Honey Bee Toxicology

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Keywords

Abstract

Insecticides are chemicals used to kill insects, so it is unsurprising that many
insecticides have the potential to harm honey bees (*Apis mellifera*). How-
ever, bees are exposed to a great variety of other potentially toxic chemicals,
including flavonoids and alkaloids that are produced by plants; mycotox-
ins produced by fungi; antimicrobials and acaricides that are introduced by
beekeepers; and fungicides, herbicides, and other environmental contami-
nants. 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 have 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
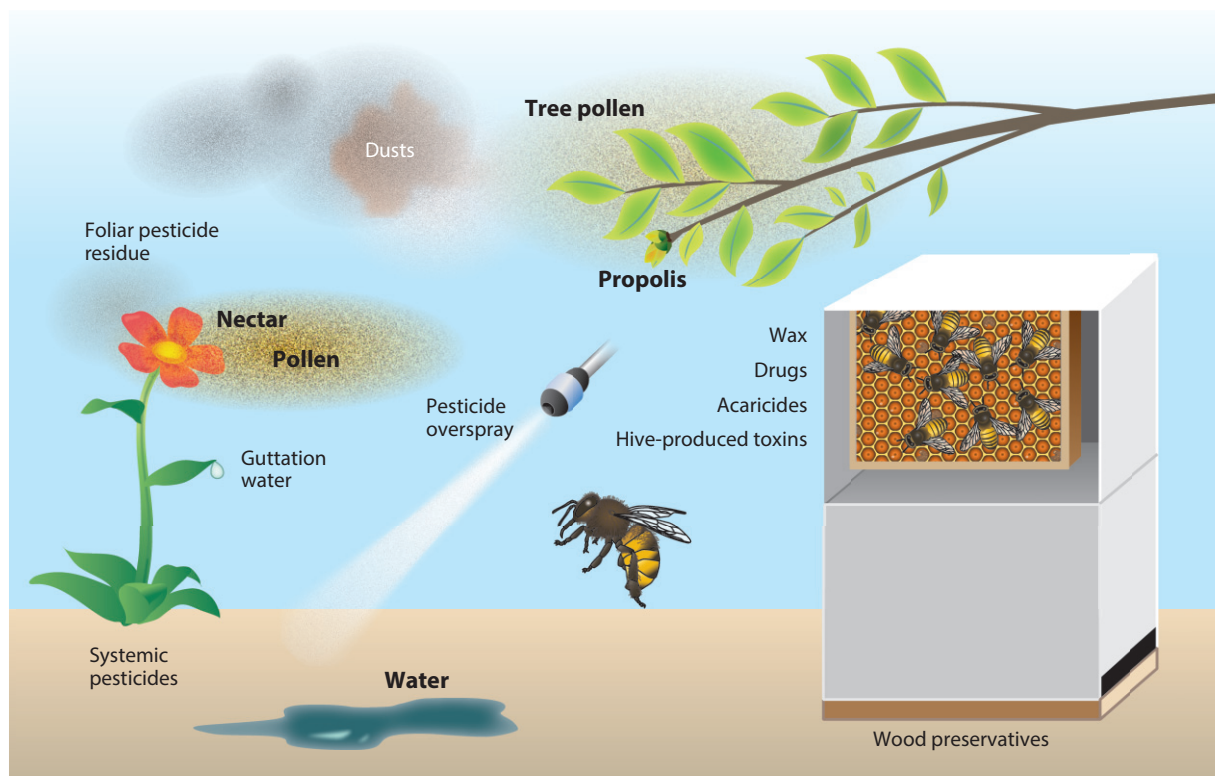

Figure 1

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 carbohydrates toxic to bees (8, 79).

INORGANIC COMPOUNDS

Gasses

Bees generate CO_2 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_2 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_2-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_2O, 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^2 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^{2+} 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 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_{50} 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_{50} for caffeine is 2,000 ppm (35), and acute mortality likely occurs through increased Ca^{2+} 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, CRY TOXINS, AND BT CROPS

The soil bacterium *Bacillus thuringiensis* is widely used as a biological insecticide. *B. thuringiensis* kills insects by producing crystalline δ-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).

Many transgenic crops today express genes from *B. thuringiensis* to produce 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 (*Paenbacillus 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 antioxidant protein in the hemolymph of younger, well-fed workers, can extend the life of bees injected with paraquat (120).

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} = 0.018–31.2 μg/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 μg/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 a 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 ($-NO_2$) or cyano ($-CN$) 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 μg/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 μg/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^{14}-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_{50} = 4.5 ng) or chronic (LC_{50} = 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 only 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_{50} = 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$^+$ Channel Agonists

Pyrethrins are terpenoids produced by pyrethrum flowers (*Chrysanthemum cinerariaefolium*) with potent insecticidal activity (36). It is possible that visitors to these flowers encounter

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).

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} = 0.05–0.21 µg/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} = 0.017–20 µg/bee; 53, 67).

Natural pyrethrins, the pyrethroids, and DDT, an organochlorine insecticide, act on the voltage-gated Na^+ channel in the axons of nerve cells, where they delay the closing of the Na^+ channel and prolong the recovery period following transmission of an action potential (24). The pyrethroids modify 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 τ-fluvalinate are used by bee-keepers 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 τ-fluvalinate are less likely to survive at both the larval and the adult stages (14, 154).

GABA-Gated Cl^- Channel Agonists

Fipronil binds to the GABA-gated 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 ng/bee; 126) and effectively blocks the GABA receptor in isolated neurons exposed to fipronil in concentrations as low as 1 µM (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 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 µg/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).

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 τ-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_{50} 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^+ channel (67, 81), as well as caffeine and ryanodine at the Ca^{2+} 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 τ-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 xenobiotics may accumulate in beeswax over time (16, 93) and 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*) (155). 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-κB 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.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Alaux C, Brunet J-L, Dussaubat C, Mondet F, Tchamitchan S, et al. 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environ. Microbiol.* 12(3):774–82
2. Aliano NP, Ellis MD. 2008. Bee-to-bee contact drives oxalic acid distribution in honey bee colonies. *Apidologie* 39(5):481–87
3. Anderson LD, Atkins EL. 1968. Pesticide usage in relation to beekeeping. *Annu. Rev. Entomol.* 13(1):213–38
4. Atkins EL, Anderson LD. 1962. DDT resistance in honey bees. *J. Econ. Entomol.* 55(5):791–92
5. Atkins EL, Kellum D. 1986. Comparative morphogenic and toxicity studies on the effect of pesticides on honey bee brood. *J. Apic. Res.* 25(4):242–55
6. Ayestaran A, Giurfa M, de Brito Sanchez MG. 2010. Toxic but drank: gustatory aversive compounds induce post-ingestional malaise in harnessed honeybees. *PLOS ONE* 5(10):e15000
7. Barbara GS, Zube C, Rybak J, Gauthier M, Grünewald B. 2005. Acetylcholine, GABA and glutamate induce ionic currents in cultured antennal lobe neurons of the honeybee, *Apis mellifera*. *J. Comp. Physiol. A* 191(9):823–36

8. Barker RJ. 1977. Some carbohydrates found in pollen and pollen substitutes are toxic to honey bees. *J. Nutr.* 107(10):1859–62

9. Barker RJ, Lehner Y. 1974. Acceptance and sustenance value of naturally occurring sugars fed to newly emerged adult workers of honey bees (*Apis mellifera* L.). *J. Exp. Zool.* 187(2):277–85

10. Barnett EA, Charlton AJ, Fletcher MR. 2007. Incidents of bee poisoning with pesticides in the United Kingdom, 1994–2003. *Pest Manag. Sci.* 63(11):1051–57

11. Basedow T, El-Shafie HAF, Al-Ajlan AM, Abo-El-Saad MM. 2012. Evaluation of *Bacillus thuringiensis aizawai* and neem for controlling the larvae of the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae). *Int. J. Agric. Biol.* 14(4):629–32

12. Belzunces LP, Tchamitchian S, Brunet J-L. 2012. Neural effects of insecticides in the honey bee. *Apidologie* 43(3):348–70

13. Bernal J, Garrido-Bailón E, del Nozal MJ, González-Porto AV, Martín-Hernández R, et al. 2010. Overview of pesticide residues in stored pollen and their potential effect on bee colony (*Apis mellifera*) losses in Spain. *J. Econ. Entomol.* 103(6):1964–71

14. Berry JA, Hood WM, Pietravalle S, Delaplane KS. 2013. Field-level sublethal effects of approved bee hive chemicals on honey bees (*Apis mellifera* L). *PLOS ONE* 8(10):e76536

15. Biondi A, Mommaerts V, Smagghe G, Viñuela E, Zappalà L, Desneux N. 2012. The non-target impact of spinosyns on beneficial arthropods. *Pest Manag. Sci.* 68(12):1523–36

16. Bogdanov S, Kilchenmann V, Imdorf A. 1998. Acaricide residues in some bee products. *J. Apic. Res.* 37(2):57–67

17. Boppre M, Colegate SM, Edgar JA. 2005. Pyrrolizidine alkaloids of *Echium vulgare* honey found in pure pollen. *J. Agric. Food Chem.* 53(3):594–600

18. Bravo A, Likitvivatanavong S, Gill SS, Soberón M. 2011. *Bacillus thuringiensis*: a story of a successful bioinsecticide. *Insect Biochem. Mol. Biol.* 41(7):423–31

19. Bromenshenk JJ, Carlson SR, Simpson JC, Thomas JM. 1985. Pollution monitoring of Puget Sound with honey bees. *Science* 227(4687):632–34

20. **Bromenshenk JJ, Gudatis JL, Carlson SR, Thomas JM, Simmons MA. 1991. Population dynamics of honey bee nucleus colonies exposed to industrial pollutants. *Apidologie* 22(4):359–69**

21. Brunet J-L, Badiou A, Belzunces LP. 2005. In vivo metabolic fate of [^{14}C]-acetamiprid in six biological compartments of the honeybee, *Apis mellifera* L. *Pest Manag. Sci.* 61(8):742–48

22. Burdock GA. 1998. Review of the biological properties and toxicity of bee propolis (propolis). *Food Chem. Toxicol.* 36(4):347–63

23. Camp HB, Fukuto TR, Metcalf RL. 1969. Toxicity of isopropyl parathion: effect of structure on toxicity and anticholinesterase activity. *J. Agric. Food Chem.* 17(2):243–48

24. **Casida JE, Durkin KA. 2013. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annu. Rev. Entomol.* 58:99–117**

25. Charpentier G, Vidau C, Ferdy J-B, Tabart J, Vetillard A. 2014. Lethal and sub-lethal effects of thymol on honeybee (*Apis mellifera*) larvae reared in vitro. *Pest Manag. Sci.* 70(1):140–47

26. Chauzat M-P, Faucon J-P. 2007. Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L.) in France. *Pest Manag. Sci.* 63(11):1100–6

27. Claudianos C, Ranson H, Johnson RM, Biswas S, Schuler MA, et al. 2006. A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. *Insect Mol. Biol.* 15(5):615–36

28. Collet C, Belzunces L. 2007. Excitable properties of adult skeletal muscle fibres from the honeybee *Apis mellifera*. *J. Exp. Biol.* 210(3):454–64

29. Corona M, Velarde RA, Remolina S, Moran-Lauter A, Wang Y, et al. 2007. Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proc. Natl. Acad. Sci. USA* 104(17):7128–33

30. Cousin M, Silva-Zacarin E, Kretzschmar A, El Maataoui M, Brunet J-L, Belzunces LP. 2013. Size changes in honey bee larvae oenocytes induced by exposure to paraquat at very low concentrations. *PLOS ONE* 8(5):e65693

31. Couvillon MJ, Schürch R, Ratnieks FLW. 2014. Dancing bees communicate a foraging preference for rural lands in high-level agri-environment schemes. *Curr. Biology* 24(11):1212–15

32. **Cresswell J. 2011. A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology* 20(1):149–57**

20. Effect of toxic metals in the environment on whole colonies.

24. Explanation of insecticide modes of action.

32. Relationship between field dose of imidacloprid and effects studies.

33. Dahlgren L, Johnson RM, Siegfried BD, Ellis MD. 2012. Comparative toxicity of acaricides to honey bee (Hymenoptera: Apidae) workers and queens. *J. Econ. Entomol.* 105(6):1895–902

34. Desneux N, Decourtye A, Delpuech JM. 2007. The sublethal effects of pesticides on beneficial arthropods. *Annu. Rev. Entomol.* 52:81–106

35. **Detzel A, Wink M. 1993. Attraction, deterrence or intoxication of bees (*Apis mellifera*) by plant allelochemicals. *Chemoecology* 4(1):8–18**

36. Dewick PM. 2009. *Medicinal Natural Products: A Biosynthetic Approach*. Chichester, UK: Wiley. 3rd ed.

37. Di Prisco G, Cavaliere V, Annoscia D, Varricchio P, Caprio E, et al. 2013. Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. *Proc. Natl. Acad. Sci. USA* 110(46):18466–71

38. Dinter A, Brugger KE, Frost NM, Woodward MD. 2009. Chlorantraniliprole (Rynaxypyr): a novel DuPont[TM] insecticide with low toxicity and low risk for honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris*) providing excellent tools for uses in integrated pest management. *Julius-Kühn-Archiv* 423:84–96

39. Duan JJ, Marvier M, Huesing J, Dively G, Huang ZY. 2008. A meta-analysis of effects of Bt crops on honey bees (Hymenoptera: Apidae). *PLOS ONE* 3(1):e1415

40. Eiri DM, Nieh JC. 2012. A nicotinic acetylcholine receptor agonist affects honey bee sucrose responsiveness and decreases waggle dancing. *J. Exp. Biol.* 215(12):2022–29

41. Elzen PJ, Elzen GW, Rubink W. 2003. Comparative susceptibility of European and Africanized honey bee (Hymenoptera: Apidae) ecotypes to several insecticide classes. *Southwest. Entomol.* 28(4):255–60

42. Engel P, Martinson VG, Moran NA. 2012. Functional diversity within the simple gut microbiota of the honey bee. *Proc. Natl. Acad. Sci. USA* 109(27):11002–7

43. Floris I, Satta A, Cabras P, Garau VL, Angioni A. 2004. Comparison between two thymol formulations in the control of *Varroa destructor*: effectiveness, persistence, and residues. *J. Econ. Entomol.* 97(2):187–91

44. Forkpah C, Dixon LR, Fahrbach SE, Rueppell O. 2014. Xenobiotic effects on intestinal stem cell proliferation in adult honey bee (*Apis mellifera* L.) workers. *PLOS ONE* 9(3):e91180

45. Forster R. 2009. Bee poisoning caused by insecticidal seed treatment of maize in Germany in 2008. *Julius-Kühn-Archiv* 423:126–31

46. Fries I. 1991. Treatment of sealed honey bee brood with formic acid for control of *Varroa jacobsoni*. *Am. Bee J.* 131:313–14

47. Giovenazzo P, Dubreuil P. 2011. Evaluation of spring organic treatments against *Varroa destructor* (Acari: Varroidae) in honey bee *Apis mellifera* (Hymenoptera: Apidae) colonies in eastern Canada. *Exp. Appl. Acarol.* 55(1):65–76

48. González G, Hinojo MJ, Mateo R, Medina A, Jiménez M. 2005. Occurrence of mycotoxin producing fungi in bee pollen. *Int. J. Food Microbiol.* 105(1):1–9

49. Grdiša M, Carović-Stanko K, Kolak I, Šatović Z. 2009. Morphological and biochemical diversity of Dalmatian pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.). *Agric. Conspec. Sci. ACS* 74(2):73–80

50. Gregorc A, Smodiš Škerl MI. 2007. Toxicological and immunohistochemical testing of honeybees after oxalic acid and rotenone treatments. *Apidologie* 38(3):296–305

51. Guez D. 2013. A common pesticide decreases foraging success and survival in honey bees: questioning the ecological relevance. *Front. Physiol.* 4:37

52. Haarmann T, Spivak M, Weaver D, Weaver B, Glenn T. 2002. Effects of fluvalinate and coumaphos on queen honey bees (Hymenoptera: Apidae) in two commercial queen rearing operations. *J. Econ. Entomol.* 95(1):28–35

53. **Hardstone MC, Scott JG. 2010. Is *Apis mellifera* more sensitive to insecticides than other insects? *Pest Manag. Sci.* 66(11):1171–80**

54. Harris JW, Woodring J, Harbo JR. 1996. Effects of carbon dioxide on levels of biogenic amines in the brains of queenless worker and virgin queen honey bees (*Apis mellifera*). *J. Apic. Res.* 35(2):69–78

55. Hawthorne DJ, Dively GP. 2011. Killing them with kindness? In-hive medications may inhibit xenobiotic efflux transporters and endanger honey bees. *PLOS ONE* 6(11):e26796

35. Repellence and toxicity for 63 toxins in nectar and pollen.

53. Broad comparison of insecticide toxicity between bees and other insects.

56. Hendriksma HP, Küting M, Härtel S, Näther A, Dohrmann AB, et al. 2013. Effect of stacked insecticidal Cry proteins from maize pollen on nurse bees (*Apis mellifera carnica*) and their gut bacteria. *PLOS ONE* 8(3):e59589

57. **Henry M, Béguin M, Requier F, Rollin O, Odoux J-F, et al. 2012. A common pesticide decreases foraging success and survival in honey bees. *Science* 336(6079):348–50**

58. Heylen K, Gobin B, Arckens L, Huybrechts R, Billen J. 2011. The effects of four crop protection products on the morphology and ultrastructure of the hypopharyngeal gland of the European honeybee, *Apis mellifera*. *Apidologie* 42(1):103–16

59. Hillier NK, Frost EH, Shutler D. 2013. Fate of dermally applied miticides fluvalinate and amitraz within honey bee (Hymenoptera: Apidae) bodies. *J. Econ. Entomol.* 106(2):558–65

60. Hladun KR, Kaftanoglu O, Parker DR, Tran KD, Trumble JT. 2013. Effects of selenium on development, survival, and accumulation in the honeybee (*Apis mellifera* L.). *Environ. Toxicol. Chem.* 32(11):2584–92

61. Hladun KR, Parker DR, Tran KD, Trumble JT. 2013. Effects of selenium accumulation on phytotoxicity, herbivory, and pollination ecology in radish (*Raphanus sativus* L.). *Environ. Pollut.* 172:70–75

62. Hong S, Pedersen PL. 2008. ATP synthase and the actions of inhibitors utilized to study its roles in human health, disease, and other scientific areas. *Microbiol. Mol. Biol. Rev.* 72(4):590–641

63. Hrassnigg N, Brodschneider R, Fleischmann PH, Crailsheim K. 2005. Unlike nectar foragers, honeybee drones (*Apis mellifera*) are not able to utilize starch as fuel for flight. *Apidologie* 36(4):547–57

64. Huang W-F, Solter LF, Yau PM, Imai BS. 2013. *Nosema ceranae* escapes fumagillin control in honey bees. *PLOS Pathog.* 9(3):e1003185

65. Iwasa T, Motoyama N, Ambrose J, Roe RM. 2004. Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*. *Crop Prot.* 23(5):371–78

66. Johansen CA. 1977. Pesticides and pollinators. *Annu. Rev. Entomol.* 22:177–92

67. Johnson RM, Dahlgren L, Siegfried BD, Ellis MD. 2013. Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). *PLOS ONE* 8(1):e54092

68. Johnson RM, Ellis MD, Mullin CA, Frazier M. 2010. Pesticides and honey bee toxicity—USA. *Apidologie* 41(3):312–31

69. Johnson RM, Mao W, Pollock HS, Niu G, Schuler MA, Berenbaum MR. 2012. Ecologically appropriate xenobiotics induce cytochrome P450s in *Apis mellifera*. *PLOS ONE* 7(2):e31051

70. Johnson RM, Wen Z, Schuler MA, Berenbaum MR. 2006. Mediation of pyrethroid insecticide toxicity to honey bees (Hymenoptera: Apidae) by cytochrome P450 monooxygenases. *J. Econ. Entomol.* 99(4):1046–50

71. Kadala A, Charreton M, Jakob I, Le Conte Y, Collet C. 2011. A use-dependent sodium current modification induced by type I pyrethroid insecticides in honeybee antennal olfactory receptor neurons. *Neurotoxicology* 32(3):320–30

72. Kalnins MA, Detroy BF. 1984. The effect of wood preservative treatment of beehives on honey bees and hive products. *J. Agric. Food Chem.* 32(5):1176–80

73. Kezic N, Lucic D, Sulimanovic D. 1992. Induction of mixed function oxidase activity in honey bee as a bioassay for detection of environmental xenobiotics. *Apidologie* 23(3):217–23

74. Köehler A, Pirk CWW, Nicolson SW. 2012. Honeybees and nectar nicotine: deterrence and reduced survival versus potential health benefits. *J. Insect Physiol.* 58(2):286–92

75. Kozak PR, Currie RW. 2011. Laboratory study on the effects of temperature and three ventilation rates on infestations of *Varroa destructor* in clusters of honey bees (*Hymenoptera: Apidae*). *J. Econ. Entomol.* 104(6):1774–82

76. Kretschmar JA, Baumann TW. 1999. Caffeine in citrus flowers. *Phytochemistry* 52(1):19–23

77. Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K. 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLOS ONE* 7(1):e29268

78. Larson JL, Redmond CT, Potter DA. 2013. Assessing insecticide hazard to bumble bees foraging on flowering weeds in treated lawns. *PLOS ONE* 8(6):e66375

79. LeBlanc BW, Eggleston G, Sammataro D, Cornett C, Dufault R, et al. 2009. Formation of hydroxymethylfurfural in domestic high-fructose corn syrup and its toxicity to the honey bee (*Apis mellifera*). *J. Agric. Food Chem.* 57(16):7369–76

80. Levot GW. 2008. An insecticidal refuge trap to control adult small hive beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae) in honey bee colonies. *J. Apic. Res.* 47(3):222–28

81. Liu M-Y, Plapp FW. 1992. Mechanism of formamidine synergism of pyrethroids. *Pestic. Biochem. Physiol.* 43(2):134–40

82. Main AR, Headley JV, Peru KM, Michel NL, Cessna AJ, Morrissey CA. 2014. Widespread use and frequent detection of neonicotinoid insecticides in wetlands of Canada's prairie pothole region. *PLOS ONE* 9(3):e92821

83. Malone LA, Burgess EPJ. 2009. Impact of genetically modified crops on pollinators. In *Environmental Impact of Genetically Modified Crops*, ed. N Ferry, AMR Gatehouse, pp. 199–224. Oxfordshire, UK: CABI

84. Mao W, Rupasinghe SG, Johnson RM, Zangerl AR, Schuler MA, Berenbaum MR. 2009. Quercetin-metabolizing CYP6AS enzymes of the pollinator *Apis mellifera* (Hymenoptera: Apidae). *Comp. Biochem. Physiol. B* 154(4):427–34

85. Mao W, Schuler MA, Berenbaum MR. 2011. CYP9Q-mediated detoxification of acaricides in the honey bee (*Apis mellifera*). *Proc. Natl. Acad. Sci. USA* 108:12657–62

86. Mao W, Schuler MA, Berenbaum MR. 2013. Honey constituents up-regulate detoxification and immunity genes in the western honey bee *Apis mellifera*. *Proc. Natl. Acad. Sci. USA* 110:8842–46

87. McIndoo NE. 1926. *Effects on Honeybees of Spraying Fruit Trees with Arsenicals*. Washington, DC: US Dep. Agric.

88. McMartin K. 2009. Are calcium oxalate crystals involved in the mechanism of acute renal failure in ethylene glycol poisoning? *Clin. Toxicol.* 47(9):859–69

89. Medrzycki P, Sgolastra F, Bortolotti L, Bogo G, Tosi S, et al. 2010. Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides. *J. Apic. Res.* 49(1):52–59

90. Meled M, Thrasyvoulou A, Belzunces LP. 1998. Seasonal variations in susceptibility of *Apis mellifera* to the synergistic action of prochloraz and deltamethrin. *Environ. Toxicol. Chem.* 17(12):2517–20

91. Mendelsohn M, Kough J, Vaituzis Z, Matthews K. 2003. Are *Bt* crops safe? *Nat. Biotechnol.* 21(9):1003–9

92. Moffett JO, Morton HL, MacDonald RH. 1972. Toxicity of some herbicidal sprays to honey bees. *J. Econ. Entomol.* 65(1):32–36

93. Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, et al. 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. *PLOS ONE* 5(3):e9754

94. Mussen EC, Lopez JE, Peng CYS. 2004. Effects of selected fungicides on growth and development of larval honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). *Environ. Entomol.* 33(5):1151–54

95. Nauen R, Ebbinghaus-Kintscher U, Salgado VL, Kaussmann M. 2003. Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pestic. Biochem. Physiol.* 76(2):55–69

96. Nauen R, Ebbinghaus-Kintscher U, Schmuck R. 2001. Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). *Pest Manag. Sci.* 57(7):577–86

97. Nicolas G, Sillans D. 1989. Immediate and latent effects of carbon dioxide on insects. *Annu. Rev. Entomol.* 34:97–116

98. Niu G, Johnson RM, Berenbaum MR. 2011. Toxicity of mycotoxins to honeybees and its amelioration by propolis. *Apidologie* 42(1):79–87

99. Patil PB, Zeng Y, Coursey T, Houston P, Miller I, Chen S. 2010. Isolation and characterization of a *Nocardiopsis* sp. from honeybee guts. *FEMS Microbiol. Lett.* 312(2):110–18

100. Pawlikowski T. 2010. Pollination activity of bees (Apoidea: Apiformes) visiting the flowers of *Tilia cordata* Mill. and *Tilia tomentosa* Moench in an urban environment. *J. Apic. Sci.* 54(2):73–79

101. Peng Y-SC, Mussen E, Fong A, Montague MA, Tyler T. 1992. Effects of chlortetracycline of honey bee worker larvae reared in vitro. *J. Invertebr. Pathol.* 60(2):127–33

102. Pettis JS, Lichtenberg EM, Andree M, Stitzinger J, Rose R, vanEngelsdorp D. 2013. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. *PLOS ONE* 8(7):e70182

103. Pettis JS, vanEngelsdorp D, Johnson J, Dively G. 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. *Naturwissenschaften* 99(2):153–58

104. Pilling ED, Jepson PC. 1993. Synergism between EBI fungicides and a pyrethroid insecticide in the honeybee (*Apis mellifera*). *Pestic. Sci.* 39(4):293–97

86. Honey, pollen, and propolis xenobiotics affect detoxification capacity.

105. Qi S, Casida JE. 2013. Species differences in chlorantraniliprole and flubendiamide insecticide binding sites in the ryanodine receptor. *Pestic. Biochem. Physiol.* 107(3):321–26

106. Ramirez-Romero R, Desneux N, Decourtye A, Chaffiol A, Pham-Delègue MH. 2008. Does Cry1ab protein affect learning performances of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae)? *Ecotoxicol. Environ. Saf.* 70(2):327–33

107. Ratnaike RN. 2003. Acute and chronic arsenic toxicity. *Postgrad. Med. J.* 79(933):391–96

108. Reinhard A, Janke M, von der Ohe W, Kempf M, Theuring C, et al. 2009. Feeding deterrence and detrimental effects of pyrrolizidine alkaloids fed to honey bees (*Apis mellifera*). *J. Chem. Ecol.* 35(9):1086–95

109. Reybroeck W, Daeseleire E, De Brabander HF, Herman L. 2012. Antimicrobials in beekeeping. *Vet. Microbiol.* 158(1–2):1–11

110. Rindfleisch JK. 1977. The use of nitrous oxide as an anesthetic for honey bees. *Am. Bee J.* 117:622

111. Robertson HM, Wanner KW. 2006. The chemoreceptor superfamily in the honey bee, *Apis mellifera*: expansion of the odorant, but not gustatory, receptor family. *Genome Res.* 16(11):1395–403

112. Robinson GE. 1987. Regulation of honey bee age polyethism by juvenile hormone. *Behav. Ecol. Sociobiol.* 20(5):329–38

113. Rortais A, Arnold G, Halm M-P, Touffet-Briens F. 2005. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36(1):71–83

114. Rose R, Dively G, Pettis J. 2007. Effects of Bt corn pollen on honey bees: emphasis on protocol development. *Apidologie* 38(4):368–77

115. Sammataro D, Weiss M. 2013. Comparison of productivity of colonies of honey bees, *Apis mellifera*, supplemented with sucrose or high fructose corn syrup. *J. Insect Sci.* 13(19):1–13

116. Schmuck R, Stadler T, Schmidt HW. 2003. Field relevance of a synergistic effect observed in the laboratory between an EBI fungicide and a chloronicotinyl insecticide in the honeybee (*Apis mellifera* L., Hymenoptera). *Pest Manag. Sci.* 59(3):279–86

117. Schneider CW, Tautz J, Grünewald B, Fuchs S. 2012. RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. *PLOS ONE* 7(1):e30023

118. Schneider S, Eisenhardt D, Rademacher E. 2012. Sublethal effects of oxalic acid on *Apis mellifera* (Hymenoptera: Apidae): changes in behaviour and longevity. *Apidologie* 43(2):218–25

119. Schulz DJ, Robinson GE. 2001. Octopamine influences division of labor in honey bee colonies. *J. Comp. Physiol. A* 187(1):53–61

120. Seehuus S-C, Norberg K, Gimsa U, Krekling T, Amdam GV. 2006. Reproductive protein protects functionally sterile honey bee workers from oxidative stress. *Proc. Natl. Acad. Sci. USA* 103(4):962–67

121. Serra Bonvehí J, Soliva Torrentó M, Centelles Lorente E. 2001. Evaluation of polyphenolic and flavonoid compounds in honeybee-collected pollen produced in Spain. *J. Agric. Food Chem.* 49(4):1848–53

122. Singaravelan N, Inbar M, Ne'eman G, Distl M, Wink M, Izhaki I. 2006. The effects of nectar-nicotine on colony fitness of caged honeybees. *J. Chem. Ecol.* 32(1):49–59

123. Singaravelan N, Nee'man G, Inbar M, Izhaki I. 2005. Feeding responses of free-flying honeybees to secondary compounds mimicking floral nectars. *J. Chem. Ecol.* 31(12):2791–804

124. Smirle MJ, Winston ML. 1987. Intercolony variation in pesticide detoxification by the honey bee (Hymenoptera: Apidae). *J. Econ. Entomol.* 80(1):5–8

125. Song C, Scharf ME. 2008. Formic acid: a neurologically active, hydrolyzed metabolite of insecticidal formate esters. *Pestic. Biochem. Physiol.* 92(2):77–82

126. Stoner KA, Eitzer BD. 2013. Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (*Apis mellifera*) in Connecticut. *PLOS ONE* 8(10):e77550

127. Suchail S, De Sousa G, Rahmani R, Belzunces LP. 2004. In vivo distribution and metabolisation of ^{14}C-imidacloprid in different compartments of *Apis mellifera* L. *Pest Manag. Sci.* 60(11):1056–62

128. Suchail S, Guez D, Belzunces LP. 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ. Toxicol. Chem.* 20(11):2482–86

129. Suzuki T, Nojiri H, Isono H, Ochi T. 2004. Oxidative damages in isolated rat hepatocytes treated with the organochlorine fungicides captan, dichlofluanid and chlorothalonil. *Toxicology* 204(2–3):97–107

130. Tahori AS, Sobel Z, Soller M. 1969. Variability in insecticide tolerance of eighteen honey-bee colonies. *Entomol. Exp. Appl.* 12(1):85–98

131. Tapparo A, Giorio C, Marzaro M, Marton D, Soldà L, Girolami V. 2011. Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds. *J. Environ. Monit.* 13(6):1564–68

132. Tasei J-N. 2001. Effects of insect growth regulators on honey bees and non-*Apis* bees: a review. *Apidologie* 32(6):527–45

133. Thompson HM, Wilkins S, Battersby AH, Waite RJ, Wilkinson D. 2005. The effects of four insect growth-regulating (IGR) insecticides on honeybee (*Apis mellifera* L.) colony development, queen rearing and drone sperm production. *Ecotoxicology* 14(7):757–69

134. Tian B, Fadhil NH, Powell JE, Kwong WK, Moran NA. 2012. Long-term exposure to antibiotics has caused accumulation of resistance determinants in the gut microbiota of honeybees. *mBio* 3(6):e00377-12

135. Tolba MF, Azab SS, Khalifa AE, Abdel-Rahman SZ, Abdel-Naim AB. 2013. Caffeic acid phenethyl ester, a promising component of propolis with a plethora of biological activities: a review on its anti-inflammatory, neuroprotective, hepatoprotective, and cardioprotective effects. *IUBMB Life* 65(8):699–709

136. Tomás-Barberán FA, Martos I, Ferreres F, Radovic BS, Anklam E. 2001. HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *J. Sci. Food Agric.* 81(5):485–96

137. Tomizawa M, Casida JE. 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annu. Rev. Pharmacol. Toxicol.* 45:247–68

138. Tomizawa M, Otsuka H, Miyamoto T, Eldefrawi ME, Yamamoto I. 1995. Pharmacological characteristics of insect nicotinic acetylcholine receptor with its ion channel and the comparison of the effect of nicotinoids and neonicotinoids. *J. Pestic. Sci.* 20(1):57–64

139. Tong F, Coats JR. 2012. Quantitative structure–activity relationships of monoterpenoid binding activities to the housefly GABA receptor. *Pest Manag. Sci.* 68(8):1122–29

140. Treutter D. 2005. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biol.* 7(6):581–91

141. Umpiérrez ML, Santos E, González A, Rossini C. 2011. Plant essential oils as potential control agents of varroatosis. *Phytochem. Rev.* 10(2):227–44

142. Underwood RM, Currie RW. 2003. The effects of temperature and dose of formic acid on treatment efficacy against *Varroa destructor* (Acari: Varroidae), a parasite of *Apis mellifera* (Hymenoptera: Apidae). *Exp. Appl. Acarol.* 29(3–4):303–13

143. van den Heever JP, Thompson TS, Curtis JM, Ibrahim A, Pernal SF. 2014. Fumagillin: an overview of recent scientific advances and their significance for apiculture. *J. Agric. Food Chem.* 62(13):2728–37

144. van der Steen JJM, de Kraker J, Grotenhuis T. 2012. Spatial and temporal variation of metal concentrations in adult honeybees (*Apis mellifera* L.). *Environ. Monit. Assess.* 184(7):4119–26

145. Vidau C, Diogon M, Aufauvre J, Fontbonne R, Viguès B, et al. 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. *PLOS ONE* 6(6):e21550

146. Wahl O, Ulm K. 1983. Influence of pollen feeding and physiological condition on pesticide sensitivity of the honey bee *Apis mellifera carnica*. *Oecologia* 59(1):106–28

147. Walz B, Baumann O, Zimmermann B, Ciriacy-Wantrup EV. 1995. Caffeine-sensitive and ryanodine-sensitive Ca^{2+}-induced Ca^{2+} release from the endoplasmic reticulum in honeybee photoreceptors. *J. Gen. Physiol.* 105(4):537–67

148. Wiermann R. 1968. Phenylpropanoid metabolism by pollen. I. Survey of flavonoid components isolated from gymnosperms and angiosperms. *Ber. Dtsch. Bot. Ges.* 81:3–16

149. Williamson SM, Moffat C, Gomersall MAE, Saranzewa N, Connolly CN, Wright GA. 2013. Exposure to acetylcholinesterase inhibitors alters the physiology and motor function of honeybees. *Front. Physiol.* 4:13

150. Wright GA, Baker DD, Palmer MJ, Stabler D, Mustard JA, et al. 2013. Caffeine in floral nectar enhances a pollinator's memory of reward. *Science* 339(6124):1202–4

151. **Wu JY, Anelli CM, Sheppard WS. 2011. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. *PLOS ONE* 6(2):e14720**

151. Effects of multiple pesticides in wax on larval development.

152. Yu SJ. 2008. *The Toxicology and Biochemistry of Insecticides*. Boca Raton, FL: CRC. 1st ed.

153. Yu SJ, Robinson FA, Nation JL. 1984. Detoxication capacity in the honey bee, *Apis mellifera* L. *Pestic. Biochem. Physiol.* 22(3):360–68

154. Zhu W, Schmehl DR, Mullin CA, Frazier JL. 2014. Four common pesticides, their mixtures and a formulation solvent in the hive environment have high oral toxicity to honey bee larvae. *PLOS ONE* 9(1):e77547

155. Simone-Finstrom MD, Spivak M. 2012. Increased resin collection after parasite challenge: a case of self-medication in honey bees? *PLOS ONE* 7(3):e34601

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**