounter these compounds (Table 1).

Effects on honey bees & ecosystems

Exposure to residues via plant products

A wide range of pesticides (including several neonicotinoids) have been detected in honey bee hive resources including bee-collected pollen, stored pollen (or bee bread) and wax collected from honey bee hives located near commercial agriculture operations [16–21]. In most cases where neonicotinoids have been documented in honey bee or hive products, annual crops grown in the vicinity have been implicated as the likely source. This may be due to deposition of contaminated soil or planting dust upon bees, plants, or both. However, many crop plants grown from treated seeds express neonicotinoid residues in pollen or nectar, which poses exposure risks to honey bees via their food resources. Pollen loads from honey bee hives placed adjacent to oilseed rape grown from thiamethoxam or clothianidin-treated seeds in Poland have shown mean residue concentrations of these active ingredients in pollen to be 6.6 parts per billion (ppb) and 0.6 ppb respectively [22]. Imidacloprid concentrations ranging between 1.1 and 5.7 ppb have been detected in honey bee-collected pollen loads in France [16,18], while thiamethoxam and clothianidin

Table 1

Summary of published literature documenting exposure routes and concentrations of neonicotinoids found in environmental matrices encountered by honey bee foragers. All concentrations are reported in parts per billion (ppb).

Exposure route	Neonicotinoids/metabolites detected	Time in season	Conc. reported in matrices	Reference
Dust	Imidacloprid	Mid-March to May	Mean: 21 (grass) Mean: 32 (flowers)	Greatti *et al*. [30]
	Imidacloprid	Mid-March to May	40–58 (grass) 22–123 (flowers)	Greatti *et al*. [33]
	Clothianidin & imidacloprid	Mid-March to May	29–3661 ng/bee	Girolami *et al*. [37]
	Clothianidin (soil); Clothianidin & thiamethoxam (dandelions)	Mid-April to early May	2.1–9.6 (soil) 1.1–9.4 (dandelions)	Krupke *et al*. [19]
	Clothianidin	Mid-March to May	0–47.8 (non-crop flowers)	Pistorius *et al*. [21]
Dew & Guttations	Clothianidin	May	1 h post planting: 17.5 and 27 24 h post planting: 6.5 and 12.5	Marzaro *et al*. [36]
	Imidacloprid, clothiandin, & thiamethoxam (field samples); imidacloprid only (lab samples)	April to May	Mean: 11,900–47,000 (field) Mean: 82,800–110,000 (laboratory)	Girolami *et al*. [23]
Pollen	Imidacloprid	Mid-April to August	1.1–5.7	Chauzat *et al*. [18]
	Imidacloprid and metabolite 6-chloronicotinic acid		0.9–1.2	Chauzat *et al*. [17]
	Thiacloprid, Imidacloprid, acetamiprid, & thiamethoxam		Mean thiacloprid: 23.8 (max: 115) Mean imidacloprid: 39.0 (max: 912) Mean acetamiprid: 59.3 (max: 134) Mean thiamethoxam: 53.3 (max: 53.3)	Mullin *et al*. [20]
	Clothiandin & thiamethoxam		Clothianidin: 3.9–88 Thiamethoxam: 1.2–7.4	Krupke *et al*. [19]
	Clothiandin & thiamethoxam		Mean clothianidin: 0.6 Mean thiamethoxam: 6.6	Pohorecka *et al*. [22]
Water	Clothianidin, thiamethoxam, imidacloprid, acetamiprid, & dinotefuran	April to March	Clothianidin: 0.0017–.257 Thiamethoxam: 0.0017–.185 Imidacloprid: 0.003–0.0427 Acetamiprid: 0–0.0111 Dinotefuran: 0–0.0027 Thiacloprid: ND	Hladik *et al*. [40••]
	Clothianidin, thiamethoxam, & imidacloprid		Clothianidin: 0.21–3.34 Thiamethoxam: 0.20–8.93 Imidacloprid: 0.26–3.34	Huseth and Groves [41••]
	Imidacloprid		Urban settings: 2–131 Suburban settings: 1–12 Rural settings: 1–25	Johnson and Pettis [43••]
	Imidacloprid, thiamethoxam, clothianidin, & acetamiprid		Mean spring 2012: 0.0083 (max: 0.184) Mean summer 2012: 0.0768 (max 3.11) Mean fall 2012: 0.004 (max: 0.101) Mean spring 2013: 0.0527 (max: 0.212)	Main *et al*. [42]
	Clothiandin & thiamethoxam		Clothianidin: 0.1–55.7 Thiamethoxam: 0.1–63.4	Samson-Robert *et al*. [44••]

concentrations ranging from 1.2 to 7.4 ppb and 3.9 to 88 ppb, respectively have been detected in honey bee-collected pollen in Indiana, USA well after planting activities ceased [19]. Maize pollen grown from seeds treated with thiamethoxam and clothianidin contained 1.7 and 3.9 ppb respectively, and bees were shown to forage upon this pollen in the field [19]. In a 3-year study conducted in France, fifty-seven percent of 185 honey bee pollen loads exhibited imidacloprid contamination with an average concentration of 0.9 ppb [17]. The neonicotinoids thiacloprid, imidacloprid, and acetamiprid have been detected in 5.4%, 2.9%, and 3.1% of 350 pollen samples collected from North American honey bee colonies located in various cropping systems [20], although very few of these samples were collected from areas where neonicotinoid-seed treated crops were grown. Although the percentages reported in this study are low, individual detections of neonicotinoids included maximum values of 115 ppb for thiacloprid, 912 ppb for imidacloprid, and 134 ppb for acetamiprid.

There is further evidence that honey bees can be intoxicated by neonicotinoid residues in guttations, exuded water droplets, produced by maize seedlings grown from treated seed. Exposure in this case is the result of the systemic movement of active ingredients from treated seeds into the seedlings. Chemical analysis of guttations collected from field and laboratory-grown maize plants seed treated with imidacloprid, clothianidin, or thiamethoxam exhibit high concentrations ranging from 11,900 to 47,000 ppb in field-collected guttations and 82,800 to 110,000 ppb in lab-collected guttations [23]. Furthermore, honey bees fed the guttations from treated maize seedlings exhibited lack of coordination, irreversible wing paralysis and death shortly thereafter. Although honey bees are known to collect guttations from winter rape [24], the extent to which honey bees utilize water resources in the form of guttations from other treated crop species requires further study.

The range of concentrations listed above generally fall below acute toxicity levels (Table 2) and represent a chronic, sub-lethal exposure route for pollinators. Effects of ingestion of food containing sub-lethal doses of neonicotinoids have recently been quantified for honey bees and bumblebees. Although beyond the scope of this article, effects of these sub-lethal exposures have included impaired navigation and learning, impaired immunity and reduced colony growth and queen rearing [1,25•,26–28,29••].

Residues in dust from planting treated seeds

Neonicotinoid seed treatments are currently a focus of scrutiny for several reasons; but chronicling their unintended environmental impacts was first initiated by the deaths of large numbers of honey bees following the planting of neonicotinoid-treated seeds in several countries, spanning the period since these products were first widely adopted [19,21,30–32]. Initial investigations determined that seed-treatment coatings can abrade and fall away from the seed surface [21,30,33]. Investigations of these acute exposures suggested that some form of 'operator error' (i.e., below standard application of seed treatment pesticides) was responsible for the observed honey bee deaths during spring seed sowing [32]. However, despite improvements in pesticide formulations and the quality of seed coat applications, additional bee die-offs have been documented in the EU, Canada and the US [19,21,31]. It is now clear that during the course of normal planting operations, exhaust systems of modern pneumatic planters deliver seed treatment active ingredients into the air, where the dusts can disperse and settle onto nearby vegetation or honey bees themselves [21,34••,35].

Efforts to quantify neonicotinoid contamination resulting from planter dust have documented the presence of residues in soil, grass, and flower blossoms following the sowing of treated seeds. Evaluations of environmental contamination by maize seed treatments containing clothianidin and thiamethoxam have found concentrations ranging between 2.1–9.6 ppb in soil samples and 1.1–9.4 ppb in dandelion blossoms collected from field margins [19]. Average concentrations of imidacloprid in grass and flower samples of 21 ppb and 32 ppb, respectively, have been documented [30], as well as higher concentrations ranging between 14–29 ppb in grass samples and 22–59 ppb in flower samples collected the day of, as well as several days following, the sowing of neonicotinoid-treated maize [33]. Variable clothianidin residue concentrations, some exceeding 40 ppb, have also been detected in flowers collected from untreated apple, dandelion, oilseed rape and other wildflowers [21]. The contamination of dew and guttation droplets by dispersing planter dust is another possible exposure route for honey bees. Evaluation of these water sources for contamination following the sowing of clothianidin-treated seeds revealed active ingredient concentrations ranging between 17.5 and 27 ppb, one hour after planting and concentrations between 6.5 and 12.5 ppb 24 h after planting [36]. Furthermore, the addition of seed

Table 2

Summary of acute toxicity levels of 5 neonicotinoids to honey bees and the environmental fate of these active ingredients in soil and water. Lethal dose (LD_{50}) values are reported in ng/bee and degradation time (DT_{50}) values are reported in days.

Neonicotinoid	Honey bee (LD_{50})		Half-life (DT_{50})	
	Oral	Contact	Soil	Water
Thiamethoxam [46,47]	5	24	5–100	8–44
Clothianidin [48]	4	43.9	148–1155	27
Imidacloprid [49]	3.7	59.7	40–124	30–162
Acetamiprid [50,51]	14,530	8090	2.6–133	13–420
Thiacloprid [52]	17,320	38,800	2.4–27.4	10–63

Note: Adapted from [46] Syngenta Crop Protection (2005) ENVIROfacts Thiamethoxam; [47] European Commission (2006) Health & Consumer Protection Directorate, review report Thiamethoxam; [48] US EPA (2003) Office of pesticide programs, factsheet Clothianidin; [49] Gervais, J.A.; Luukinen, B.; Buhl, K.; Stone, D. (2010) NPIC Imidacloprid Technical Fact Sheet; [50] European Commission (2004) Health & Consumer Protection Directorate, review report Acetamiprid; [51] US EPA (2002) Office of pesticide programs, factsheet Acetamiprid; [52] US EPA (2003) Office of pesticide programs, factsheet Thiacloprid.

lubricants such as graphite or talc (a recommended practice for planting with most pneumatic planters) can exacerbate the abrasion of seed coatings in the planter, such that lubricants also become contaminated with active ingredients and further contribute to environmental contamination when expelled with exhaust air [19].

Direct contact with neonicotinoid-contaminated dust clouds has been shown to occur for honey bees foraging in and around fields during planting activities, and in fact individual foragers exposed to dust clouds during flight subsequently suffer mortality within hours, particularly in cases of high humidity [35–37]. Chemical analysis of bees following their exposure to planter-emitted dusts demonstrate that foragers may acquire 29–3661 ng/bee of imidacloprid and 118–674 ng/bee of clothianidin [37]; well in excess of concentrations sufficient to cause acute intoxication for honey bees (Table 2). Furthermore, the characteristic pubescence of honey bees causes them to become electrostatically charged during flight as a result of friction with air; this is generally an adaptive trait that increases the attraction of small particles like pollen to the body surface as bees visit flowers [38]. In conditions where insecticide-laden dusts are found, however, this same mechanism may render bees more likely to accumulate residues as they fly near areas where planter dust is present.

Exposure to residues via contaminated water

Several recent publications have documented contamination of water sources with neonicotinoids used in seed treatments [39••]. Sampling of surface waters in the US has revealed frequent contamination of stream waters with clothianidin, thiamethoxam, and imidacloprid. Of 79 water samples collected across 9 sites of high maize and soybean production in the US, 75% were contaminated with clothianidin, 47% with thiamethoxam, and 23% with imidacloprid [40••]. Furthermore, documented concentration fluctuations corresponded with planting of neonicotinoid-treated maize seed and subsequent rainfall. These findings implicate neonicotinoid-seed treatments as likely sources of contamination and also reflect the very high water solubility of these compounds [8]. Similarly, thiamethoxam was detected in groundwater samples collected from intensively-managed agricultural regions in Wisconsin, USA from 2008 to 2012 [41••]. In this case, leaching of thiamethoxam applied during potato planting was implicated as a key contributor to groundwater contamination in and around crop production areas, both in-season and beyond. Neonicotinoids were also frequently detected in water samples collected in a repeated sampling of 136 Canadian wetlands spanning the provinces of Alberta, Saskatchewan, and Manitoba with 36% of wetlands showing evidence of contamination with at least one neonicotinoid before seed sowing and 62% of wetlands exhibiting contamination following seed sowing [42]. Furthermore, the same study found that the percentage of wetlands contaminated with neonicotinoids increased to 91% before seeding in the following year, suggesting that movement of residues from seed-treated fields to wetland areas occurs via run-off from melting snow. Finally, imidacloprid concentrations evaluated in water samples potentially used by bees in urban, suburban, and rural areas of Maryland, USA have documented values between 7 and 131 ppb [43••]. A similar study in Quebec, Canada evaluated pesticide residue concentrations in field puddles during the planting of treated-maize seed and detected clothianidin and thiamethoxam at values between 0.01 and 63 ppb [44••], which can exert sublethal effects on honey bees.

Quantifying impacts at the ecosystem level

Although the levels of neonicotinoids applied to each seed are readily available, there is almost no knowledge about the efficiency of translocation (i.e., the uptake and circulation of active ingredients by seedlings from the treated seed) or the concentration of active ingredients in various plant tissues after germination and during the growth and maturation of crop plants. This represents a key gap in our understanding of the environmental fate of these compounds. The degree to which these compounds may remain in crop soils and later translocate into flowering weeds or subsequent crops in the same field is also unclear. The potential for abraded seed treatments to move across the landscape has also not been quantified. Given that these compounds are highly water soluble and act systemically, there is the potential for dispersing residues (e.g., in planter dust) to be absorbed by plant tissues or dissolved in surface or ground water. This is of particular importance in many North American crop fields, where fields are drained using a system of perforated, buried pipes that convey excess water to drainage ditches at field margins.

Synthesis and future directions

The additive effects of these various exposure routes are still being quantified. However, given the area devoted to production of crops grown from neonicotinoid-treated seeds, it is clear that a great degree of temporal and spatial overlap exists between neonicotinoids and pollinators and other non-target organisms. Exposure can take place through various matrices — including air-borne and stationary dusts, soil, plant products, and water. For honey bees, where most current research is focused, future estimates of individual and colony-level effects of these exposures should incorporate these multiple routes into assessments of risk posed by neonicotinoid residues. Of particular interest is the typical period of sowing of many annual crops grown from neonicotinoid-treated seeds, which corresponds closely with flowering of spring blossoms and the concomitant increase in honey bee foraging activity across the landscape [45].

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