

Does the Honey Bee “Risk Cup” Runneth Over? Estimating Aggregate Exposures for Assessing Pesticide Risks to Honey Bees in Agroecosystems

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ABSTRACT: Honey bees (*Apis mellifera*) are uniquely vulnerable to nontarget pesticide impacts because, as ubiquitous managed pollinators, they are deliberately transported into areas where crops are grown with pesticides. Moreover, attributes making them excellent managed pollinators, including large long-lived colonies and complex behavior, also make them challenging subjects for toxicity bioassays. For over 150 years, improvements in formulation and delivery of pesticides, increasing their environmental and temporal presence, have had unintended consequences for honey bees. Since 1996, the Environmental Protection Agency has used “aggregate risk”—exposure risks to all possible sources—to set tolerances; once a “risk cup” is filled, no new pesticide or use can be approved unless risks are reduced elsewhere. The EPA now recommends a modeling approach for aggregating all exposure risks for bees, with differential lifestage sensitivity and exposure probabilities. Thus, the honey bee is the first insect with its own “risk cup”—a technological innovation that may not have unintended consequences for this beleaguered beneficial species.

KEYWORDS: *Apis mellifera*, colony collapse disorder, eusociality, Food Quality Protection Act, pesticide residue, systemic

■ INTRODUCTION

Certain biological characteristics of the western honey bee *Apis mellifera* make this species uniquely well-suited to function as a managed pollinator.¹ The eusocial honey bee lives in large perennial colonies with tens of thousands of workers capable of collecting nectar and pollen from a tremendous diversity of plant species. By virtue of a symbolic language, a forager can communicate to nestmates the location of particular floral resources, increasing the likelihood of revisitation of conspecific flowers and thus increasing the odds of successful pollination and fertilization. They are also by nature cavity-nesters, building nests in such natural cavities as hollow tree trunks, and thus adjust readily to establishing nests in human-made structures.

Although humans have engaged in beekeeping since biblical times, little success was achieved in improving upon the basic biology of *A. mellifera* as a pollinator until the onset of the Industrial Revolution. A transformation unprecedented in the history of apiculture took place in the second half of the 19th century. Among the first improvements was in the design of man-made housing for honey bees, culminating in the invention of the moveable frame hive in 1852.² In addition to increasing efficiencies associated with reuse of wax comb and reducing the susceptibility of managed bees to diseases, the wooden box-style hive allowed beekeepers to transport entire colonies reliably to meet time-sensitive pollination needs. Improvements in transportation allowed hives to be moved over increasingly longer distances, initially by train and subsequently by trucks in the early 20th century, and contributed to the development of large-scale American tree and row crop agriculture.³

At the same time apiculture was undergoing a transformation, American agriculture, too, was being remade by the Industrial Revolution with the introduction of chemical

pesticides for crop protection. Improvements in pesticide technology have a long history of unintended consequences for beneficial insects in general and for the honey bee in particular. Honey bees are uniquely vulnerable to nontarget impacts of agricultural pesticide use largely because, as the world’s premier managed pollinator species, they are deliberately transported by beekeepers into areas where crops are grown (often with the help of chemical pesticides) to provide pollination services.

■ INORGANIC INSECTICIDES AND THE BEGINNINGS OF HONEY BEE TOXICOLOGY

Large-scale commercial agriculture received a significant boost from technology aimed at improving pesticide efficiency and safety in the final quarter of the 19th century. The inorganic pesticides of the era, based on heavy metals such as arsenic and lead, posed significant health threats to applicators, which constrained their use. In 1874, the invention of the backpack sprayer enhanced the ability of growers to dispense lower volumes of pesticides over greater distances while reducing the risk of personal exposure. Subsequent inventions, including the barrel spray pump in 1880 and the cyclone spray nozzle in 1887, expanded the range and accuracy of sprays even further without significantly increasing applicator risk. Paris Green, or copper(II) acetate triarsenite, was introduced into the United States in 1867 initially for use against the Colorado potato

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beetle (*Leptinotarsa decemlineata*), but by 1879 it was being used in U.S. orchards to combat codling moths (*Cydia pomonella*) and plum curculio (*Conotrachelus nenuphar*), among other tree pests.⁴ With the widespread adoption of pesticide application technology that made pesticide delivery over large geographic areas feasible, copper-based inorganic insecticides were replaced with cheaper alternatives, including lead arsenate, on a wide range of field and orchard crops.

Almost immediately, a debate emerged on whether broadcast sprays presented a threat to beneficial species, specifically, to the honey bee. According to F. M. Webster,⁵ the “prevailing opinion seems to favor the theory, that if arsenical mixtures are sprayed or dusted upon fruit trees while the latter are in bloom, the bees which frequent them will be destroyed”. Yet Webster took pains to point out that not all fruit-growers subscribed to this viewpoint, citing “good negative evidence” from one “Mr. Edwin Yenowine”, a “fruit-grower near New Albany, Ind.”, to counter the prevailing wisdom. Yenowine claimed to have sprayed “all sorts of fruits freely...both in and out of the blooming season, and instead of destroying his bees they have increased from 8 to 17 strong, healthy colonies”. Such anecdotal accounts introduced, if not doubts, then at least confusion as to whether “judicious and cautious use” of arsenicals could spare bees from destruction.

Notwithstanding anecdotal evidence, legislation to protect bees from agricultural chemicals was passed by the Ontario Legislature in April 1890, according to which “No person in spraying or sprinkling fruit trees during the period within which such trees are in full bloom shall use, or cause to be used, any mixture containing Paris green, or any other poisonous substance injurious to bees” (Annual Report of the Department of Agriculture of the Province of Ontario 1893, Volume 1). Some harbored few doubts even in the absence of definitive evidence. Professor J. H. Panton, of the Ontario Agricultural College, remarked that “Although there has been no analysis of the bodies of the dead bees for the purpose of ascertaining the presence of arsenic, still the death of the bees is so intimately associated with spraying that there seems but little reason to believe otherwise than that the bees have been poisoned by Paris green used in spraying”.

In August 1891, at their annual meeting in Washington, DC, members of the Association of Economic Entomologists tackled the question of nontarget impact of arsenical pesticides on bees. According to the meeting report, published in *Insect Life*, Prof. J. A. Lintner (then State Entomologist of New York) called for experiments “to prove that bees were ever killed by the spraying of fruit trees”.⁶ He doubted if they were ever killed in this way. To the credit of those attending the meeting who felt that there was “little room for doubt as to the injury done by spraying during fruit bloom”, the group collectively agreed that definitive evidence was in fact lacking and appointed a committee to take up the issue.

F. M. Webster of the Agricultural Experiment Station of Ohio was designated to head the committee of three to conduct the definitive experiments on whether arsenicals sprayed on fruit trees can kill bees. Yet obtaining irrefutable evidence of what seemed obvious to many was an elusive goal. Webster commenced a series of experiments enclosing a hive under a sprayed plum tree with netting; although bees died, whether the cause of death was arsenic exposure or their desperate struggles to escape the netting could not be determined. Professor A. J. Cook conducted experiments in which bees were fed sweetened water dosed with arsenic; although they, too, died, the

objection could be raised that “in his experiments...the bees were fed in his laboratory within a small cage. Bees are known to die very soon in confinement, even without an arsenical diet”. Reviewing the experiments, Lintner⁷ remained unconvinced, and the work of the committee was “not deemed conclusive”. Documenting adverse impacts of pesticide exposure on honey bees had proved to be scientifically more challenging than had been initially imagined.

More extensive experiments, particularly those conducted in orchards at the Cornell Agricultural Experiment Station in Geneva, NY, USA, were sufficiently compelling to convince the majority of entomologists that, in fact, spraying fruit trees while in bloom was ill-advised. Root and Root,⁸ in their “bible” of beekeeping, *The ABC and XYZ of Beekeeping*, decisively stated that “Now that spraying with various poisonous liquids has come to be almost universal among fruit-growers, the question arises, Shall such spraying be done during the time the trees are in bloom...? If it is administered when the petals are out, bees are almost sure to be poisoned, much brood will be killed, and many times valuable queens are lost”. Regional laws prohibiting spraying fruit trees during bloom had already been instituted in Ontario in 1892, in Vermont in 1896, in New York in 1898, and in Michigan in 1905; Nebraska and Colorado in turn enacted such a ban in 1913, with Kentucky following suit in 1915 and Utah in 1919. Their impact, however, was severely limited by virtue of the fact that no enforcement agency existed to monitor compliance and penalties for violations were trivial.⁹

■ AERIAL SPRAYING, LAWSUITS, AND EVIDENTIARY ISSUES

After World War I, pesticide application technology changed dramatically with the availability of airplanes for pesticide delivery on a hitherto unprecedented scale. The first aerial application of an insecticide was the delivery of lead arsenate dust to control catalpa sphinx caterpillars (*Ceratomia catalpae*) in Ohio.¹⁰ Combined with the adoption of (cheaper) calcium arsenate and mechanized improvements in pesticide delivery systems (including those deploying airplane propellers to enhance dispersal), bee losses mounted dramatically and lawsuits began to proliferate.¹¹ One of the early suits was over damage caused to an apiary by drift from aerial spraying of nearby lettuce fields by an independent contractor (*S. A. Gerrard Co. v. Fricker*, 42 Ariz. 503, 27 P.2d 678 (1933); *S. A. Gerrard Co. v. Fricker*, Az S Ct 27 P. 2d 678, (1933)). The Arizona Supreme Court supported the plaintiff, finding that, although lettuce farmers have a legal right to rid their fields of pests, hiring a contractor to spray pesticides from an airplane does not release the grower from liability “because of the very great likelihood of the poisonous dust or spray spreading to adjoining or nearby premises and damaging or destroying valuable property thereon”. A beekeeper was also awarded damages in the case of *Miles v. A. Arena & Co.*, 73 P.2d 1260, 1263 (Cal. Ct. App. 1937), having lost 56 hives to pesticide drift from the defendant’s farm, based on the rule that “no landowner has the right to use his property in such a way that damage to a neighbor is foreseeable...the defendants should have foreseen the damage to plaintiff’s bees and were liable for damages”.¹² But recovery of damages was not a given; in *Lenk v. Spezia*, 213 P.2d 47, 51 Cal. Ct. App. 1949, the beekeeper could not recoup his losses after his bees were killed by arsenical insecticides because they encountered this pesticide while “trespassing on the fields of other owners of land”.

Throughout this period, the challenge of quantifying toxicity was helped enormously by the introduction of statistical measures for standardizing dose–responses and, apparently, the concept of an experimental control. Trevan¹³ pioneered the concept of the median lethal dose (LD_{50}), the individual dose that can kill 50% of a test population within a specified time, which soon became the standard for assessing toxicity. Expressed in units of mass of substance per unit mass of test animal, the LD_{50} allowed for comparisons of relative toxicity adjusted for differences in size of the test animal. Another key statistical innovation introduced in this era was probit analysis, which provided a way to understand toxicity as a function of the percentage of a pest population killed by a pesticide (in his case, insecticides being compared for their efficacy against grape pests).¹⁴ Probit analysis is a type of regression analysis that converts sigmoid response curves to linear response. Probit analysis also facilitated comparisons of toxicity across organisms and was an important method for standardizing dose–response relationships.

In apparent contrast with previous advances in pesticide technology that seemed inevitably to ratchet up exposure risks for bees, another technology change appeared at least at first to bode well for bees. In August 1945, after the end of World War II, the synthetic organic insecticide DDT became widely available for civilian use.¹⁵ A chlorinated hydrocarbon first synthesized in 1874, dichlorodiphenyltrichloroethane was regarded as an enormous improvement over the heavy metal inorganic insecticides at least in part due to its low acute vertebrate toxicity and its low costs of production. As part of the effort to protect allied troops (as well as civilian populations in conflict areas) from insect-borne disease, government scientists, including entomologists in the Bureau of Entomology and Plant Quarantine, developed new methods for cheap production and rapid, efficient broadcast delivery, including aerosol “bombs” and refined aerial spraying techniques. DDT was chemically well-suited for aerial application—effective against some pests at application rates of ounces per acre, a single aircraft could deliver sufficient quantities to treat enormous acreages. Shipped as a concentrate, it could be diluted in the field with cheap, available solvents, and its solubility meant that it rarely clogged nozzles and other spray equipment. Along with DDT itself, these new tools and equipment for pesticide delivery were also released to civilian markets. DDT was immediately embraced for a variety of agricultural uses, not only because of its relatively low cost and its low vertebrate toxicity, but also because it was perceived as less likely to cause major acute mortality to invertebrate nontarget species (general broad-spectrum efficacy against arthropods notwithstanding). Shifting from the “very destructive” arsenicals to DDT for boll weevil control in Texas cotton in fact reduced bee losses from 10000 colonies in 1945 to negligible levels for the next five years.⁹

Despite the initial optimism, the use of DDT in an extensive aerial spray program against gypsy moths beginning in 1945 immediately revealed numerous and extensive adverse impacts on a wide range of beneficial insects. Attitudes toward DDT began to sour. In 1957, lawsuits aimed at halting the USDA spray program to eradicate gypsy moths were filed in New York by 15 beekeepers and other plaintiffs from Long Island and elsewhere, claiming that the spray program deprived the plaintiffs “of property and possibly lives without due process of law and [took] their private property for public use without just compensation” (Murphy v. Benson, 151 F.Supp. 786

(E.D.N.Y.,1957), 789; Murphy v. Benson, 164 F.Supp. 120 (E.D.N.Y., 1958), 128; Murphy v. Benson, 270 F.2d 419 (second Cir., 1959)). The plaintiffs were unsuccessful, the court ruling that the needs of the public trump the rights of individuals. Ultimately, however, these beekeepers may have had a greater impact by virtue of the fact that the gypsy moth spray program in Long Island and beekeeper losses figured prominently in Rachel Carson’s *Silent Spring* (e.g., “It is a very distressful thing...to walk into a yard in May and not hear a bee buzz...”), a profoundly influential evaluation of adverse impacts of indiscriminate pesticide use that changed the nature of the public conversation about environmental consequences of pesticide use.

■ NEW ROUTES OF EXPOSURE AND NEW STANDARDS OF RISK ASSESSMENT

The 1960s were followed by repeating cycles of new insecticides and new waves of bee deaths.¹⁶ The Environmental Protection Agency, created in 1970, with its mandate to evaluate environmental risks of pesticides, provided a mechanism for restricting or canceling pesticides with adverse impacts on nontarget organisms. After DDT was banned for domestic use in 1972, replacement pesticides continued to cause problems for beekeepers, and lawsuits continued unabated (e.g., chlordane in *Brown v. Sioux City IA*, Iowa App., 49 N.W. 2d.853, 1951 and parathion in *Hall v. C&A Navarra Ranch Inc.*, 24 Cal App.3d 74 1972). Only three years after the DDT ban, a technological innovation introduced in 1975 presented new unanticipated risks to bees. Methyl parathion, an organophosphate insecticide, was supposed to be an improvement on the banned, highly persistent organochlorines in that it is environmentally short-lived; sprayed before sunrise, it can kill target pests and then break down before bees begin foraging. To prolong its persistence in the field, however, the Pennwalt Corp. introduced the process of microencapsulation, packaging the pesticide Penn-Cap M in capsules, approximately 30 μm in size, that can be dispersed by airplanes and that slowly release pesticides over time.^{17–19} As it turned out, the microcapsules were about the size of pollen grains and bees encountering microencapsulated methyl parathion processed the material as if it were pollen (and, in fact, it posed a greater risk to bees than did more conventional powder formulations because the microcapsules adhered more strongly to hairs). Incorporated into beebread and stored in the hive, the microencapsulated insecticide continued to kill bees long after it was initially collected by foragers.^{20–22}

In the meantime, the National Research Council, the research arm of the National Academy of Sciences, had been conducting consensus studies on pesticides and pesticide regulations and the then-new science of risk assessment. In 1983, a report titled *Risk Assessment in the Federal Government: Managing the Progress* (generally called the “Red Book”, by virtue of the color of the report’s cover) provided a framework for conducting risk assessment to allow the use of research results in setting guidelines to manage risks. The framework was initially used to assess toxicity risks to humans but later it was adapted to ecological risk assessments in which the primary focus was other than human health. In 1993, a National Academy of Sciences report from the Committee on Pesticides in the Diets of Infants and Children changed how pesticides are regulated and how risks to human populations are assessed (this report is generally known as the “Blue Book”, again, in recognition of the color of its cover). This committee

determined that children are dramatically different from adults qualitatively and quantitatively in both the frequency and type of exposures and their physiological and toxicological reactions to pesticides. The recommendation was made to take into consideration all forms of exposures, dietary and nondietary, to pesticides in evaluating risks to infants and children, recognizing life cycle differences in sensitivity and life cycle differences in exposure risk. In the wake of this compelling report, Congress unanimously approved the Food Quality Protection Act (FQPA), effective August 1996, requiring the Environmental Protection Agency to retest tolerance levels for all existing pesticides and, in doing so, incorporate the concept of “aggregate risk”—risk of exposure to all possible sources—amending in the process the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which regulates pesticide registration and use and the Federal Food, Drug, and Cosmetic Act (FFDCA), which sets guidelines for pesticide tolerances in food.

Under FQPA, to set tolerances, EPA was required for the first time to calculate aggregate exposure for all compounds with a common toxicity mechanism. Aggregate exposures include dietary residues as well as nonfood residues (resulting, e.g., from lawn care, crop protection, pest control in public parks, home use, and the like). Once a “risk cup” is filled, potential registrants cannot add a new pesticide or new use for an existing pesticide without reducing risk somewhere else. Similarly, the concept of “cumulative risk” dictates that pesticides that affect human health by the same mechanism must share a risk cup. In summarizing the seismic impact of this change in approach, Novak²³ wryly remarked, “We now find ourselves in a strange world where the regulatory focus of FQPA is aimed at nonfood uses of a pesticide”.

At first glance, the “risk cup”/“aggregate exposure” concept seems uniquely appropriate for humans. Many nontarget insect species are at relatively low risk of exposure by any route other than diet. Some herbivorous lepidopterans, for example, are univoltine, with larval stages restricted to particular habitats, with diet intake limited to a single food item, and adult stages not even capable of feeding (e.g., *Callosamia angulifera*, the tuliptree silkmoth). The risk cup concept, however, may be especially applicable for eusocial beneficial insects, particularly the honey bee. The size and perennial nature of honey bee colonies necessitate the collection of nectar and pollen from a remarkably broad diversity of flower species. Foragers must fly through many landscapes to obtain sufficient quantities of food to sustain 30000+ nestmates.

A forager may make up to 30 foraging trips in a single day, visiting 50–100 flowers each trip.²⁴ Over the course of her month-long lifespan, a forager may thus visit up to 90000 flowers. Pollen and nectar sources vary spatially and seasonally, so nestmates within a colony can be exposed to pesticides across both a broad geographic area and a substantial time span. In addition to foraging for nectar and pollen on flowers, some workers also visit other parts of their environment to collect water for colony thermoregulation (up to 100 trips per day²⁵) and plant resins for production of propolis (“bee glue”). Honey bees, like humans, thus encounter pesticides via a diversity of dietary and nondietary routes.

Unlike other insects (and most other animals), bees process and store food for later use: they convert pollen into beebread by microbial fermentation²⁶ and nectar into honey by water removal, biochemical processing, and storage prior to capping at hive temperatures of 35 °C. This processing means that what

bees encounter in the environment is not necessarily what they eat. From pollen to beebread, food processing results in changes in multiple compounds; generally, compounds with higher molecular weights are more likely to be present in pollen than in beebread, suggesting that microbial fermentation may play a role in predigesting pollen constituents (Liao, unpublished data). The possibility exists that fungal symbionts in beebread contribute to colony-level detoxification of pesticides, as they do in some solitary species (e.g., Shen and Dowd²⁷).

That honey bees consume a highly specialized diet of processed food (in the form of honey and beebread) has other toxicological implications.²⁸ Although in most insects substrates of cytochrome P450 detoxification enzymes up-regulate the genes encoding those enzymes, in the honey bee this pattern does not always apply. Quercetin, for example, is a virtually universal flavonoid constituent of honey and beebread that is detoxified by CYP6AS enzymes; it does not, however, induce those enzymes.²⁹ Certain ubiquitous constituents of honey and beebread function like nutraceuticals in that they influence the rate and efficiency with which xenobiotics are metabolized. A diet of honey enhances survival in the presence of aflatoxin B1, a mycotoxin produced by *Aspergillus*, relative to high-fructose corn syrup or sucrose, and *p*-coumaric acid added to a diet of sucrose enhanced pesticide metabolism by 60%.²⁹

Evaluating the toxicity of pesticides to honey bees presents challenges that in many ways are unique within insect toxicology. As in Lintner’s day, designing toxicity assays for honey bees is not like designing assays for other insects (Table 1). Due to its highly eusocial nature, for the honey bee the

Table 1. Toxicology of Most Insects versus Toxicology of Bees

	most insects	honey bees
unit of replication	individual	colony
size	unidirectional increase with time	variable
genotype	constant through life	constantly changing
lifespan	fairly predictable	varies seasonally
food	collector is consumer	collector is rarely consumer

colony, not the individual worker, is the unit of replication.³⁰ The colony is effectively a superorganism; in view of the extent to which individual workers cooperate in obtaining nectar and pollen, converting them into honey and beebread, and distributing those products to other colony members, it is difficult to interpret the significance of an individual forager’s ability to withstand exposure to a particular pesticide. As well, the size of the colony can get larger or smaller over time; individuals, typical subjects for insect toxicity testing, rarely grow smaller. The genetics of a colony change over the course of its lifetime, because the single queen that produces all eggs (and hence offspring) for the colony mates with multiple males on her initial nuptial flight and uses stored sperm from that flight randomly for the rest of her life (which may last several years). Physiological traits (including longevity) vary seasonally such that workers that live throughout the winter months display very different physiological traits from those of foragers that live less than a month during summer months.³¹ Finally, food consumption in the honey bee is unlike that of most organisms—the collector is not generally the consumer and consumed materials are shared (trophallaxis)—so dietary

exposures to pesticides are almost certainly unlike those of most organisms.

COLONY COLLAPSE DISORDER AND THE CHALLENGE OF MEASURING COLONY IMPACTS OF PESTICIDES

The unique nature of honey bee toxicology took on new importance in October 2006, when the first reports of “sudden and alarming colony losses” appeared in beekeeping publications and forums. These losses shared certain unusual attributes, chief among which was the precipitous disappearance of adult workers, without the accompanying appearance of cadavers in or near the colony. Soon thereafter, this syndrome was dubbed “colony collapse disorder” by a working group comprising academic faculty, USDA-ARS and Pennsylvania Department of Agriculture scientists, and beekeepers. At a meeting convened by the USDA-ARS at the Beltsville Agricultural Research Center, attendees prioritized hypotheses for scientific investigation. Pesticides, particularly the relatively new neonicotinoids, were from early stages considered likely causative or contributing factors, along with novel pathogens or parasites, nutritional challenges, and management practices (including cross-country transport for delivery of pollination services). Neonicotinoids were suspected in part because, as past experience had repeatedly demonstrated, new pesticide application technologies tend to have unintended consequences for honey bees. What was noteworthy about the neonicotinoids is that they were developed for systemic use, targeting seed and seedling pests as well as vascular sap feeders. Systemic use of neonicotinoids via seed dressing (e.g., clothianidin, as the systemic seed dressing product Poncho) was introduced in 2002 to target root- and phloem-feeding pests.³² Whether it might affect bees via pollen and nectar ingestion was not immediately clear; initial efforts to identify nontarget risks focused on terrestrial and aquatic arthropods in “tests with simulated field exposure conditions”.

Whether honey bees might be particularly sensitive to neonicotinoids, or any other new class of pesticide, was impossible to predict a priori. An abundance of LD₅₀ data for multiple classes of pesticides based on topical application to adult workers had accumulated over the years but, even within specific pesticide classes, sensitivity varied dramatically (and not predictably in any obvious way).³³ Importantly, little information on environmental exposure levels or on colony-level impacts was available. At the time as well, life cycle studies that evaluated sublethal chronic effects for all life stages along with systematic residue analysis were unavailable for any pesticide; in a memo, EPA scientists (in the Environmental Fate and Effects Division) expressed concerns over “the possibility of chronic toxic exposure to honey bees. Considering the toxicity profile and reported incidents of other neonicotinoids (e.g., imidacloprid), the proposed seed treatment with clothianidin has the potential for toxic risk to honey bees, as well as other pollinator insects. As a result of this concern, EFED is asking for additional chronic testing on bee hive activity (e.g., effects to queen, larvae, etc.)”.³⁴

Among the earliest efforts to identify factors contributing to CCD were chemical analyses of pesticide residues in beehives. Initial findings revealed startling frequency of contamination by pesticides other than neonicotinoids in the hive. Two acaricides used by beekeepers to control varroa mites, fluralinate and coumaphos, contaminated almost all hives tested.³⁵ These two compounds had already been demonstrated to interact

synergistically to enhance toxicity,³⁶ and their co-occurrence in virtually all beehives suggested a potential source of pesticide-related mortality that had not hitherto been considered. A more comprehensive residue analysis using more sensitive techniques revealed even more types of contaminants: “121 different pesticides and metabolites within...wax, pollen, bee and associated hive samples...Almost all comb and foundation wax samples (98%) were contaminated...with an average of 6 pesticide detections per sample and a high of 39”.³⁷ Essentially every type of agrochemical could be detected in hives—herbicides, fungicides, and insecticides—and, among insecticides, almost every structural class was represented among the residues (chiefly organophosphates, cyclodienes, pyrethroids, and organochlorines). Neonicotinoids, however, were not frequently detected. Subsequent studies documented a wide range of synergistic interactions in both adults and larvae among pesticide classes (as well as formulation solvent),^{38–41} demonstrating a need for pesticide risk assessment beyond acute toxicity of individual pesticides to adults to encompass evaluation of chronic toxicity and mixture toxicities of all chemical classes and “inert” components.

Ironically, the greatest technological innovation in beekeeping in the past 500 years—the widespread adoption of the moveable frame hive in the 19th century, which allows beekeepers to remove frames of honey without killing bees, to extract honey, and to reuse the wax comb—may have contributed to the 21st century problem of massive contamination by (highly wax-soluble) agrochemicals outside and inside the hive. The almost universal practice of reusing wax comb was a logical means for improving honey bee efficiency in the mid-19th century before the widespread deployment of agrochemicals; in the context of 21st century chemical-intensive agriculture, recycling wax comb after honey extraction allows for accumulation of unprecedented levels of contaminants. Indeed, Wu et al.^{42,43} demonstrated that larvae reared in brood comb contaminated with pesticides experienced profoundly delayed development and increased susceptibility to disease.

Although neonicotinoids were not particularly well-represented among hive contaminants, their use as seed-dressings provided the possibility of new avenues of exposure via systemic transport into pollen. Moreover, planter dust from coated corn seed turned out to be a route of environmental exposure with toxic consequences.^{44,45} Guttation water from seed-treated plants can also potentially contain pesticides, raising the possibility of yet another oral route of exposure.^{46–48} Yet very few quantitative data were available on the frequency of encountering corn dust or the amount of guttation water consumed or carried by bees; even less was known about the colony-level impacts of these new routes of exposure. Testing for colony-level effects proved to be experimentally challenging, and often results were subject to different interpretations. Schneider et al.⁴⁹ used RFID tracking to demonstrate sublethal effects of neonicotinoid ingestion on foraging behavior, but changes in foraging activity and duration of foraging flights did not persist beyond the first day. Although Henry et al.,⁵⁰ also using RFID tracking, documented sublethal effects of the neonicotinoid thiomethoxam on homing behavior “that could put a colony at risk of collapse”, admonitions to consider seasonally appropriate colony-level responses in projecting colony fate were made soon thereafter.⁵¹

Another problem with the vast majority of studies on both individual bees and on colonies is that pesticides were generally

force-fed to test subjects, despite the extraordinarily sophisticated behavior exhibited by *A. mellifera* in foraging behavior. On the one hand, it has been abundantly clear for over 40 years that bees do collect and store pollen that is contaminated with pesticides, suggesting an inability to detect or avoid chemical pesticides,^{52–55} but, on the other hand, the phenomenon of “entombed pollen” whereby bees “entomb” or seal off cells containing pollen with higher fungicide content suggests they may be able to recognize contaminants in the hive.⁵⁶ Some insights on the discriminatory capabilities of foragers can be gained by examining the substantial literature on responses to chemicals; in short, honey bees display structure- and concentration-dependent responses to phytochemicals.⁵⁷ In terms of nectar, studies suggest responses to nectar phytochemicals⁵⁸ are idiosyncratic and dose-dependent, with bees avoiding certain substances (e.g., phenolics⁵⁹) but displaying a paradoxical preference for some neurotoxins (e.g., nicotine and caffeine⁶⁰). Whether free-flying bees discriminate against toxin-laden nectar or pollen while foraging has long been a question that, with the rising use of systemic pesticides, has become even more pressing to address.

Any discriminatory behavior has implications for experimental design in assessing risk.⁶¹ Yet the vast majority of field toxicity studies involve no-choice designs (e.g., Henry et al.^{50,62}), with pesticides administered in sucrose solutions rather than in honey or beebread, which contain phytochemicals that regulate detoxification gene expression.^{40,63} Force-feeding is certainly appropriate for determining effects of concentrations and side effects, but estimating risks is a little more complicated, particularly in view of the fact that some of the compounds in question have behavioral effects.⁶⁴ How repellency, aversive responses, induced “malaise”, choice behavior, and communication figure into colony-level responses is not well understood, despite their relevance to assessing exposure risks. Captive or immobilized bees, or bees given no choice of foods to eat, behave differently from bees permitted their full behavioral spectrum.⁶⁵ As well, the standard behavioral bioassay, the proboscis extension reflex (PER), involves chilling and immobilizing individual bees and then exposing them to potential toxins under no-choice conditions, a scenario that bears little relationship to natural behaviors. Tolerances in free-flying bees are much higher than those determined by PER. For that matter, such circumstances may also introduce physiological artifacts; refrigeration and immobilization preceding PER assays may reduce metabolic rates and thereby affect detoxification capabilities, possibly giving rise to misleading toxicity estimates.⁶⁶

Just as they can display idiosyncratic and dose-dependent responses to phytochemicals, free-flying honey bees can display idiosyncratic and dose-dependent responses to synthetic pesticides. Moreover, the extraordinary complexity of honey bee behavior, relative to the behavior of most insects, makes a prior prediction of impacts of ingestion of neuroactive compounds difficult. The alkaloid caffeine, for example, known to have neurotoxic effects on some insects, increases motivation and cognitive performance in free-flying bees,⁶⁷ and ingestion of sublethal concentrations of imidacloprid and coumaphos can even enhance olfactory learning and memory.⁶⁸

Risk of exposure to pesticides for honey bees, then, cannot simply be equated with the presence of the pesticide in the environment. A Scientific Advisory Panel convened by EPA in September 2012 to evaluate its Pollinator Risk Assessment Framework identified a myriad of challenges in assessing risks

presented to honey bees by pesticides.⁶⁹ Several of the data gaps identified by the SAP involved incomplete knowledge of feeding and food-processing behavior; other gaps exist in identifying and quantifying additional routes of exposure (dust, drinking water) and behavioral interactions in a colony context. According to Goulson,⁷⁰ environmental risks are hard to assess without more quantitative data on levels of residues that are encountered and behavioral responses to those residues; Goulson’s own work⁷¹ demonstrates that pollinating syrphid flies and beetles are repelled by imidacloprid at ecologically realistic concentrations, yet comparable data for honey bees are in short supply.

In summary, as in 1890, definitively characterizing the impacts of pesticides on honey bees remains experimentally challenging. Knowledge of honey bee behavior, physiology, and toxicology has expanded exponentially in the past 125 years; indeed, scientific publications indexed by Web of Science by the search terms “honey bee” and “pesticide” ballooned from 4 in 1913 to 242 in 2013 (accessed August 1, 2014). Evaluating risks of pesticides to honey bees and other pollinators has improved markedly. The EPA published its final guidance for assessing risks of pesticides to bees in June 2014,⁷² recommending a modeling approach for summing all exposure risks, with different risk factors for non-systemic foliar, systemic seed, foliar, and soil applications. Like FQPA, the recommendations take into consideration life cycle differences in sensitivity as well as life cycle differences in exposure risk. Thus, the honey bee has in effect become the first insect with its own “risk cup”—one technological innovation that may not actually have unintended consequences for the honey bee. In fact, the risk cup concept can help enormously, by clearly identifying and quantifying risks, to inform pest management practices to protect and preserve bees. In their ability to find and evaluate multiple types of raw materials, to process raw materials into food that they can allocate to others and store for future use, and to carry out all kinds of activities in human-altered landscapes, honey bees are more like humans than they are like other insects (target or nontarget). Maybe they deserve a “risk cup” as well?

■ AUTHOR INFORMATION

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