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Forum

Routes of Pesticide Exposure in Solitary, Cavity-Nesting Bees

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Abstract

Declines of pollinator health and their populations continue to be commercial and ecological concerns. Agricultural practices, such as the use of agrochemicals, are among factors attributed to honey bee (*Apis mellifera* L. (Hymenoptera: Apidae)) population losses and are also known to have negative effects on populations of managed non-*Apis* pollinators. Although pesticide registration routinely requires evaluation of impacts on honey bees, studies of this social species may not reveal important pesticide exposure routes where managed, solitary bees are commonly used. Studies of solitary bees offer additional bee models that are practical from the aspect of availability, known rearing protocols, and the ability to assess effects at the individual level without confounding factors associated with colony living. In addition to understanding bees, it is further important to understand how pesticide characteristics determine their environmental whereabouts and persistence. Considering our research expertise in advancing the management of solitary bees for crop pollination, this forum focuses on routes of pesticide exposure experienced by cavity-nesting bees, incorporating the relative importance of environmental contamination due to pesticide chemical behaviors. Exposure routes described are larval ingestion, adult ingestion, contact, and transovarial transmission. Published research reports of effects of several pesticides on solitary bees are reviewed to exemplify each exposure route. We highlight how certain pesticide risks are particularly important under circumstances related to the cavity nesters.

Key words: alfalfa leafcutting bee, insecticide, mason bee, pesticide, pollinator

Relevance and Rationale

Meeting the demand for healthy honey bee (*Apis mellifera* L. (Hymenoptera: Apidae)) populations for large commercial pollination events has been particularly challenging since colony collapse disorder (CCD) was recognized in 2006 (vanEngelsdorp et al. 2009). According to a 2016 report, winter colony losses were at 28%, which followed a summer loss also reported to be 28% (Steinhauer et al. 2016). Concerns over CCD and other major stressors contributing to chronic honey bee losses have been elicited by bee researchers and the media. Such concerns also have highlighted and strengthened the global recognition of perils for all pollinators. Nonetheless, it is difficult to document pollinator declines, in part due to the paucity of baseline data for wild bees that are not used in managed systems (Klein et al. 2003, Goulson et al. 2015). Causes of pollinator declines include singular and interacting stress factors: habitat loss, nutritional deficiencies, and exposure to pests, pathogens, and pesticides.

In response to the importance and complexity of solving a multifaceted bee health dilemma, the research community has been actively focusing on one of the most scrutinized and debated impact factors, which is bee exposure to chemical pesticides. Most academic and government agency studies to date only have considered pesticide effects on honey bees (Kubik et al. 1999, Wu et al. 2011, DeGrandi-Hoffman et al. 2013, Cutler and Scott-Dupree 2014, USEPA, PMRA, and CDPR 2014, Berenbaum 2016, Fisher et al. 2017), although new attention has been given to some species of non-*Apis* bees (EFSA 2013; Godfray et al. 2014, 2015; APVMA 2015; Biddinger and Rajotte 2015; Jin et al. 2015; Lundin et al. 2015), of which there are at least 20,000 species globally (Michener 2000). Goals of new efforts address the ability to assure pollinator health, abundance, and conservation, and to mitigate factors that harm or diminish pollinator populations and their habitats. As a result, better documentation of needed research actions, knowledge gaps, regulatory requirements, and suggested paradigms for pesticide risk assessments have begun to emerge (EFSA 2012, 2013, 2014; EMBRAPA 2013; USEPA, PMRA, and CDPR 2014; White House 2014, 2015).

Whether pesticides are used in cropping systems to control arthropod pests, fungal pathogens, and weeds or in residential areas to control mosquitoes or garden and lawn pests, bees are exposed to chemicals in many contexts (Johnson 2015, Hladik et al. 2016). Most non-*Apis* bees are solitary and short-lived with limited foraging ranges and restricted geographic distributions compared with social bees. We are particularly interested in the exposure routes to managed, solitary bees that may experience the agricultural landscape differently than do honey bees. We choose to focus on these bees because of their major current and potential roles in North American and Eurasian agriculture. These are cavity-nesting bees of genera *Megachile* and *Osmia* (Hymenoptera: Megachilidae) that can be easily purchased for crop pollination while they are in diapause, and later incubated to produce mature adults for pollination and nesting in artificial bee tunnels in the fields. These bees have similar exposures as honey bees when they come into direct contact with pesticides during applications or by collecting and feeding on pollen and nectar. But on account of their biology, ecology, physiology, and genetics (Kapheim et al. 2015), they can differ from honey bees in their exposures to pesticides via plant materials, soil, and water, and in their susceptibility to some chemistries and ability to recover from contact or ingestion (Hooven et al. 2014, Heard et al. 2017). Differences that distinguish solitary lifestyles from social ones necessitate the exploration of potential pesticide impacts that are not considered when studying honey bees. Nesting behavior, habitat locations and types, seasonality, immune responses, and mechanisms of detoxification each may render differential routes, intensities, and effects of pesticide exposure.

This article describes both the known and probable routes of pesticide exposure in managed, cavity-nesting bee species. We hope to enrich the conversation that defines routes of exposure not only to these bees, but also consequently to wild solitary bees that nest both above and below ground. In a forum style, we address critical components of cavity-nesting bee life histories that may expose them to pesticides that persist in the environment due to key characteristics of pesticides, regardless of when those pesticides were applied for pest, pathogen, and weed control. We deliver the details of four routes of exposure: larval ingestion, adult ingestion, adult contact, and transovarial transmission (Figs. 2–5). For each route for several agrochemicals, we also provide recent examples of studies that reveal effects of pesticides on cavity-nesting bees and techniques for examining them. We discuss the interactions between the specific dangers to cavity-nesting bees due to chemical properties of some pesticides and the ecology and behavior of the bees.

Comparison of Managed Bee Life Histories: Solitary, Cavity-Nesting Bees Versus Social Honey Bees

Solitary, cavity-nesting bees make brood cells in old holes in tree trunks and other woody stems, in reeds, and other various aboveground vacancies that exist naturally, but also readily use artificial tunnels provided by bee managers (Fig. 1A). Commercial tunnels are frequently made of cardboard or wood, which are placed in protective shelters. Bees will nest in these shelters *en masse*, creating artificial aggregations (Fig. 1B). Each female is a reproductive individual and builds her own nest, with one bee occupying one cavity at a time in the aggregation (Fig. 1C). Solitary bees use various materials to partition brood cells within the nest, such as soil, cut or masticated plant tissue, resin, or a combination of such materials (Cane et al. 2007). Unlike colonies of honey bees where larvae are fed progressively by workers, solitary bee mothers create a mass provision in 1 d or less from pollen and nectar she collects from flowers. She then lays an egg on the provision mass, and a larva develops to adulthood on this sole source of food (Bosch and Kemp 2001) (Fig. 1C). The process is repeated to make multiple nest cells per cavity. Usually, nesting bees live for about 4–6 wk, and brood spend a year in nests to develop and overwinter before emerging as adults in the next season.

Honey bees live in colonies that may include >20,000 worker bees, seasonal males, and a queen. Only the queen can produce new worker daughters who perform all hive tasks including feeding larvae, storing food, and building new nest cells. A new colony is started by the swarming of the old queen plus some of the workers. They identify and move into a new nest site to continue the colony cycle. The daughter queen that remains inherits the old hive and workers, where she continues the colony by producing her own offspring. Therefore, honey bee colonies are perennial and never exhibit a solitary phase (Winston 1987).

Fig. 1. (A) An *Osmia lignaria* nest box hanging in an almond tree in a California orchard, with close-up of mud-plugged nest tubes. (B) Commercial tunnels are made of cardboard or wood, and bees will nest in them, creating aggregations at protective shelters. (C) Mother bees use pollen and nectar to make mass provisions upon which she lays her eggs.

The greatest risk to a solitary female is the loss of potential offspring, because she is the sole reproductive entity of her nest. Depending on the timing of her death in the nesting season, only the already completed nest cells will represent her total reproductive output. The loss of nesting bees due to direct sprays or bee handling of contaminated forage may kill adult bees and could lead to a local population decline due to low reproductive success. On the other hand, the sociality of honey bees affords the advantage of the resilience of a superorganism (Johansen and Mayer 1990, Straub et al. 2015). As long as a lethal dose of a pesticide does not penetrate the hive, the loss of some of a colony's workers in the field does not affect the honey bee queen, who can replace worker daughters, if she remains healthy and reproductive, and if the number of workers remains above a critical threshold (Dennis and Kemp 2016).

Chemical Characteristics

The chemical properties of a pesticide are important for a product's ability to contact or penetrate the target pest, and these same properties will also contribute to how and where the pesticide may eventually settle in the environment. Lipophilicity, hydrophilicity, and soil adsorption are three characteristics of agrochemicals that are pertinent to understanding their environmental persistence and potential to facilitate routes of exposure of pesticides to bees freely foraging in an agricultural landscape.

Lipophilicity is a chemical's affinity for lipids. Attraction to lipids allows a pesticide to permeate the cuticular lipid layers of both plants and insects, aiding in the distribution of the desired toxin and its effect on pests. Hydrophilicity is a chemical's affinity for water. It affects the accumulation of the chemical in the environment and its bioavailability for uptake by a plant, allowing some pesticides to act systemically. Systemic pesticides can be distributed throughout the plant as it grows, which means it can be found not only in vegetative material, but also potentially in the pollen and nectar (Godfray et al. 2014, Larson et al. 2015).

Lipophilicity and hydrophilicity of a substance are determined using the octanol:water partition coefficient (K_{ow}). This coefficient describes the distribution of a compound between a lipophilic phase (*n*-octanol) and an aqueous phase of the test system. A lipophilic pesticide has a high K_{ow}, and a hydrophilic chemical has a low K_{ow} (Table 1). K_{ow} also indicates the compound's bioaccumulation potential in animal fats and plant lipids plus its adsorption potential in organic matter of soil (Russel 1995). Pesticides with a high K_{ow} are capable of translaminar movement through plant cuticular lipid layers, which might also move across a bee's lipid layer and into the body through simple cuticular contact during foraging and nesting, as has been suggested for bumble bee workers exposed to various chitin synthesis inhibitors (Mommaerts et al. 2006).

Soil adsorption, or K_{oc}, is the soil organic carbon:water partitioning coefficient. It indicates a chemical's soil binding propensity. Specifically, this coefficient is the concentration of chemical in soil per concentration of chemical substance in water divided by the percent of organic carbon in the soil. A high value for the K_{oc} of a pesticide means that it is more likely to accumulate in the soil; a low K_{oc} value indicates that the pesticide will move with water and leach out of the soil (Fisk 1995, Klaasen 2007).

Chemical characteristics and their interactions with the environment affect their half-lives, i.e., the time it takes for an amount of a pesticide to be reduced by half from being broken down by environmental factors. In general, one half-life indicates that a pesticide has been broken down to 50% of the original amount, and two half-lives mean 25% breakdown, and so forth. The amount of a pesticide applied may increase its half-life and repeated applications that add to the amount of chemical in a matrix. Factors that break down pesticides include sunlight, temperature, oxygen, soil composition, pH of soil and water, microbial activity, and metabolism or elimination by the insects themselves (Cresswell et al. 2014). As environmental factors change, so can the duration of a half-life (National Pesticide Information Center 2017).

Pesticides can immediately enter an ecosystem through such avenues as application sprays, dust in the soil or air from seed treatments (Corn Dust Research Consortium 2015, Tsvetkov et al. 2017, Woodcock et al. 2017), additives in irrigation systems, or incidental run off and spray drift beyond intended targets. However, because soil and water are ultimate sinks for pesticides, chemicals can be present in bees' foraging landscapes long before bees are actively visiting a crop in bloom (Kubik et al. 1999, Larson et al. 2015, Long and Krupke 2016, Tsvetkov et al. 2017, Woodcock et al. 2017). Soil is adsorbent with its hydrophobic domains, and chemicals having high K_{ow} and K_{oc} allow them to cling to the soil and persist in this matrix (Fisk 1995, Klaasen 2007, Palmquist et al. 2012). Water acts as solvent and can displace chemicals from hydrophobic domains of soil. Therefore, water disperses chemicals with low K_{ow} and K_{oc} across the environment or allows them to accumulate in a local water source or move beyond the immediate application area (e.g., runoff).

Major Pesticide Classes and Properties

Organochlorines are very persistent nerve toxins that bioaccumulate, such as dichlorodiphenyltrichloroethane (DDT). After extensive use as an important insecticide, DDT was banned by the U.S. Environmental Protection Agency (USEPA) in the early 1970s, because its pervasive and negative environmental and human impacts were realized (Carson 1962, Heberer and Dünnbier 1999). Currently used organochlorines also are environmentally persistent due to low water solubility (Saldalgo 2013) (Table 1).

Organophosphates and carbamates are also nerve toxins, but with a different mode of action than the organochlorines (Table 1). Organophosphates were originally developed as nerve gases for use in chemical warfare, and many are now banned due to their high human toxicity. Carbamates, used as insecticides and fungicides, have similar modes of action as organophosphates. Although much less widely used now than when popular from 1950s to 1980s, carbamates are still applied as broad-spectrum insecticides that protect large commodity crops (e.g., fruit trees, cotton, vegetable, and row crops), and their field use remains a concern for bee safety. Like organophosphates, carbamates can have high vertebrate toxicity. Although some organophosphates are water soluble and can leach into ground water, other organophosphates and carbamates that adhere to soil matter can move into water along with soil sediment (Singh 2012, Saldalgo 2013). However, they are easily degraded in nature and not considered persistent or likely to biomagnify (Saldalgo 2013). Carbamates have high lipophilicity, which facilitates their ability to reach an insect's nervous system simply by crossing the lipid-coated cuticle (Ishaaya and Degheele 1998).

Pyrethroids are synthetic derivatives of the naturally occurring pyrethrins from chrysanthemums. They are neurotoxins like organophosphates and carbamates, but they are much less persistent than organochlorines, largely due to degradation mechanisms that are catalyzed by ultraviolet light, water, and oxygen (Palmquist et al. 2012, Saldalgo 2013). Pyrethroids might offer a potentially reduced risk insecticide option if the spray occurs at night when bees are not on the crop and if the chemicals are degraded under the morning

Table 1. Examples of modes of action on pests and environmental characteristics of various agricultural insecticide families and fungicide classes

Family/Class	Mode of Action	Active ingredient	Log *K*ow[a]	Activity in environment[b]
Organochlorine	GABA-gated chloride channel antagonists	Endosulfan	3.83	High persistence
Organophosphate	Acetylcholine esterase inhibitors	Dimethoate	0.78	Low persistence (degradation by microbes), low biomagnification; some with high soil adsorbance; some soluble in water and in runoff
Carbamate	Acetylcholine esterase inhibitors	Carbofuran	2.32	Low persistence (degradation by hydrolysis), low biomagnification
Pyrethroid	Axonic excitoxins (prevent closure of sodium channels)	Bifenthrin	6.00	Quick degradation due to UV, water, and oxygen; environmental residuals mostly absent; high soil adsorbance; lipophilic and insoluble in water
Neonicotinoid	Nicotinic acetylcholine receptor agonists or antagonists	Imidacloprid	0.57	High water solubility; systemic; prone to leach into groundwater; moderately persistent; does not biomagnify
Spinosyn	Nicotinic acetylcholine receptor agonists; metabolite of soil actinomycete (bacteria)	Spinosad	2.80–5.20	Low persistence due to photo- and microdegradation; low leaching potential
Sulfoxaflor	Agonists of acetylcholine receptors, by mimicking action of acetylcholine	Sulfoxaflor	0.80	Hydrophilic; rapidly degraded in soil and water
Pyridinecarboxamide	Molecular target not yet identified; Antifeedant effect due to action of compounds on chordotonal organs, proprioceptive sensory organs present throughout the insect body important in hearing, gravity perception, and fine motor coordination	Flonicamid	0.30	Degrades rapidly in soil; low risk of groundwater contamination
Anthanilic diamide	Modulation of ryanodine receptor to cause calcium channel to remain open leading to lethargy, feeding cessation, and death	Chlorantraniliprole	2.90	Persistent and mobile in terrestrial and aquatic environments; residue accumulation in soil after extended use; degradation by hydrolysis, light, leaching, and runoff
Benzoylurea	Chitin biosynthesis inhibitor, type 0	Novaluron	5.27	Translaminar; lipophilic; low water solubility; strong soil adsorption; low leaching potential; persistent
Juvenile hormone mimic	Juvenile hormone and ecdysone analogues	Fenoxycarb	4.30	Lipophilic
Fungicide1[c]	Aniline pyrimidine: inhibits methionine biosynthesis and secretion of hydrolytic enzymes	Pyrimethanil	2.84	Strong soil adsorption; moderately persistent; possible surface runoff with soil particles
Fungicide2	Sterol biosynthesis inhibitor	Iprodione	3.00	Strong soil adsorption; moderately persistent; possible surface runoff with soil particles
Fungicide3	Succinate dehydrogenase inhibitor	Boscalid, Pyroclostrobin	2.96, 3.99	Strong soil adsorption; highly persistent; possible surface runoff with soil particles

[a]Log K_{ow} values from http://www.pubchem.ncbi.nlm.nih.gov.
[b]Characteristics from the following: Thompson et al. (2000), Cutler and Scott-Dupree (2007), Wightwick et al. (2010), Singh 2012, Saldalgo (2013).
[c]Fungicide classifications: http://www.frac.info/working-group.

sun before bees begin their forays into the field. However, many pest insects have developed resistance to this insecticide family (Ishaaya and Degheele 1998). Pyrethroids also do not biomagnify because of their low soil mobility (i.e., their propensity to adhere to soil particles), which reduces a tendency to leach (Saldalgo 2013).

Neonicotinoids are pesticides that overstimulate insect nerve receptors, which eventually cause paralysis and death. Formulations of this relatively new pesticide family are the most widely used insecticides in the world (Goulson 2013, Lundin et al. 2015). Neonicotinoids currently arouse contentious discussion within and outside of the scientific community because of their widespread use and sometimes conflicting claims of negative effects on bees. They are used as seed, soil, and trunk treatments, are painted onto plants, and are applied as foliar sprays (Saldalgo 2013). They are systemic insecticides, being highly water soluble with a low K_{ow} so that they are absorbed and stored in plant tissue (Ishaaya and Degheele 1998) and occur in nectar and pollen, all of which are major sources of exposure to bees (Goulson 2013, Godfray et al. 2014, 2015; Stewart et al. 2014; Botías et al. 2015; Rundlöf et al. 2015; David et al. 2016; Long and Krupke 2016; Tsvetkov et al. 2017). Neonicotinoids are also prone to leaching, are moderately persistent in the environment, but do not biomagnify (Saldalgo 2013). Due to their hydrophilicity,

common neonicotinoids have been detected in macro-ecosystems, such as wetlands of Canada and the Netherlands where invertebrates and vertebrates alike could be exposed (Hallmann et al. 2014, Main et al. 2014, Samson-Robert et al. 2014, Schaafsma et al. 2015), and in micro-ecosystems, such as in guttation fluid of cantaloupe plants that honey bees may imbibe (Hoffmann and Castle 2012, Fairbrother et al. 2014).

Anthranilic diamide insecticides are unique ryanodine receptor modulators. Ryanodine binds to the ryanodine receptor, which locks the calcium channel in a partially open condition. By leading to the loss of calcium regulation, a chewing insect that has ingested a diamide insecticide becomes lethargic or paralyzed, ceases to feed, and eventually dies (Teixeira and Andaloro 2013). Diamides, such as chlorantraniliprole (Cordova et al. 2006, USEPA 2008), are used as foliar sprays and in drip irrigation. Recent widespread global use of diamides raises concerns of insect resistance (Teixeria and Andaloro 2013), and extended use may result in soil accumulation (USEPA 2008). Persistence in some environments is mitigated by degradation via hydrolysis, light, leaching, and runoff (USEPA 2008).

Insect growth regulators (IGRs) and juvenile hormone mimics are biorational (reduced risk) pesticides. They are designed to attack immature insects because they prevent molting by inhibiting chitin synthesis or by mimicking molting hormones at the molecular level by binding with receptors (but being ineffective at gene regulation of ecdysis; Retnakaran et al. 2003). Such effects result in a soft exoskeleton, deformed appendages and sexual organs, and incomplete larval and pupal molts. IGRs work slower than the other 'knock-down' pesticides but are more effective at reducing an entire pest population because affected insects never reach the reproductive adult stage. Due to very low water solubility, most IGRs are unlikely to leach through the soil, and some persist in the environment with activity at very low levels (Saldalgo 2013). Furthermore, translaminar movement into plant tissue extends the duration of the efficacy of some IGRs, such as the product novaluron (Cutler and Scott-Dupree 2007).

Fungicides can be divided into classes by their chemical structure or by their mode of action. Such classes include the aniline pyrimidines, sterol biosynthesis inhibitors, and succinate dehydrogenase inhibitors (http://www.frac.info/working-group) (Table 1). Fungicides are widely used in agriculture, and there is recent evidence of their sublethal, and perhaps lethal, impact on bees (Ladurner et al. 2005, 2008; Artz and Pitts-Singer 2015; Fisher et al. 2017). Because they are regarded as safe for bees, these chemicals are sprayed during bloom when bees are present as managed and wild pollinators. Although care is often taken to only spray at night, direct, indirect, and synergistic effects on bees have been demonstrated in the field and laboratory (Pettis et al. 2013, Sanchez-Bayo and Goka 2014, Artz and Pitts-Singer 2015, Sgolastra et al. 2016, Fisher et al. 2017). Effects on honey bees include worker mortality (Fisher et al. 2017), possibly through inhibition of detoxification mechanisms (Pilling et al. 1995), and effects on solitary bees include disorientation and dispersal from nest sites (Ladurner et al. 2008, Artz and Pitts-Singer 2015).

Herbicides also are among the pesticides detected in wax and pollen in honey bee hives (Johnson et al. 2010, Mullin et al. 2010). Recently, certain herbicides have been shown to affect the bee carotenoid-retinoid system, which is critical for larval development, bee vision, and antioxidant capacity, and may increase bee foraging activity (Helmer et al. 2015). The herbicide glyphosate has been shown to affect conditional learning and also navigation in honey bees (Herbert et al. 2014, Balbuena et al. 2015). Although sublethal effects of herbicides may affect bee health, we will not be discussing them specifically in this article.

Routes of Bee Exposure to Pesticides

The accumulation of pesticides in both soil and water, and the presence of contaminated nesting materials and food sources within bee foraging ranges create conditions under which cavity-nesting bees are particularly vulnerable to many potential sources of contamination and the consequences that follow exposure. How pesticide and bee behaviors interact is discussed in the following routes of pesticide exposure for cavity-nesting bees.

Route 1: Larval Ingestion

The routes that pesticides travel to the limited food stores of solitary bee larvae can be attributed to the intersection of pesticides present in the environment and bee nesting behavior (Fig. 2). A single pollen-nectar mass provision created from naturally occurring resources is the sole source of food consumed by a larva for development to adulthood. If pollen, nectar, or both harbor pesticides through systemic uptake by the plant, from direct topical application, or dust clouds and residuals from planting of pesticide-treated seeds, then there is no mechanism for the larva to avoid ingestion of contaminants (except to cease feeding), and any potential detrimental effects of pesticides on larval survival or later adult fecundity will be suffered. Another means of larval exposure via ingestion may originate from the nest-building material (usually soil or leaves) fashioned by the mother bee into cell linings or partitions. Leaf material may be contaminated at the surface or internally through translaminar and systemic actions of pesticides. Soil can be contaminated with persistent, soil-bound chemicals that land directly on the soil surface and also temporarily contaminated by pesticides that move with water deeper into or through the soil matrix. Soil also may be contaminated by agricultural aqueous runoff that contains pesticides (Russel 1995, Klaasen 2007). Pesticide residues in nest cell materials may leach from the material into the soft, wet provision. Because nectar is aqueous and contains water and carbohydrates (sugars; Cane et al. 2011), and because pollen contains lipids and proteins (Dobson 1988, Roulston and Cane 2000), the nectar in the provision mass could attract agrochemicals with a low K_{ow}, and the pollen could attract chemicals with a high K_{ow}. Therefore, the interface between provision mass and contaminated nest material may allow a slow, passive transference of toxins that a larva will eventually encounter through contact or ingestion.

Studies that focus on the effects of pesticides on bee larvae and how those larvae are exposed remain less common than studies on adult bees (Huntzinger et al. 2008b, Sgolastra et al. 2017). Within the hive, it is difficult to follow individual honey bee larvae through development, and even more difficult to know exactly what larval foods are gathered and processed by workers for progressive feeding of each larva. Individual solitary bee larvae in cavity nests are more amenable than honey bee larvae to studies of contamination of larval food and subsequent effects, but studies of solitary bee larvae of ground-nesting species are lacking, due to the absence of techniques for managing these bees in artificial nests or rearing them in the laboratory so that they can be observed over time.

Route 1 Examples

A. Huntzinger et al. (2008b): In a laboratory study, *Megachile rotundata* F. (Hymenoptera: Megachilidae) nest cells were uncapped, and provisions remained intact after being placed into plastic well plates. The provisions were injected with four fungicide formulations (1 µl solution under the egg of each provision) to examine their effects on the fungal pathogen *Ascophaera aggregata* (Skou) and evaluate nontarget effects on bee larvae. Fungal spores contaminate larval

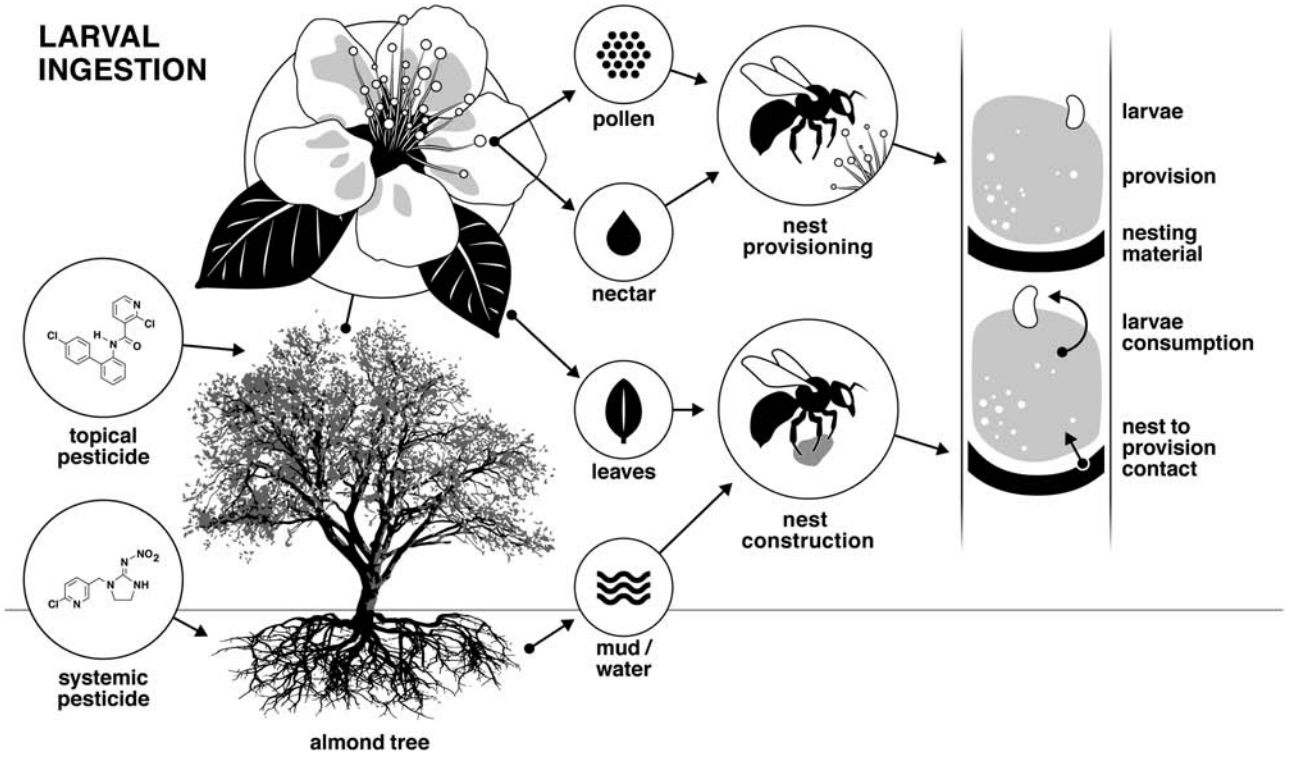

Fig. 2. Larval ingestion exposure route with almond orchard example. Developing larvae ingest: 1) contaminated pollen and nectar, 2) contaminated soil or plant material used in nest construction, or 3) pesticides leached from nest partition into provisions. Illustration by James Bradford.

provisions, and the fungus develops inside larval guts after being eaten. The resulting lethal fungal disease of larvae is called chalkbrood. Three of the four fungicides reduced *A. aggregata* hyphal growth. Interestingly, the fungicide captan (concentration of 700 g a.i./liter) was ineffective at controlling *A. aggregata* and was lethal to the bee larvae.

B. Hodgson et al. (2011): Using similar techniques to Huntzinger et al. (2008b), *M. rotundata* provisions were dosed with 0.5–10 times the field rate (745 ml/ha) of the chitin synthesis inhibitor novaluron (Table 1) recommended for control of the seed predator, *Lygus hesperus* Knight (Hemiptera: Miridae). In treated bee cells at all dose rates, *M. rotundata* eggs and early instars suffered very high mortality (>85%) compared with controls (>60%). Such consequences for pollinator reproduction (here and in other examples) raise serious concerns for growers that must rely on commercially managed *M. rotundata* for alfalfa seed production.

C. Pitts-Singer and Barbour (2016): *M. rotundata* exposure to novaluron was also studied in large cages placed over a blooming alfalfa plot in which mother bees made nest cells from leaf pieces that had been sprayed with a hand-held sprayer (at full field rate, 745 ml/ha) with novaluron 7–14 d before nesting commenced. Compared to survival of larvae (average mortality approximately 10%) in cages where no novaluron was ever sprayed, significantly more larvae died as eggs or first instars (average mortality approximately 54–74%) in nests from the cages with novaluron-treated alfalfa. Results suggested the possibility that novaluron-treated alfalfa leaf pieces used to make cell linings were the source of contaminates that could leach into the larval provision that, when fed upon, interrupted larval development. Because alfalfa flowers wilt within a few days after opening (Carlson 1928), those that had gotten sprayed would have already closed by the time that bees were introduced to cages. Therefore, only newly opened flowers would have been present, and the nectar and pollen from flowers present at the time of treatment could not have been the source of novaluron contamination.

Abbott et al. (2008); Nicholls et al. (2017): By dosing *Osmia lignaria* Say (Hymenoptera: Megachilidae) mass provisions (natural and ones made of pulverized honey bee pollen) with the neonicotinoid imidacloprid, and *M. rotundata* provisions with clothianidin, larvae were monitored for lethal and sublethal effects (Abbott et al. 2008). No lethal effects were observed in either species at any concentration tested (range = 3–300 ppm). This outcome was explained by the presumed degradation of the products before enough provision had been consumed to cause an effect. However, one sublethal effect was detected: *O. lignaria* larval development and cocoon spinning took longer at the higher doses of imidacloprid (30–300 ppm). A similar type of study that dosed natural provisions of *Osmia bicornis* L. (Hymenoptera: Megachilidae) with clothianidin (0–10 ppb) showed no effect on larval development time, overwintering survival, or adult weight (Nicholls et al. 2017).

Route 2: Adult Ingestion

Although adult bee ingestion is a well-established risk assessment parameter for honey bees and bumble bees, some studies also confirm that contaminated adult bee food, nectar and pollen, can have a detrimental impact on solitary bees (Mommaerts et al 2006, Gill and Raine 2014) (Fig. 3). Active solitary adult bees regularly ingest nectar to maintain their energy, and newly emerged female bees also consume pollen to aid in ovary maturation and egg development (Cane 2016). Likewise, during the solitary founding phase of bumble bee colony cycles, queen bumble bees also risk exposure to contaminated nectar and pollen that negatively impacts survival, nest initiation, and ovary development (Baron et al. 2017, Wu-Smart and Spivak 2018)

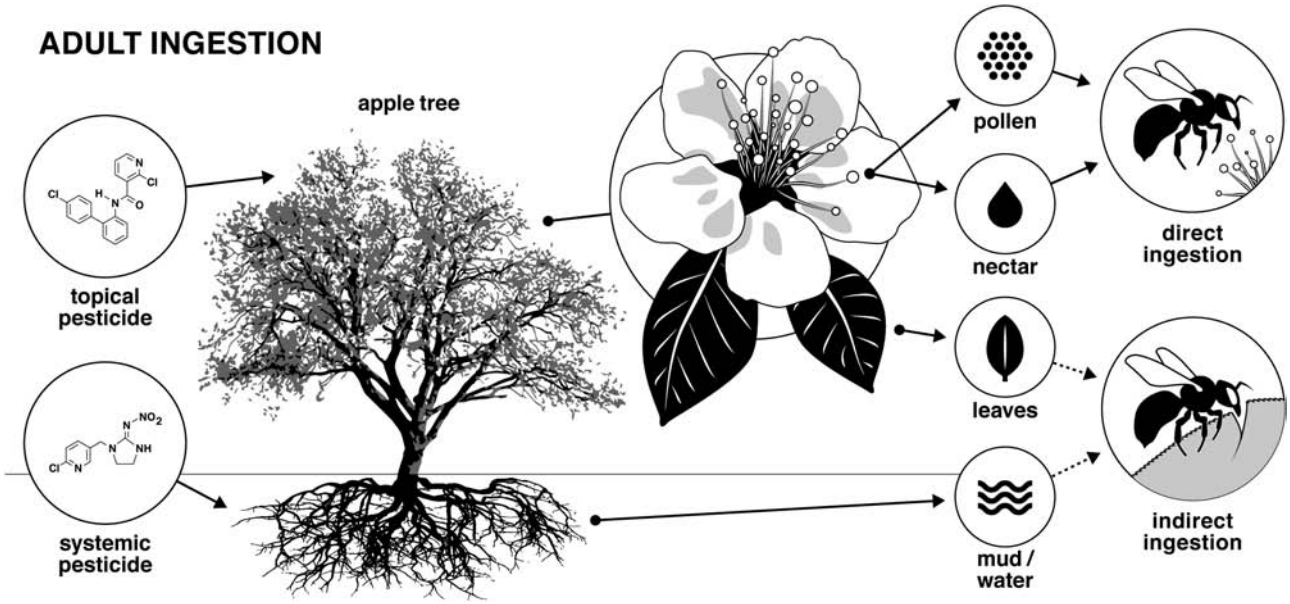

Fig. 3. Adult ingestion exposure route with apple orchard example. Adults ingest contaminated: 1) nectar and pollen while feeding or provisioning a nest and 2) plant material when cutting or masticating leaves or soil when collecting for nest-building. Illustration by James Bradford.

Use of the mandibles and tarsi to manipulate and move soil and leaf material may prove another means of adult pesticide ingestion. When constructing nests, bees such as *M. rotundata* females may incidentally ingest masticated leaf material and plant juices, and mason bees such as *O. lignaria* may ingest water or particles from moist soil. Furthermore, bees groom their bodies, which includes use of mouthparts for cleaning body parts, and they may imbibe contaminants or contaminated materials by performing this behavior. To date, no studies have revealed negative effects of contaminated nesting-building materials on solitary female bees nor quantified the amount of pesticide residues (i.e., pesticides and their metabolites) that may exist on or in nest-building materials for direct or indirect bee exposure. It is not clear to what extent solitary bees encounter pesticides by actively collecting standing water, but honey bee workers collect water to make honey and cool the hive (Gary 1992, Free 1993).

Route 2 Examples

A. Ladurner et al. (2005): Using a laboratory feeding technique that incorporates a real flower with a false, fillable ampule that replaces the corolla (Ladurner et al. 2003), *O. lignaria* and honey bee adults were offered 10 µl of five different sucrose plus fungicide solutions. The fungicide propiconazole (65.0 µg a.i./liter) was found to be lethally toxic to both bee species, and captan (122.5 µg a.i./liter) also was lethal to *O. lignaria*.

B. Artz and Pits-Singer (2015): A study was performed in cages, and the probable direct ingestion of (rather than contact with) fungicides sprayed at night on blooming forage using a hand-held sprayer (full field rates: iprodione = 2.2 kg/ha, pyraclostrobin + boscalid = 1.6 kg/ha) resulted in a change in bee nesting behavior. Before foraging on the sprayed flowers, nesting *O. lignaria* and *M. rotundata* females had readily oriented to their nesting tunnels in provided bee boards, but the morning after the spray, they appeared to be confused and unable to find their nests. This behavioral change was sublethal, but in an open-field situation would likely have resulted in bees eventually abandoning their nests, as has been reported anecdotally when managed *O. lignaria* were used in cherry and almond pollination (Ladurner et al. 2008).

C. Peach et al. (1995): Sublethal effects of carbaryl (a carbamate) were evaluated for *M. rotundata* after female adults were fed carbaryl bran bait in honey water or plain wheat bran mixed in honey water. Uniquely marked females were flown in a greenhouse where white clover was offered as a resource for making nests, which were collected and assessed for revealing reproduction by treatment. There was no effect of treatment on adults, adult nesting behavior, or progeny survival, size, and sex ratio.

D. Sandrock et al. (2013): Based on field-realistic trace residue amounts, the neonicotinoids thiamethoxam (2.87 µg/kg) and clothianidin (0.45 µg/kg) were mixed into sugar water, and the solutions were offered to *O. bicornis* in the controlled environment of flight cages to examine chronic adult bee exposure. No effect was found on nesting female longevity, but reproduction was significantly affected. In the flight cage with the neonicotinoid treatment, reproduction was decreased, offspring mortality was increased, and sex ratio was more male-biased. However, no pesticide residues were found in larval provisions or adult offspring.

E. Rundlöf et al. (2015); Woodcock (2017): In two studies performed in oilseed rape fields planted with neonicotinoid-treated seeds, reproduction for honey bees, bumble bees, and *O. bicornis* was impaired. *O. bicornis* females that foraged in treated fields produced fewer brood in trap-nests adjacent to treated fields compared with trap-nests at control fields. The mechanisms by which bee nesting is affected by the presence of residues of insecticides in fields have yet to be discerned.

Route 3: Contact

Physical contact between adult bees and toxins on contaminated resources is the simplest and most direct exposure route assessed for solitary bees (Ladurner et al. 2005, Huntzinger et al. 2008a, Biddinger et al. 2015) (Fig. 4). Toxins that contact the bee cuticle may penetrate it directly or may pass (actively or passively) into the body through such orifices as spiracular openings or pores. Besides being directly sprayed during pesticide applications, bees can land on or walk about on contaminated surfaces of soil, lawns, flowers, foliage, or artificial nest materials and even water located in treated fields or gardens.

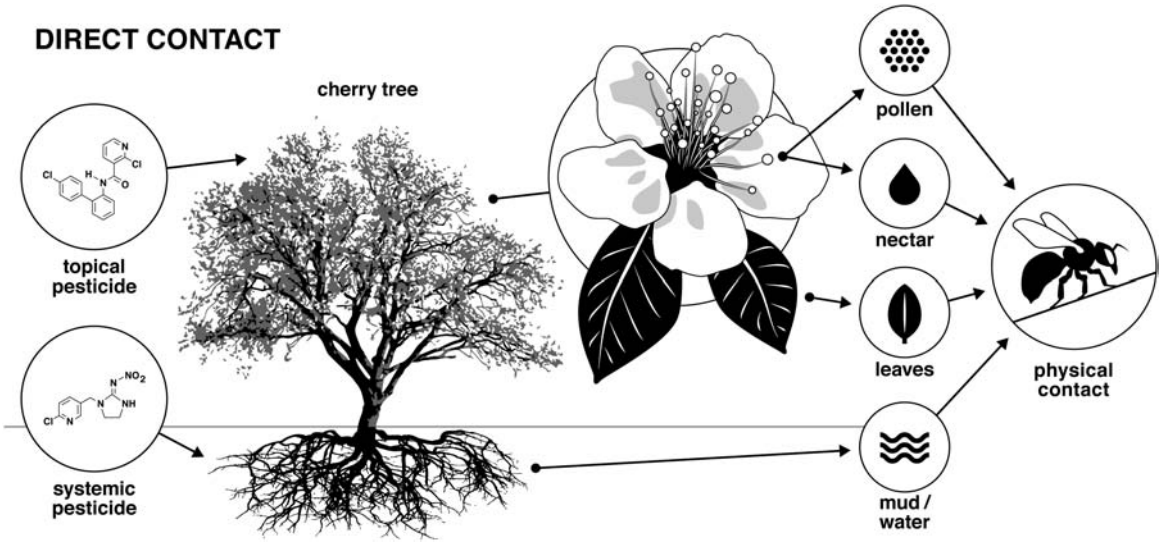

Fig. 4. Contact exposure route with cherry orchard example. Upon contact, the lipophilic properties of pesticides allow them to enter a bee directly through the cuticle. Illustration by James Bradford.

Route 3 Examples

A. Ladurner et al. (2005): In a study of the effects of five fungicides, an effect was observed immediately after a 1 µl topical dose (or ingestion; 122.5 µg a.i. per bee) of captan. *O. lignaria* females exhibited abnormal behaviors, such as inactivity, regurgitation of the ingested sucrose solution, extension of proboscis, abdomen, and genitalia. No similar effects were observed for similarly tested honey bees. The other fungicides had neither acute nor delayed toxic effects on the two bee species.

B. Huntzinger et al. (2008a): Topical doses of the same fungicides used in Huntzinger et al. (2008b) were applied to *M. rotundata* adults. Results showed significantly reduced survival of males treated with captan at 684 g a.i./liter. Female survival was reduced at the lesser amount of 342 g a.i./liter, but inexplicably, not at the higher rate like for males. Other fungicides did not appear to harm the adult bees.

Route 4: Transovarial Transmission

The transovarial transmission of pesticides results when chemicals taken in by the mother bee have a deleterious effect on her offspring, resulting in the suppression of targeted pest populations (Fig. 5). Transovarially transmitted pesticides are ingested by an adult female or they penetrate her cuticle. Although the intended use of these pesticides is to reduce pest insect reproduction and protect a crop, they may also reduce pollinator reproductive success and effect the availability of future pollinators. The direct effect of this route of exposure on reproduction is manifested as low or no survival of eggs or reduced egg production (Ishaaya and Degheele 1998, Mommaerts et al. 2006, Hoffmann et al. 2008, Trostanetsky and Kostyukovsky 2008).

Route 4 Examples

A. Hodgson et al. (2011): *M. rotundata* females were fed a sugar water + novaluron solution or simply sugar water in the laboratory. Novalruon was diluted to represent a full field rate (745 ml/ha) in the sugar solution. Females then were allowed to forage on uncontaminated alfalfa for nesting in field cages. Almost all (97%) of the eggs failed to hatch if they were laid by females that fed upon the novaluron-treated solution, while females fed only sugar water laid many eggs that hatched and survived to full larval development (mortality of 12–20%).

B. Pitts-Singer and Barbour (2016): In a follow-up study to Hodgson et al. (2011), caged *M. rotundata* females foraged on alfalfa that had just been sprayed with novaluron (delivered with a hand sprayer at full field rate, 745 ml/ha) or that had been sprayed with this same IGR 1 or 2 wk prior to bee presence. Compared with controls (0%), significantly more of the resulting nest cells contained pollen balls with dead eggs (5–26%). A pollen ball is a provision mass with an unhatched egg or no egg at all (Pitts-Singer 2004). The ovicidal effect may have been from the mother bees' ingestion of contaminated nectar just after application, or ingestion of chemicals when cutting leaf pieces more than a week post-spray.

Highlights, Areas of Concern, and Research Needs

The routes of exposure that we describe here are certainly not the first to be proposed. However, our scenarios are distinct in their focus on solitary cavity-nesting bees. Other diagrammatic conceptual models heavily emphasize pesticide risks to honey bees, and to a lesser extent to bumble bees, while the few models that depict exposure for other bees offer scant details (Cutler et al. 2014, Purdy 2014, USEPA, PMRA, and CDPR 2014, Heard et al. 2017). Although current pesticide evaluations for bee safety include ingestion and contact with honey bee adults and larvae, by testing only honey bees as the surrogate for all bees, we achieve an incomplete assessment of pesticide safety for all wild and managed pollinators and are left with many unanswered questions (Johansen and Mayer 1990, Biddinger et al. 2013, Arena and Sgolastra 2014, Sanchez-Bayo and Goka 2014).

Our models for solitary bees reveal areas where we lack an understanding of how and at what levels these bees may incur higher exposure risks than honey bees or bumble bees due to differences in nesting, foraging, and social behaviors. A solitary bee may experience different exposure routes, have dissimilar pesticide susceptibility and immune response, and present different or unexpected sublethal symptoms and effects (Sandrock et al. 2013, Arena and Sgolastra 2014, Gill and Raine 2014, Jin et al. 2015). Awareness of the interaction and fate of pesticides in the environment on account of their physical properties

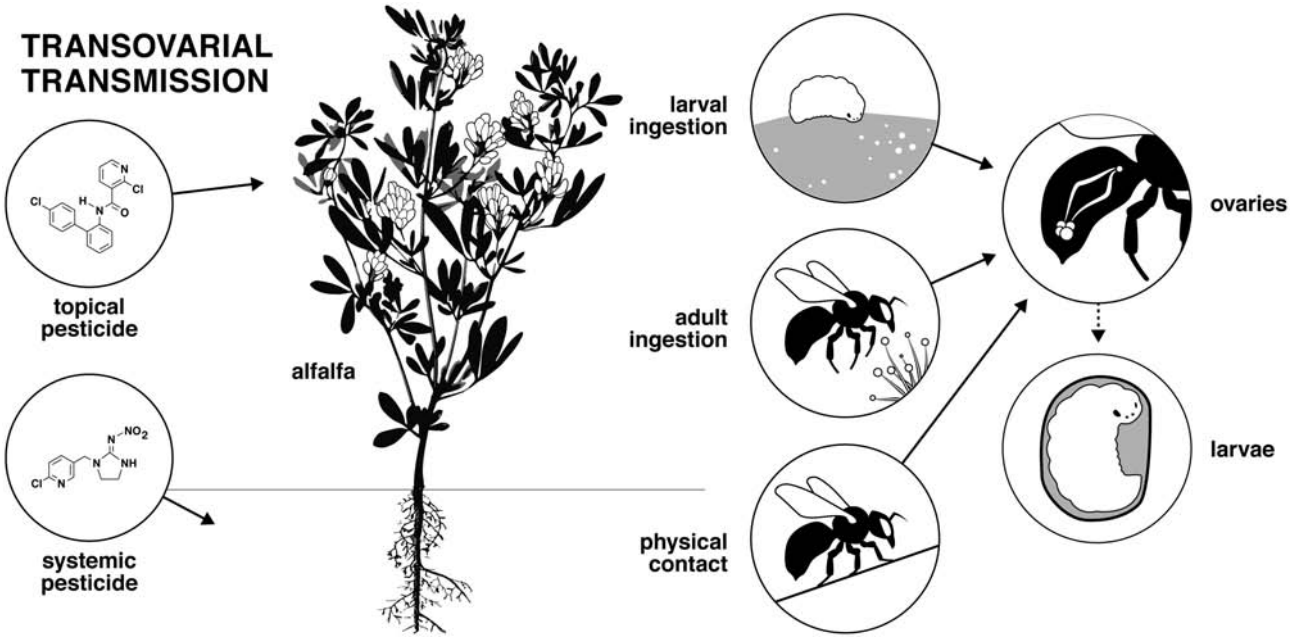

Fig. 5. Transovarial transmission exposure route with alfalfa plant example. Pesticides in the mother's system affect (often kill) her eggs, health of her offspring, or reproductive output. Illustration by James Bradford.

will help in formulating hypotheses about the probability and extent of risk in a bee's foraging range and activity portfolio.

Pesticides of most concern for exposure risk to all bees include those that easily contaminate pollen and nectar, affecting both adult and larval stages. Additionally important for solitary bee exposure are those pesticides that are expressed in leaves and are persistent in soils. Not all pesticides are equally relevant in their persistence and movement in the environment, and therefore their likelihood of coming into contact with bees via the various routes of exposure can be predicted by their chemical properties. Systemic and translaminar pesticides (e.g., neonicotinoids and benzoylureas, respectively) will provide a route of exposure for bees that use vegetative materials in nest construction. Chemicals persistent in the soil (e.g., pyrethroids, spinosyns, and anthanilic diamides) can be present year-round in soils collected by orchard bees for use during nesting.

Using products with specific targets, modes, or action on immatures only, or low environmental persistence may indeed reduce risk to pollinators in some cases. However, in other cases such as for *M. rotundata* used as a pollinator in alfalfa seed production fields treated for *Lygus* control with an IGR, the simple act of cutting leaf pieces exposes these bees both topically and orally, which results in all four possible routes of pesticide exposure.

Some government agencies (e.g., United States, European Union, and Australia) are moving toward pesticide evaluations for not only honey bees, but also for bumble bees and some solitary bees (e.g., the European red mason bee, *O. bicornis*; Haskell and McEwen 1998, EFSA 2014). New techniques and protocols are needed across the globe for making standard assessments on non-*Apis* bees and for performing bioassays that better explore the kinds of exposure routes we describe, especially those that extend beyond the worst case scenarios described for honey bees by USEPA, PMRA, and CDPR (2014). Expectations of lethal, sublethal, and synergistic effects need to be based on a thorough understanding of all exposure routes, including the levels of potential contamination in each route under various conditions and how each route contributes to varying amounts of bee exposure through contact, ingestion, transmission, and their combinations. Beyond the routes already investigated under current guidelines for honey bees, additional important routes may be realized using an ecosystem approach that examines a representative set of bees to consider situations unique to non-*Apis* wild and managed bees, and how ecosystem services may be disrupted as a consequence (Stanley et al. 2015). With a robust understanding of routes of pesticide exposure in pollinators, more realistic and effective studies can be conducted to better grasp what direct and indirect factors might lead to pollinator stress, decline, or extinction.

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