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# Honey Bee Toxicology

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## Keywords

xenobiotics, insecticides, fungicides, *Apis mellifera*, interactions

## Abstract

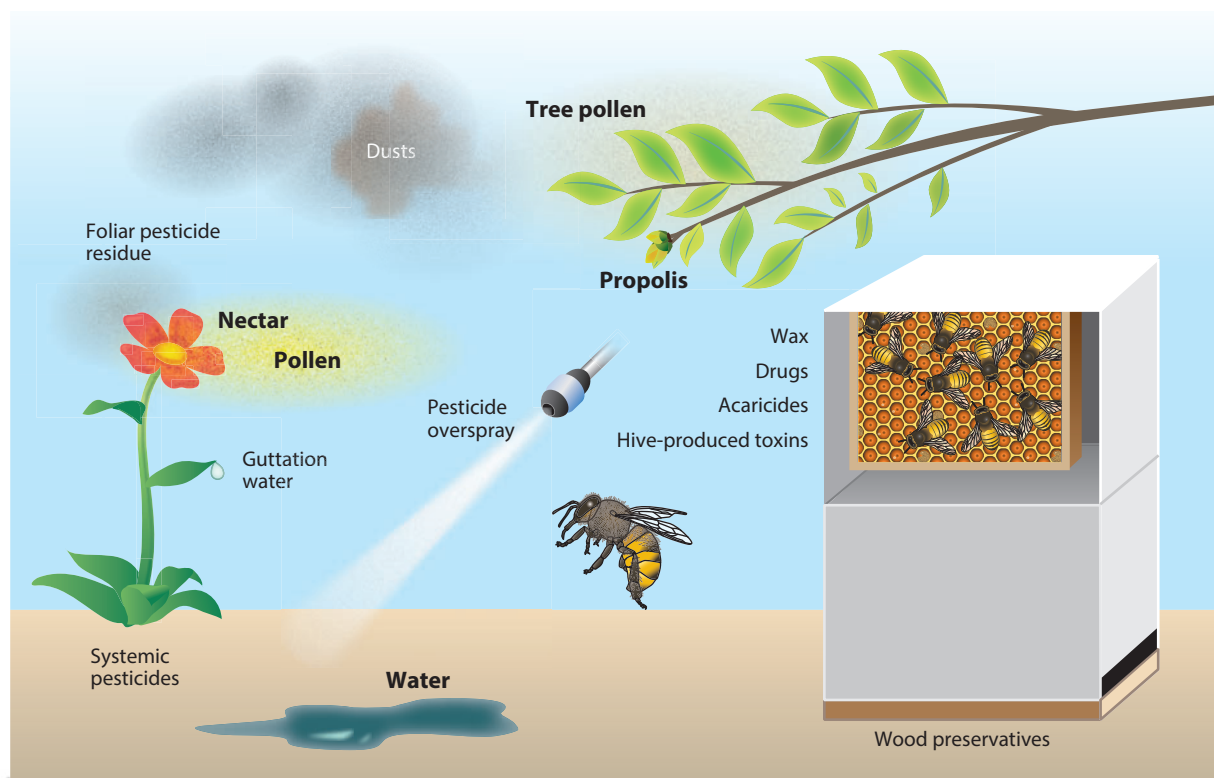
Insecticides are chemicals used to kill insects, so it is unsurprising that many insecticides have the potential to harm honey bees (*Apis mellifera*). However, bees are exposed to a great variety of other potentially toxic chemicals, including flavonoids and alkaloids that are produced by plants; mycotoxins produced by fungi; antimicrobials and acaricides that are introduced by beekeepers; and fungicides, herbicides, and other environmental contaminants. Although often regarded as uniquely sensitive to toxic compounds, honey bees are adapted to tolerate and even thrive in the presence of toxic compounds that occur naturally in their environment. The harm caused by exposure to a particular concentration of a toxic compound may depend on the level of simultaneous exposure to other compounds, pathogen levels, nutritional status, and a host of other factors. This review takes a holistic view of bee toxicology by taking into account the spectrum of xenobiotics to which bees are exposed.

## INTRODUCTION

Honey bees (*Apis mellifera*) are exposed to an ever-changing array of xenobiotics from both natural and synthetic sources. Previous comprehensive reviews of honey bee toxicology focused on the effects of pesticide, particularly insecticide, exposure on bees (3, 66). The importance of the interaction between bees and insecticides has not diminished despite continual discovery of new active ingredients and new modes of action that can be used to control insect pests. The late twentieth century also saw drastic changes in the toxicology of practical beekeeping, with beekeepers beginning to use pesticides inside the colony in the effort to control pests and pathogens. Although the natural xenobiotics present in plants and the environment has changed little, much has been learned over the last decades about the interaction between bees and the natural toxins that exist in their environment.

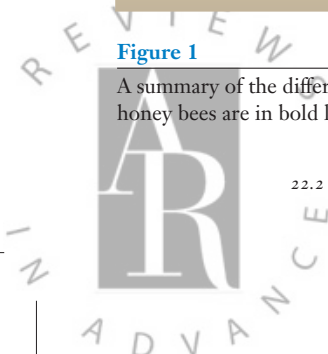
## EXPOSURE TO XENOBIOTICS

Thousands of older foraging worker honey bees travel as far as 10 km from the hive (31) in the course of collecting the nectar, pollen, water, and propolis needed to sustain a colony of tens of thousands of young adult workers, immature bees, and reproductives. While foraging over this large area, foragers encounter toxic materials of both natural and synthetic origin, and they may bring these xenobiotics back to the colony (Figure 1). Bees collect nectar to satisfy the



**Figure 1**

A summary of the different routes by which honey bees may be exposed to potentially toxic xenobiotics. Materials collected by foraging honey bees are in bold letters.



carbohydrate needs of the colony, but this food source is not entirely innocuous. From 9% to 55% of nectars produced by flowers also contain plant-synthesized xenobiotics (76, 122, 123, 150), and the sugars present in some nectars are indigestible (100). Bees collect pollen as their principal source of amino acids and sterols, but most pollen also contains phenolic xenobiotics with potentially toxic biological activity (8, 69, 121, 148).

Nectar and pollen may contain environmental pollutants (20, 60, 73) or systemic pesticides (113) drawn from the soil, or they can be contaminated from topical pesticide applications or drift from such applications (66, 77). Insecticidal toxins expressed in genetically engineered crops may also be present in pollen (39, 83, 91).

Bees also collect propolis from tree buds to use as a sealant, glue, and antimicrobial agent within the hive. Propolis contains a rich suite of phenolics with biological activity (22). Bees also collect water from environmental sources to dilute honey and cool the colony. Among these environmental sources, surface water (60, 66, 82) or guttation water produced by plants at the leaf margins may be contaminated with high concentrations of systemic insecticides (131).

Xenobiotics can appear inside the colony through other means, including fungi and bacteria that produce toxic compounds (48, 99). Beekeepers also add drugs to the colony environment to control pathogens and parasites, including the devastating mite *Varroa destructor* (68, 109, 143). Both drugs and agricultural pesticides can persist over many years in the wax combs (93). In addition, when forage is scarce beekeepers may feed sugar and protein supplements containing a fraction of carbohydrates toxic to bees (8, 79).

## INORGANIC COMPOUNDS

### Gasses

Bees generate CO<sub>2</sub> through metabolic processes and can achieve concentrations as high as 4.25% in summer and 6% in winter (75). When adult workers are briefly exposed to 80% CO<sub>2</sub> narcosis is achieved, which is useful in some experimental situations, but narcosis comes at the cost of a reduction in worker life span and premature transition from nursing to foraging tasks (97). CO<sub>2</sub>-induced narcosis induces ovary development and oviposition in queens while suppressing ovary development in workers and causing a reduction in the concentration of biogenic amines in the brains of both castes (54). Another anesthetic gas, N<sub>2</sub>O, can cause a similar reduction in the life span of workers but does not promote egg laying in queens (110).

### Metals and Environmental Contaminants

Honey bees have been used to assess the level of environmental contamination within the colony's 7-km<sup>2</sup> foraging range (19). Contaminated soil particles may stick to bees, or contaminants may be taken up by plants and incorporated into pollen and nectar (19, 60, 144). Such contaminants include the so-called heavy metals, which can occur naturally in the environment but are often encountered by bees because of human activities.

Arsenic (As), which was applied as an agricultural insecticide in the early 1900s and also released as an industrial pollutant, causes acute mortality at 400–500 µg/bee—a field-relevant dose in orchards sprayed with lead arsenate during bloom (87). Arsenic interferes with cellular metabolism and can produce oxidative stress (107). Hives placed along a gradient of industrial As and cadmium (Cd) pollution from a smelter contained fewer bees and produced less honey in areas where metal contamination of pollen and bees was higher (2–20 ppm body burden) (20). Cd can block the Ca<sup>2+</sup> channel and impair the function of muscles in honey bees (28).



Selenium (Se) is an industrial and agricultural pollutant that accumulates in some plants and is present in pollen collected by bees at levels as high as 2,830 mg/kg (61). Se is toxic because it replaces sulfur in amino acids, which changes protein conformation. However, oral exposure to inorganic forms of Se is more toxic to adult bees ( $LC_{50} = 1$  mg/L) than exposure to the selenoamino acids ( $LC_{50} = 5$  mg/L) (60). Honey bee larvae are about 30 times more sensitive to selenoamino acids and 50 times more sensitive to inorganic Se than adults (60).

Honey bees may bear metals from environmental sources external to the hive at concentrations as high as 80 ppm (144), but they are also exposed to chromium (Cr), copper (Cu), tin (Sn), and As owing to wood preservatives used on hive equipment (72). Sn- and As-based preservatives, but not copper naphthenate, have been associated with increased winter mortality of colonies (72). However, copper naphthenate use has been associated with reduced brood survival and increased queen replacement (14). Compared with use of other wood preservatives, copper naphthenate use is associated with greater contamination of bees (21 ppm) (144).

## NATURAL TOXINS IN FOOD

### Toxic Carbohydrates

Although bees live longest when fed sucrose, the sugars fructose, glucose, maltose, melezitose, and trehalose are safe for bees to consume. In contrast, the monosaccharides arabinose, mannose, xylose, and galactose and oligosaccharides containing galactose (melibiose, raffinose, stachyose, and lactose) reduce the life span of adult bees at concentrations as low as 2% in syrup or nectar (8). Nectar of linden trees (*Tilia* spp.) contains mannose and may cause paralysis in bees because they lack the enzyme phosphomannose isomerase, which is needed to metabolize mannose (100). Other toxic sugars may harm bees through interference with trehalose metabolism (9).

Pollen contains pectins, polysaccharides containing galacturonic acid, in the pollen wall. Although toxic to bees at high concentrations (8), pectins may be metabolized by microorganisms living in the bee gut (42). Starches, which are polysaccharides of glucose, are reputed to be toxic to bees, but adult workers have the enzymes amylase and saccharase to break down starch and can utilize starches to power flight (63).

High-fructose corn syrup (HFCS) may be used to supplement colony nutrition (86), but it does contain some potentially toxic oligosaccharides and pectins (79) that may contribute to reduced colony performance (115). Soy-based protein supplements may also contain toxic saccharides, as approximately 40% of the sugars present in soybeans are toxic to bees (8). Hydroxymethylfurfural (HMF) is toxic to bees and is produced in a predictable manner over time when fructose is present in an acidic aqueous solution, such as honey or HFCS, and temperatures are above 40°C (79). Caged adult bees fed HFCS with 250 ppm HMF were less likely to survive to 26 days (79). The mechanism of HMF toxicity to bees is unknown.

### Phenolics

The flavonoids present in plant tissues can deter feeding or reduce the digestibility of the leaf material in folivorous insects other than bees (140). Honey and pollen contain a variety of flavonoids as well; these compounds are ubiquitous and distinctive in composition, such that flavonoid profiles can be used to validate the floral source of honey and pollen (136, 148). The yellow flavonoid quercetin and its glycoside rutin are the most common and abundant flavonoids in pollen, with combined concentrations reaching 300 ppm (121). Quercetin affects energy production through the inhibition of mitochondrial ATP synthase (62). Bees tolerate high levels of dietary quercetin



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through metabolism by CYP6AS subfamily cytochrome P450 monooxygenases (84). Moreover, bees may benefit from the presence of quercetin and other flavonoids in food because of their antioxidant or antimicrobial activity (140). Additionally, exposure to *p*-coumaric acid, pinobanksin 5-methyl ether, and pinocembrin—phenolics present in honey—increased the expression of detoxification genes (86).

Some of the phenolics present in honey may be derived from the propolis bees use to seal and line the hive (86). Research on propolis has focused on the activity of caffeic acid phenethyl ester, which has demonstrable antibiotic, antifungal, antiviral, and antitumor activity (135). Whole propolis extracts also extend the life of bees exposed to naturally occurring aflatoxins (98).

### Cyanogenic Glycosides

The cyanogenic glycoside amygdalin is produced by bee-attractive trees in the genus *Prunus* and is present in almond nectar at concentrations as high as 10 ppm (123); this may pose a hazard for bees, because the LC<sub>50</sub> of amygdalin for adult bees is 30 ppm (35). Hydrolysis of amygdalin yields toxic hydrogen cyanide, which interferes with energy production through inhibition of cytochrome *c* oxidase in the mitochondria (36).

### Alkaloids

Alkaloids are a diverse group of low-molecular-weight, nitrogen-containing compounds with many different modes of action (36). Many alkaloids are produced by plants to defend against herbivory, and alkaloid content is generally higher in leaves, anthers, and flowers than in the nectar and pollen that bees collect (35).

The pyrrolizidine alkaloids (PAs) are produced by approximately 3% of angiosperm plant species, particularly those in the family Asteraceae. PAs are hepatotoxic to mammals, but the mechanism of their toxicity to insects is not clear. Pollen can contain PAs or amine oxides at concentrations as high as 14,000 ppm PAs (17), which may be sufficient to harm bees, as sugar water containing 20,000 ppm PA caused acute mortality (108). Bees are incapable of active detoxification of PAs using amine oxidation (108).

The purine alkaloid caffeine, along with the related compounds theophylline and theobromine, is present in the nectar of *Citrus* spp. and *Coffea* spp. at concentrations from 10 to 95 ppm (76, 150) and in pollen up to 1,300 ppm (76). The LC<sub>50</sub> for caffeine is 2,000 ppm (35), and acute mortality likely occurs through increased Ca<sup>2+</sup> release from the endoplasmic reticulum that occurs at concentrations greater than 500 ppm (147). However, at lower concentrations caffeine appears to act as an antagonist of the adenosine receptor in the bee brain (150), and bees show a preference for sugar water with caffeine at 25 ppm (123), although it is repellent at 150 ppm (35, 123). At concentrations as low as 0.02 ppm, caffeine enhances the long-term memory of a sugar water reward in trained adult bees (150). There is evidence that bees can metabolize or remove caffeine from food through some mechanism, as honey made from caffeine-rich *Citrus* nectar contained only 5% of the expected caffeine (76).

### Natural Toxins Used in Beekeeping

Formic acid and oxalic acid are naturally present in honey in low concentrations and are applied by beekeepers at higher concentrations to control *Varroa* mites (68). Formic acid is used as a fumigant within the hive, where it kills mites by binding cytochrome *c* oxidase and inhibiting mitochondrial respiration, and possibly through neuroexcitatory activity as well (125). When used at therapeutic



rates, formic acid can, depending on conditions, reduce the survival of larvae (46), workers, and queens (47, 142), as well as reduce colony size and productivity (47).

Oxalic acid is a crystalline solid that is usually dissolved in water and trickled over the colony bees in a sugar solution (68). Oxalic acid forms needle-shaped calcium oxalate monohydrate crystals that cause kidney damage in mammals, although the mechanism of oxalic acid toxicity to arthropods is not well understood (88). Crystals of oxalic acid appear on the cuticle of treated bees, which likely stimulates grooming and removal of mites (118). Although bees avoid consumption of oxalic acid (2), some may be ingested, which may lead to the death of midgut cells (50) and reduce bee activity, nursing behavior, and longevity (118).

Bees are exposed to small amounts of the volatile monoterpenoids that advertise flowers, but monoterpenoids, principally thymol, are also used as fumigants for *Varroa* control (141). Thymol likely interacts with the GABA receptor in bees and mites (139). Treatment with thymol can contaminate honey at levels up to 8 ppm (43) and wax at levels up to 575 ppm (16). Thymol treatment can cause brood removal (43), and larval diet with 500 ppm thymol results in reduced brood survival (25). Adult bees from colonies treated with thymol for mite control or adult bees reared as larvae on diet with thymol (50 ppm) demonstrated reduced transcription of the gene encoding the vitellogenin protein (25).

### Natural Toxins Produced Within the Colony

*Aspergillus* fungi grow on the stored pollen, or beebread, within the hive and produce ochratoxins and aflatoxins (48). Animals that are not adapted for mycotoxin consumption bioactivate these compounds through cytochrome P450s to an epoxide form that intercalates into DNA, thereby preventing RNA synthesis and chromosomal replication (36). In culture conditions *Aspergillus* spp. from pollen produced mycotoxin concentrations of 1–5 ppm. Adult bees fed aflatoxin B1 or ochratoxin A in queen candy at 5 ppm or higher survived for 24 h but died over the subsequent three days (98). Honey bees appear to be adapted for mycotoxin exposure because cytochrome P450 activity detoxifies, rather than bioactivates, aflatoxin B1 and ochratoxin (98).

The diverse microbiota living within the guts of the bees (42) is another potential source of natural toxin exposure. An actinomycete in the genus *Nocardiopsis* is found in bee guts and produces phenazines, a family of nitrogenous heterocyclic compounds that act as respiratory electron acceptors and that also have demonstrated antibiotic activity against *Bacillus* spp. and other bacteria (99). Any harmful or beneficial effect of phenazines on bees has yet to be explored.

### BACILLUS THURINGIENSIS AND BT TOXINS

The soil bacterium *Bacillus thuringiensis* is widely used as a biological insecticide. *B. thuringiensis* kills insects by producing crystalline  $\delta$ -endotoxins (Cry) that are cleaved by proteases inside the insect gut; these cleavage products form pores in the midgut epithelium and cause osmotic shock to gut cells and, ultimately, septicemia and death (18). Different strains of *B. thuringiensis* produce different Cry toxins with high selectivity for particular insect groups. One strain of *B. thuringiensis*, var. aizawai, is used to control infestations of wax moths (*Galleria mellonella*) on stored comb (11). Cry toxins derived from wild *B. thuringiensis* populations can be detected in the guts of bees with little exposure to anthropogenic sources of Cry toxins (56).

Impacts of genetically engineered crops on bees depend not only on which toxins plants are engineered to produce but also on which tissues express the transgene. Most transgenic crops today express Cry toxins to control lepidopteran (Cry1, Cry2, and Cry9) and coleopteran (Cry3) pests (18). Generally, expression of Cry proteins in pollen of transgenic plants is very low

(<90 ng/mg) compared with Cry protein expression in leaf or root tissue (83, 91). Nectar, as a plant secretion rather than a plant tissue, contains few proteins of any kind (83). Laboratory and field tests with honey bees have not found harmful effects of pure Cry toxins or Cry toxins in pollen at field-relevant levels (39, 83). However, bees fed sugar syrup containing 5,000 ppb Cry1Ab, approximately 50 times the concentrations likely to be encountered in pollen, did consume less food and showed learning effects, as measured by the proboscis extension response assay (106). Newer crop varieties express multiple Cry toxins, but pollen from these plants has shown no effect on adult survival rate, body weight, pollen digestion, or gut microbial community (56).

## ANTIMICROBIAL COMPOUNDS

Beekeepers may apply antibacterial compounds inside the hive to suppress or control the bacterial pathogens causing American foulbrood (*Paenibacillus larvae*) and European foulbrood (*Melissococcus plutonius*) (109). Oxytetracycline hydrochloride, commonly applied as a dusting mixed with powdered sugar, has been used for the foulbrood diseases since the 1950s (134). The tetracyclines are broad-spectrum antibiotics produced by cultures of *Streptomyces* species that kill bacteria by preventing protein synthesis at the ribosome (36). Tetracyclines are transported through interactions with the P-glycoprotein transporter (55). Intestinal stem cells in the midguts of bees replicated more slowly in bees fed tetracycline at the recommended rate (44). A related antibiotic, chlortetracycline, slowed growth and caused precocious pigmentation in larvae reared in vitro with concentrations greater than 0.0025% (101). Other antibacterial compounds are used in hives, including sulfonamides and tylosin (109), but use of these has not yet been associated with bee toxicity.

Fumagillin is a naturally occurring compound isolated from *Aspergillus fumigatus* that is applied by beekeepers in a sugar solution to control the microsporidian gut parasites *Nosema ceranae* and *Nosema apis*. Fumagillin kills microsporidia by inhibiting protein modification by the enzyme methionine aminopeptidase 2 (64, 143). Studies with fumagillin at recommended treatment levels show no harmful effects on bees (143), but very low, subtherapeutic doses (1/1,000 recommended concentration) have been associated with increased *N. ceranae* infection and with altered gut protein expression (64).

## HERBICIDES AND FUNGICIDES

Herbicides and fungicides are not intended to kill insects, and their acute toxicity to adult bees is generally low. These pesticides do not carry label restrictions to reduce bee exposure, so bees may encounter high concentrations when they are applied to bee-attractive crops during bloom (102).

Bees likely suffer the greatest harm from herbicides through an indirect route—the loss of flowering plants producing nectar and pollen (66). Some herbicides may also have direct effects on bees; for example, paraquat is a model for the induction of oxidative stress in mammals (29) and has demonstrable effects on bees in laboratory studies. Caged workers sprayed with paraquat at the rate of 4.5 kg AI/ha died within three days (92), and workers injected with 15 µg paraquat experienced a tenfold reduction in median life span (29). Larvae may be affected as well, because reductions in the growth of larval oenocytes occurred when paraquat was present in larval diet at concentrations as low as 0.001 µg/kg (30). Vitellogenin, an abundant protein in the hemolymph of younger, well-fed workers, can extend the life of bees injected with paraquat (120), consistent with its known antioxidant properties.

Fungicides are widely detected in honey bee colonies (93). However, the effects of fungicide exposure are generally observed in honey bee brood rather than adults (5, 94, 154). The fungicide



chlorothalonil is detected at particularly high levels, up to 300 ppm, in bee-collected pollen and wax (93, 102). Chlorothalonil kills fungi through multiple modes of action and is metabolized by cytochrome P450s in mammals to a metabolite that causes oxidative stress (129). Larvae reared on a diet containing 34 mg/L chlorothalonil suffered 60% mortality (154). Captan, ziram, and iprodione caused elevated larval mortality when incorporated into diet at predicted field concentrations (94).

## INSECTICIDES WITH NERVE AND MUSCLE ACTION

### Acetylcholinesterase Inhibitors

Both organophosphate (OP) and methylcarbamate (MC) insecticides act on the insect nervous system by inhibiting the activity of acetylcholinesterase (AChE), the enzyme that inactivates the neurotransmitter acetylcholine in the synapses of the insect central nervous system (24). Both classes of AChE-inhibiting insecticides have an extremely broad range of toxicity to bees (topical  $LD_{50} = 0.018\text{--}31.2 \mu\text{g}/\text{bee}$ ; 53, 67). Some of the tolerance to particular MCs and OPs may be explained by poor inhibitory activity against the honey bee AChE (23) or by detoxification, rather than bioactivation, through cytochrome P450 activity (85). However, the subset of highly toxic OPs and MCs poses a substantial hazard to bees—of 117 bee-poisoning incidents investigated in the United Kingdom between 1994 and 2003, 57 were attributed to dimethoate, an OP, or bendiocarb, an MC (10).

The OP coumaphos is of such low acute toxicity ( $LD_{50} = 31.2 \mu\text{g}/\text{bee}$ ) that it is used by beekeepers to control *Varroa* mites (68). With repeated use, coumaphos builds up in the wax of colonies to concentrations as high as 90 ppm (26, 93). Use of coumaphos in colonies is associated with increased larval mortality of both queens and workers (14, 52). Larvae reared on diet containing 8 mg/L coumaphos were more likely to die during development than control larvae (154).

### Nicotinic Acetylcholine Receptor Agonists

The pyridine alkaloid nicotine is produced by plants in the genus *Nicotiana*. Nicotine mimics the neurotransmitter acetylcholine, activates the nicotinic acetylcholine receptor (nAChR), and promotes the generation of action potentials in postsynaptic nerve cells (24). Whereas leaves of *Nicotiana tabacum* contain up to 90,000 ppm nicotine, pollen may contain 23 ppm and nectar 0.1–5 ppm alkaloid content (35, 122). Bees appear to be capable of detoxifying nicotine in nectar through an unknown mechanism: Bees fed sugar water containing 50 ppm nicotine produced honey with only 3.2 ppm nicotine (122).

The median lethal concentration of nicotine for adult workers is 2,000 ppm (35). Adult bees in caged colonies fed 50 ppm nicotine in sugar syrup experienced no reduction in longevity (122), and survival of caged bees was extended when they were fed sugar syrup containing 0.5–50 ppm nicotine (74). Larvae, however, were sensitive to nicotine and were much more likely to die at the third or fourth larval instar when nicotine concentrations in syrup fed to colonies was greater than 5 ppm (122).

The neonicotinoids (also known as chloronicotinyls) are synthetic analogs of nicotine with much greater affinity than nicotine for the nAChR in the bee brain (96, 138). The selectivity of the neonicotinoids is determined by the pharmacophore—either the nitro ( $-\text{NO}_2$ ) or cyano ( $-\text{CN}$ ) group (138). The nitroguanidine neonicotinoids, including imidacloprid, clothianidin, thiamethoxam, and dinotefuran, are highly toxic to bees, with acute  $LD_{50}$ s from 0.004 to 0.075  $\mu\text{g}/\text{bee}$  (32, 65). The cyanoguanidine neonicotinoids, thiacloprid and acetamiprid, are much less toxic, with contact  $LD_{50}$ s in the range 7.1–14.6  $\mu\text{g}/\text{bee}$  (65).



The nitro- and cyanoguanidine groups of neonicotinoids display similar binding affinity for honey bee nAChR (96, 138). The relative tolerance of bees toward the cyanoguanidines is likely due to rapid cytochrome P450 detoxification because the toxicity of both acetamiprid and thiacloprid can be increased from 250- to 1,100-fold in the presence of a P450 inhibitor (65), and most C<sup>14</sup>-labeled acetamiprid was metabolized within 30 min of dosing (21). Imidacloprid, a nitroguanidine, is metabolized more slowly, with a half-life of 4 h (127), possibly through P450 activity (65). However, two of the five metabolites of imidacloprid, 5-hydroxyimidacloprid and imidacloprid olefin, have high affinity for the honey bee nAChR (96) and may contribute to bee mortality (127). The metabolites of acetamiprid, in contrast, demonstrate greatly reduced toxicity to honey bees (21, 65). Thiamethoxam has comparatively poor affinity for the insect nAChR (137), but it is rapidly converted to clothianidin, which has great affinity (95, 137).

Except for incidents where colonies are exposed to very high doses of neonicotinoids, enough to cause acute toxicity (45, 77), there is considerable controversy surrounding the effects of real-world neonicotinoid exposure on honey bee colonies. The field-relevant dose in a nectar load from a bee-attractive, flowering crop treated with imidacloprid (0.02–0.30 ng per nectar load or 0.7–10 µg/L) is not expected to cause toxic effects under either acute (LD<sub>50</sub> = 4.5 ng) or chronic (LC<sub>50</sub> = 1,760 µg/L) exposure conditions (32). Although acute lethal effects are not expected at this field-relevant level of exposure, a 6–20% reduction in the performance of adult bees is predicted (32). The nitroguanidine insecticides imidacloprid, thiamethoxam, and clothianidin all may impair the ability of foraging honey bees to return to the hive, but at doses higher than are expected when nectar is collected from treated flowering crops (51, 57, 117) (see sidebar, Sublethal and Colony-Level Effects of Insecticides).

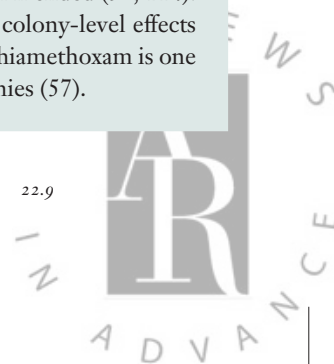
Spinosyns are naturally derived insecticides made by fermenting the soil bacterium *Saccharopolyspora spinosa*, and they are approved for use in organic agriculture. The spinosyns act as allosteric modulators of the nAChR, but at a site different from that targeted by the neonicotinoids (15, 24). Spinosad in sugar syrup fed to caged bees is relatively toxic (LC<sub>50</sub> = 7.34 ppm), but in field and semifield tests spinosad appears relatively safe for bees because of its low toxicity when dried on foliage (15).

### Voltage-Gated Na<sup>+</sup> Channel Agonists

Pyrethrins are terpenoids produced by pyrethrum flowers (*Chrysanthemum cinerariaefolium*) with potent insecticidal activity (36). It is possible that hymenopteran pollinators of these flowers

#### SUBLETHAL AND COLONY-LEVEL EFFECTS OF INSECTICIDES

Bees exposed to a high dose of an insecticide can be killed outright, but exposure to lower, sublethal doses is more common (89) and may affect the cognitive function, behavior, and physiology of bees (12, 34). Whereas sublethal effects for many insecticides are well documented using individual bees in laboratory (12, 32, 34, 40, 149) or field settings (57, 117), direct determination of colony-level effects of sublethal exposure is elusive (14, 103, 114). A major barrier to conducting whole-colony experiments is the natural variation between colonies that dictates large sample sizes: Depending on the effect being measured, 24 to 80 colonies per treatment group are recommended (32, 114). The use of colony modeling in silico provides a potential solution. A prediction of long-term colony-level effects from data on the homing behavior of foragers exposed to sublethal levels of the neonicotinoid thiamethoxam is one of the first reports of computer modeling being used to address sublethal effects in whole colonies (57).



encounter pyrethrins in the course of pollination. Pyrethrins make up as much as 3% of the flower's dry weight, though most of that content is associated with the seeds (49). Despite their natural origin, pyrethrins are highly toxic to bees ( $LD_{50} = 0.05\text{--}0.21 \mu\text{g}/\text{bee}$ ; 53). The pyrethroids are a large class of synthetic insecticides based on the structure and insecticidal activity of the pyrethrins, with a broad range of toxicity to bees ( $LD_{50} = 0.017\text{--}20 \mu\text{g}/\text{bee}$ ; 53, 67).

Natural pyrethrins, the pyrethroids, and DDT, an organochlorine insecticide, act on the voltage-gated  $\text{Na}^+$  channel in the axons of nerve cells, where they delay the closing of the  $\text{Na}^+$  channel and prolong the recovery period following transmission of an action potential (24). The pyrethroids modify  $\text{Na}^+$  channels in honey bee neurons with potency similar to that seen in other insects (71). Bees are more tolerant of some pyrethroids because of rapid detoxification by cytochrome P450s (59, 70, 85). The pyrethroids flumethrin and  $\tau$ -fluvalinate are used by beekeepers to control *Varroa* mites, but with repeated application they may also contaminate wax to levels as high as 200 ppm (26, 93). Larvae exposed to  $\tau$ -fluvalinate are less likely to survive at both the larval and the adult stages (14, 154).

### GABA-Gated $\text{Cl}^-$ Channel Agonists

Fipronil binds to the GABA-gated  $\text{Cl}^-$  channel in insect neurons, where it maintains the channel in an open state, leading to hyperpolarization of the neuron and an inability to transmit action potentials (24). Fipronil is highly toxic to bees (oral  $LD_{50} = 4 \text{ ng}/\text{bee}$ ; 126) and effectively blocks the GABA receptor in isolated neurons exposed to fipronil in concentrations as low as  $1 \mu\text{M}$  (7). Fipronil is a systemic insecticide and is detectable at low levels (1–4 ppb) in pollen (13). However, fipronil is also used by beekeepers inside traps for a hive pest, the small hive beetle (*Aethina tumida*). This use leads to very low contamination of honey (1 ppb) and no apparent negative effect on colony success (80).

### Octopamine Receptor Agonists

Amitraz, which is widely used by beekeepers to control *Varroa* mites (68), is an agonist of the octopamine receptor (152). Upon stimulation with octopamine, a second messenger is released, causing neuroexcitation and leading to behavioral effects in honey bees (119). The tolerance of bees to amitraz is unexplained but does not appear to be due to detoxification (59, 67). Amitraz is not detected in wax after use as an acaricide, but its breakdown product, 2,4-dimethylphenyl formamide (DMPF), is present in wax at concentrations as high as 43 ppm (93). The toxicity of the amitraz breakdown products DMPF and 2,4-dimethylaniline to honey bees has never been directly tested.

### Ryanodine Receptor Agonists

Members of the new anthranilic and phthalic diamide class of insecticides activate the ryanodine receptors in muscles by stimulating the release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum (38, 105). The ryanodine receptor in honey bees has a low binding affinity for chlorantraniliprole (105), which has a correspondingly low acute toxicity (oral  $LD_{50} > 104 \mu\text{g}/\text{bee}$ ; 38). Pollen collected by honey bees after chlorantraniliprole treatment contained as much as 2.6 ppm insecticide (38). Although there are no published reports on the effects of ryanodine receptor agonists on honey bees, application of chlorantraniliprole to blooming clover in lawns had no effect on bumble bee (*Bombus impatiens*) colonies (78).

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## INSECT GROWTH REGULATORS

The insect growth regulator insecticides are more toxic to larval bees than adult bees, as they specifically target insect development by mimicking the hormones juvenile hormone (JH) or the ecdysteroids, or by interfering with molting through inhibition of chitin synthesis (132, 152). Diflubenzuron, a chitin synthesis inhibitor, affects larval survival at concentrations as low as 1 ppm when directly administered to either larvae or whole colonies (132), but colonies did appear to recover following exposure (133).

Fenoxycarb is a JH mimic that is toxic to insects undergoing metamorphosis (152). Fenoxycarb may also affect adult worker honey bees, as an increase in JH titer accompanies the behavioral transition from nursing to foraging in aging adult workers (112). Fenoxycarb treatment appears to age adults prematurely (58). Whole colonies treated with a field rate of fenoxycarb immediately experienced high mortality of immature bees and reduced brood abundance a year following treatment (133).

## VARIABILITY IN TOXICITY

Contact with a xenobiotic is required for it to produce a toxic effect, but many natural toxins in nectar and pollen (6, 9, 35, 74, 108, 123) as well as pesticides (32, 106) are repellent to bees. However, bees appear to be poor at detecting the presence of toxic compounds in food (35), possibly because of the reduced complement of gustatory receptors in the bee genome (111). Instead, repellency may be the result of learned avoidance as a consequence of the negative physiological effects caused by exposure to a toxic compound (6).

The toxic effect produced by a particular xenobiotic also depends on the physiology and experience of an individual bee. Life stage (154), caste (33), age (120, 146), season (124, 146), temperature (89), feeding history (146), and concurrent or past exposure to other toxic compounds (67, 86, 154) can all modulate toxicity.

Individual bees from different colonies vary in their susceptibility to insecticides, possibly because of genetic differences (4, 41, 130). Widespread use of DDT throughout the 1950s may have selected for more tolerant populations of honey bees in California (4). Beekeepers' widespread use of  $\tau$ -fluvalinate to control *Varroa* mites in managed colonies may be responsible for the elevated pyrethroid tolerance observed in European honey bees (41). Susceptibility to imidacloprid varied dramatically among adult workers taken from different colonies, with LD<sub>50</sub> values ranging from 5 to 50 ng/bee (128), possibly owing to genetic differences.

Overwintered, older, or poorly fed bees were most susceptible to a suite of pesticides (124, 146). This sensitivity suggests that the antioxidant capacity of vitellogenin, a hemolymph protein with antioxidant properties that is abundant in young, well-fed bees, decreases the effect of exposure to toxic compounds with prooxidant properties (29, 120).

Bee colonies are exposed to multiple pesticides and natural xenobiotics simultaneously. An average of 6.5 pesticides were detected in North American colonies (93). Exposure to different xenobiotics that work through the same mode of action or target site would be expected to exhibit additive toxicity in bees (152, 154). Mixtures of xenobiotics exhibiting different modes of action may also produce synergistic or antagonistic effects and unexpected increases or reductions in toxicity (67). Combinations of compounds known to interact synergistically at the target site include amitraz and the pyrethroids at the voltage-gated Na<sup>+</sup> channel (67, 81), as well as caffeine and ryanodine at the Ca<sup>2+</sup> channel (147).

Other interactions between xenobiotics in mixtures occur by inhibition of or competition for detoxification enzymes. The honey bee genome includes fewer genes in the detoxificative gene families, including the cytochrome P450s, than the genomes of many other insects (27).



However, this reduction in detoxification genes does not prevent bees from carrying out the enzymatic functions associated with these gene families (153). The detoxification activity of the cytochrome P450 enzymes in bees can be inhibited by the sterol biosynthesis-inhibiting (SBI) group of fungicides. The SBI fungicides inhibit the P450-mediated detoxification of some pyrethroids (104) and neonicotinoids (116), with a resulting increase in insecticide toxicity to bees (65, 67). Competitive inhibition can also occur when xenobiotics compete for a limited pool of detoxification enzyme, as with  $\tau$ -fluvalinate and coumaphos, resulting in elevated toxicity (67, 85, 154). Detoxificative cytochrome P450 activity is also known to change seasonally (90, 124) and with the phytochemicals present in food (86). Multiple chemicals resident in old wax may interact to cause a delay in the development of larvae reared in old combs (151).

The interaction between toxic compounds and pathogens is a new area of research. Exposure to xenobiotics can, in some cases, make bees more resistant to pathogens. Colonies supplemented with propolis resin showed decreased infection with the fungal parasite that causes chalkbrood (*Ascosphaera apis*). Exposure to some pesticides may be more harmful to bees in combination with pathogen infection. In laboratory experiments adult bees infected with the gut parasites *N. apis* and *N. ceranae*, while being chronically exposed to the insecticides imidacloprid (1), thiacloprid, or fipronil (145), experienced reduced longevity but did not necessarily show elevated *Nosema* infection. Bees taken from whole colonies exposed to imidacloprid, however, did suffer from elevated *Nosema* infections (103). Pesticide exposure may also increase the susceptibility of bees to viral infection: Replication of deformed wing virus was enhanced in bees fed clothianidin and imidacloprid, likely because of reduced production of antiviral proteins resulting from negative modulation of the NF- $\kappa$ B immune signaling pathway, which in turn may have been a consequence of neonicotinoid effects on the neurons controlling the bees' immune response (37).

## CONCLUSION

Honey bees have been exposed to toxic compounds in their environment throughout their evolutionary history. Some natural toxins are well tolerated by, or even beneficial to, bees, whereas high concentrations of others can cause harm. Similarly, some synthetic pesticides can be used therapeutically by beekeepers, but others, particularly some insecticides, can have devastating effects when used carelessly. Continued research into the interactions between bees and the xenobiotics they encounter will serve as a basis to promote bee health as new drugs and pesticides are developed and evaluated.

### SUMMARY POINTS

1. The honey bee colony is a nexus for all of the toxic compounds that exist in an environment. Bees are simultaneously exposed to natural toxins from plants and microorganisms, pesticides, environmental contaminants, and apicultural drugs applied by the beekeeper.
2. Honey bees do not appear to be uniquely susceptible to toxic compounds in general but may be highly susceptible to particular compounds, especially certain insecticides. Exposure to some xenobiotics may be beneficial either through direct toxicity to pathogens and parasites or through modulation of detoxification or immune function.
3. Toxicity in honey bees is labile and varies depending on the particular circumstances of a colony or individual bee. Age, nutritional status, genetics, pathogens, and concurrent chemical exposure may all influence the toxic effect that is observed.



## FUTURE ISSUES

1. How can short-term toxicological experiments with individual bees over several days be used to predict colony-level effects over entire seasons or years? Colony modeling shows promise in bridging this gap, but much work remains to improve and validate model performance.
2. The number of possible interactions between different toxic compounds and a host of other factors (including but not limited to pathogen infection, phenology, and colony genetics) is astronomically large. Research is needed to understand the mechanistic basis of xenobiotic toxicity and interactions so that harmful situations can be predicted and avoided.
3. Further development of therapeutic drugs is needed to help beekeepers control *Varroa* spp., *Nosema* spp., viral infection, and the other pests and parasites in honey bee colonies. However, careful testing on bees is needed to ensure that these drugs are beneficial in field situations.
4. New pesticides will continue to be developed, and biotechnology will be applied to develop crops dependent on new methods to kill insect pests. New pest-control strategies, even if they appear safe for bees, may cause harm in ways that have not yet been considered.

## DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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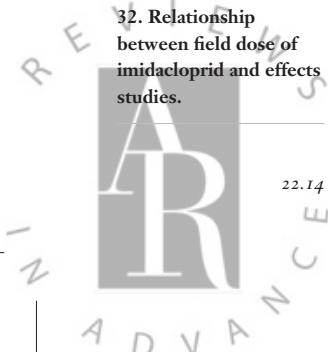
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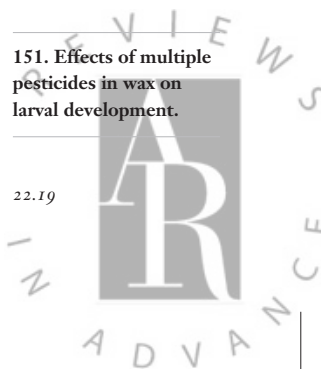
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#### RELATED RESOURCES

Agence. Natl. Secur. Sanit. Aliment. Environ. Trav. (ANSES). AGRITOX, Maisons-Alfort, Fr. <http://www.agritox.anses.fr>

US Environ. Prot. Agency. ECOTOX Database. Washington, DC. [http://cfpub.epa.gov/ecotox/ecotox\\_home.cfm](http://cfpub.epa.gov/ecotox/ecotox_home.cfm)

