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MECHANISTIC MODELING OF PESTICIDE EXPOSURE: THE MISSING KEYSTONE OF HONEY BEE TOXICOLOGY

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Abstract: The role of pesticides in recent honey bee losses is controversial, partly because field studies often fail to detect effects predicted by laboratory studies. This dissonance highlights a critical gap in the field of honey bee toxicology: there exists little mechanistic understanding of the patterns and processes of exposure that link honey bees to pesticides in their environment. We submit that 2 key processes underlie honey bee pesticide exposure: (1) the acquisition of pesticide by foraging bees and (2) the in-hive distribution of pesticide returned by foragers. The acquisition of pesticide by foraging bees must be understood as the spatiotemporal intersection between environmental contamination and honey bee foraging activity. This implies that exposure is distributional, not discrete, and that a subset of foragers may acquire harmful doses of pesticide while the mean colony exposure would be appear safe. The in-hive distribution of pesticide is a complex process driven principally by food transfer interactions between colony members, and this process differs importantly between pollen and nectar. High priority should be placed on applying the extensive literature on honey bee biology to the development of more rigorously mechanistic models of honey bee pesticide exposure. In combination with mechanistic effects modeling, mechanistic exposure modeling has the potential to integrate the field of honey bee toxicology, advancing both risk assessment and basic research.

Keywords: Behavioral toxicology, Pesticide risk assessment, Environmental modeling, *Apis mellifera*, Pollinator, Foraging

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INTRODUCTION

The potential risk that some pesticides pose to honey bees is universally acknowledged, but the extent to which specific chemistries can be blamed for particular patterns or incidents of colony damage is controversial. Most recently, this controversy has surrounded the neonicotinoid insecticides and their possible role in honey bee losses in Europe and North America [1]. While laboratory experiments have clearly established the potential for both lethal and sublethal effects of neonicotinoids on individual bees [1, 2, 3], field studies have often failed to detect colony-level effects [4, 5, 6, 7, 8, 9, 10], and where colony-level effects have been observed [11, 12, 13], their biological significance is unclear.

Any putative link between a toxic compound and a toxic effect is necessarily predicated on some model, whether stated or implied, of toxic exposure. At present, though, there exists little mechanistic understanding of the patterns and processes of honey bees pesticide exposure [14], and this might account for much of the dissonance between laboratory predictions and field observations [15] and the controversy surrounding the design and interpretation of field studies [16].

Mechanistic modeling of toxic exposure is not a novel task in the larger field of ecotoxicology [17, 18], and sophisticated exposure models have been developed for many organisms, including humans [19]. Honey bees, however, present unique challenges to exposure modeling due to their complex social biology [14, 20]. A healthy honey bee colony is composed of 3 castes: a single reproductive female (the queen), up to several hundred males (drones), and many thousands of sterile females (workers). The worker caste, which is responsible for all colony tasks except reproduction, is further subdivided into loose, age-based functional guilds: new workers initially clean and cap cells, then progress to brood and queen tending, then to

comb construction and food handling, and finally to the outside tasks of ventilation, guarding, and foraging [21]. As foragers, they may collectively survey over 100 km^2 of the environment surrounding their hive [22], collecting nectar, pollen, resin, and water from a vast array of sources. Foraged materials are then returned to the hive where they are processed and utilized in various ways by other colony members. In this complex economy, all castes and life stages are vulnerable to toxic exposure from multiple routes, and parsing this system into tractable components for modeling is no trivial challenge.

Constraining our discussion to pesticide exposure initiated by foraging in a contaminated environment (i.e. excluding in-hive pesticide applications), we identify 2 main challenges of honey bee exposure modeling: (1) predicting the acquisition of pesticide by foraging honey bees (primary exposure) and (2) tracing the in-hive distribution of pesticide returned to the hive by foragers (secondary exposure).

For concision, we will refer to these 2 challenges, respectively, using Purdy's [23] terminology of "primary" and "secondary" exposure. Within this framework, we explore the biological mechanisms underlying exposure, review existing efforts to capture these mechanisms through quantitative modeling, and discuss ways in which future models can achieve greater predictive and heuristic power. We conclude that both primary and secondary exposure are governed by aspects of honey bee behavior and environmental complexity that have not been adequately addressed in existing models, and that these oversights are manifest principally in the failure to represent exposure as a fundamentally individual-based phenomenon that cannot be subsumed by colony-level approaches to honey bee toxicology (Figure 1).

PRIMARY EXPOSURE

Pesticide exposure begins with the foraging of bees in a contaminated environment. This

results both in the exposure of the foragers themselves and, perhaps more importantly, the delivery of pesticide to the rest of the colony. Though not considered here, bees may also be exposed to pesticides applied inside the colony by the beekeeper to control parasites and pathogens.

Biological background

Honey bees gather resources from their surrounding landscape within a foraging range that routinely extends a few kilometers from the hive [24] and can extend considerably farther under conditions of local scarcity and distant reward [25]. Foragers integrate their individual knowledge of resource patches through a unique "dance language" [22, 26] that communicates, among other things, the odor and location of valuable forage [27]. This generates colony-level knowledge of a vast foraging environment, which, in combination with private information [28, 29], enables the colony to focus its foraging effort on the most rewarding resource patches [30, 31].

Because flowering plants are heterogeneous in their spatial distribution and bloom phenology, honey bee foraging is characterized by marked spatiotemporal heterogeneity [24, 32, 33, 34]. Spatiotemporal heterogeneity similarly characterizes environmental pesticide contamination, since pesticide application is normally restricted to discrete landscape components during discrete time intervals. Primary exposure, therefore, must be understood as the spatiotemporal intersection of environmental contamination and honey bee foraging activity, jointly determined by environment and behavior. This means that a mechanistic exposure model must consist of 2 basic components: (1) a submodel of environmental contamination, and (2) a submodel of honey bee foraging behavior.

Existing models

Most models of primary exposure have not attempted mechanistic representations of both environmental contamination and honey bee foraging behavior, and some attempt neither. Here, we present a summary of existing models in order of increasing mechanistic realism (Table 1).

Contact exposure models for foliar sprays. A traditional model for estimating contact exposure to foliar sprays is the Atkins model, which uses a simple conversion factor, originally derived from a large empirical data set, to estimate the critical field application rate needed to reach LD50 contact exposure (dose needed to kill 50% of exposed bees) in bees foraging on a treated crop (assuming early morning, pre-foraging application) [35].

LD50 (µg a.i/bee) x 1.12 = critical field application rate (g a.i./ha) (Equation 1)

Algebraic conversion yields a prediction of contact exposure per bee given a known application rate.

field application rate (g a.i./ha) / 1.12 = bee exposure (µg a.i/bee) (Equation 2)

Poquet et al. [36] propose an approach that estimates per-bee exposure (assuming that bees are foraging in the field at the time of application) by multiplying field application rate (g/ha) by the effective exposure surface area of a honey bee (1.05 cm^2). The latter value they calculated by exposing bees to controlled spray applications in the laboratory and taking the average ratio of the application rate (mass/area) and the resulting residues detected on treated bees (mass/bee).

As tools for screening-level risk assessment, these models meet the demand for simplicity and ease of use. They are not, however, designed to model spatial or temporal heterogeneity of

environmental contamination or foraging behavior.

Bee-REX contact and dietary exposure model. The U.S. Environmental Protection Agency (USEPA) has recently developed the Bee-REX model to predict both contact and dietary exposure of foraging honey bees under a variety of pesticide application scenarios [37]. Analogous to the Atkins model, contact exposure for aerial spray is estimated as a simple conversion factor based on the field data of Koch and Weißer [38]. Dietary exposure (contamination of nectar and pollen) via foliar spray is estimated using the contamination rate determined for tall grass vegetation in the terrestrial residue exposure model (T-REX), a model based on the work of Hoerger and Kanega [39] and originally designed to estimate pesticide residues in avian and mammalian food items. Dietary exposure via systemic translocation of seed treatment pesticides is assumed to be the peak estimate (1 ppm) recommended by Alix et al. [40]; dietary exposure via systemic translocation of tree trunk injections is estimated as the mass of injected pesticide divided by the tree's combined mass of leaves and flowers; and dietary exposure via systemic translocation of soil treatment is estimated using the fugacity model of Briggs et al. [41, 42]. All dietary exposures are converted from concentration to mass-per-bee doses using estimates of feeding rates for each caste and life stage.

The Bee-REX model is comprehensive in scope while retaining the ease of use needed to be an effective screening-level exposure model. Nevertheless, Bee-REX suffers from the same key shortcomings as the Atkins and Poquet models: all exposure estimates ignore variability in environmental contamination and the behavioral patterns of honey bee foraging.

Barmaz drift model of dietary exposure. When a treated crop itself is not attractive to foraging bees or not in bloom at the time of treatment, field application rate is no longer a meaningful determinant of honey bee exposure. To account for this, Barmaz et al. [43, 44] model

a scenario in which a pesticide sprayed on a crop drifts into off-crop habitat [43, 44]. In this model, environmental contamination is modeled by a function relating active ingredient deposition to distance from crop edge. Temporal dynamics are also accounted for by calculating rates of pesticide movement and decay. Honey bee foraging is assumed to occur only in off-crop vegetation, which is subject to a gradient of pesticide contamination determined by the drift function, and predicted exposure is taken to be the mean of the contamination gradient in the off-crop habitat.

The Barmaz model addresses the issue of variation in environmental contamination by calculating a drift gradient of pesticide deposition. For simplicity, though, this gradient is collapsed into its mean, which effectively removes the element of spatial heterogeneity from the resulting exposure estimates. A unique strength of the Barmaz model, though, is that it incorporates an additional dimension of heterogeneity by modeling pesticide movement and decay through time. As with all the models discussed so far, though, the Barmaz model attempts no mechanistic treatment of honey bee foraging, except to acknowledge that it does not occur in an unattractive/non-blooming crop.

EFSA landscape model of dietary exposure. In its guidance document on bee risk assessment [45], the European Food Safety Authority (EFSA) presents a preliminary model designed to estimate the average concentration of pesticide in nectar and pollen entering a honey bee colony from a heterogeneously contaminated landscape. The model is highly generalized, designed to accept as input any discrete pattern of environmental contamination and any estimate of pesticide concentration in floral nectar or pollen. Its basic form is given by Equation 3.

$$PEC_{hive} = \frac{\sum_{n=1}^{N} f_n a_n PEC_n}{\sum_{n=1}^{N} f_n a_n} \qquad \text{(Equation 3)}$$

where PEC_{hive} is the average concentration of pesticide entering the hive in pollen or nectar from N patches, f_n is a coefficient representing the attractiveness of patch n, which has surface area a_n and a nectar/pollen pesticide concentration of PEC_n. By dividing the area- and attractiveness-weighted sum of all patch concentrations by the attractiveness-weighted total patch area, an average concentration of pesticide entering the hive in nectar or pollen is calculated.

The EFSA model is remarkable in its versatility; given an estimate of pesticide concentrations in floral nectar or pollen and an estimate of the relative attractiveness of relevant floral patches, the average concentration entering a honey bee colony can be calculated for any landscape and any application scenario, and this concentration can be converted to a per-bee dose using feeding rate estimates. This design allows the EFSA model to accommodate virtually any degree of complexity and mechanistic realism in the representation of environmental contamination, provided that contamination is assumed to be spatially discrete (i.e. patch-based, not gradient-based). A major weakness of the model, which its authors acknowledge, is that it is extremely sensitive to errors in estimating a colony's effective foraging range, since this value defines the spatial scale of the model and has a strong effect on the area term in the denominator of the exposure equation. The model also relies heavily on estimates of the attractiveness of different flora to honey bees, and such estimates are scarce and difficult to verify across different contexts. Perhaps most importantly, though, the EFSA model represents exposure only as a colony-level mean and does not deal with the distributional nature of individual-level exposure.

Baveco dilution model of dietary exposure. The recent model of Baveco et al. [46] is by far the most mechanistic with respect to honey bee foraging behavior. In the basic ("single optimal") version of this model, a virtual colony selects a single optimal forage patch, based on the

optimization of energetic efficiency (a function of floral properties and patch distance), from within a heterogeneous landscape composed of potentially treated mass-flowering crops and untreated non-crop features. Patch selection is then iterated over hourly time steps to incorporate the effects of nectar depletion on patch selection. The more complex ("recruitment limited") version of the model adapts a previous model of honey bee foraging [30, 47] to simulate the dynamic allocation of foragers across multiple resource patches, regulated by rates of recruitment and abandonment. Both of these mechanistic approaches to simulating patch selection avoid imposing an assumed foraging range as in the EFSA model. The net concentration of pesticide in foraged nectar (pollen is not considered) over the simulation period is determined by the proportion of foragers that collected from treated crop vs. alternative habitat. Thus, the potential diluting effect of uncontaminated forage are taken into account.

The strengths of the Baveco model are that it (1) explicitly accounts for spatial heterogeneity of environmental contamination, (2) incorporates the mechanistic role of honey bee foraging behavior in determining pesticide exposure, and (3) simulates the pesticide collected on individual foraging trips rather than just the colony average. This last point, while not emphasized by the authors in the paper (since the focus was on the dilution of the colony average by uncontaminated forage), is perhaps the most important, as will be discussed in the *Future steps* section. A weakness of the Baveco model is that it cannot be expanded to include exposure via contaminated pollen because its energetics-based patch selection mechanism is relevant only to nectar foraging. The authors also acknowledge that the model relies on somewhat speculative parameters related to floral resource properties and landscape composition, but there is no reason why, in principle, the model could not parameterized more rigorously with empirical data from a particular study area.

Future steps

Modeling environmental contamination. Real landscapes–even intensively cultivated ones–are composed heterogeneously of treated and untreated habitat, and thus contain a range of pesticide contamination levels. A honey bee colony's foragers, therefore, are not exposed to a uniform pesticide dose but rather to a distribution of doses, likely ranging all the way from null to some maximum [14, 45]. Nevertheless, existing models that acknowledge the heterogeneity of environmental contamination [45, 46] still present exposure estimates in terms of colony average (though the Baveco model [46] does, in fact, calculate exposure on an individual basis).

Little is gained and much obscured by collapsing a distribution of exposure levels into some central tendency, for *average exposure* and *exposure to the average* are not interchangeable concepts [48, 49]. Consider the situation represented in the Baveco model in which a colony forages either on or off a uniformly contaminated crop, and compare the exposure predictions of the Atkins, Poquet, Bee-REX, EFSA, and Baveco ("single optimal" version) models, respectively (excluding the Barmaz model because we are assuming the treated crop is attractive). To make the models directly comparable, we assume the following: (1) application is by foliar spray at a uniform rate of 80 g/ha, with no off-field drift, (2) the application rate of 80 g/ha translates into a uniform concentration of 80 ppb in floral nectar, (3) the treated crop and alternative forage are equally attractive to honey bees, equal in floral density and nectar concentration, and never depleted, (4) patches of treated crop and alternative forage are equidistant from the hive, and (5) honey bees choose randomly between equally suitable forage patches (this is to account for the fact that, in the Baveco model, patch selection is based on a deterministic evaluation of patch reward, but under our assumptions foraging patches do not differ in reward).

Figure 2 summarizes this comparison when performed separately for contact exposure (Atkins, Poquet, and Bee-REX models) and dietary nectar exposure (Bee-REX, EFSA, and Baveco models), and repeated under 3 scenarios with differing abundance of treated vs. untreated foraging habitat. Comparing predictions of contact exposure underscores the fact that the Atkins, Poquet, and Bee-REX models are similar in that they estimate exposure by applying a simple coefficient to field application rate. None of these models is designed to account for heterogeneity of contamination, so each, respectively, yields the same exposure prediction under all 3 scenarios. Comparing predictions of dietary exposure shows that, in each scenario, the EFSA model estimates exposure to be the mean of the field contamination distribution while the Bee-REX model, not accounting for heterogeneity of contamination, performs just as it did in the prediction of contact exposure. The Baveco model is unique in that it has the potential to represent the field contamination distribution as a distribution. In each of the scenarios presented, the Baveco model would distribute foragers across the 2 levels of contamination in proportion to the abundance of each; so, for example, under Scenario A, 50% of simulated foragers would collect 0 ppb and 50% would collect 80 ppb. It is important to note, though, that if the distribution of exposure levels encountered by foragers is collapsed to its mean, as it is presented in Baveco at al. [46], then the Baveco model effectively reduces to the EFSA model under the simplifying assumptions of our comparison. The problem with any approach that collapses a distribution of exposure into a mean is that the mean concentration may actually be quite rare in the environment and experienced by few individual bees. For example, in a strongly bimodal distribution of environmental contamination, such as the one depicted in Figure 1B, the mean level of exposure is rare, and both lower and higher levels of exposure would be much more commonly encountered. The mean of a distribution, without both its form and variance, reveals

neither the proportion of foragers that acquire a potentially dangerous dose nor the range of doses that enter the hive.

The heterogeneity of contamination and the distributional nature of toxic exposure have been more fully explored outside of honey bee biology [49, 50]. For example, Schipper et al. [51] and Loos et al. [52] modeled the exposure of terrestrial vertebrates to levels of cadmium contamination that were heterogeneous both in terms of spatial distribution and concentration in different food items. In a model of human pesticide exposure, Leyk et al. [53] used a "dynamic hazard surface", a cellular automata model combining land use data with rates of pesticide deposition and decay, to simulate both the spatial and temporal distribution of pesticide levels in a patch-based landscape. In principle, there is no reason why similar models of heterogeneous environmental contamination could not be applied to pesticide exposure in honey bees.

Modeling honey bee foraging behavior. Honey bee foraging biology has been studied extensively, and many mechanistic models already exist (reviewed in [54]). The challenge for exposure modeling is not to break new theoretical ground but simply to apply existing knowledge to pesticide exposure scenarios.

Two principals of honey bee foraging–neither of which have been seriously discussed in the context of toxicology–should be addressed in future exposure models. First, and most importantly, colony-level foraging is the collective activity of thousands of individual bees, each of which interacts uniquely with the distributions of floral resources and pesticide contamination in the foraging landscape. While dance language recruitment creates a degree of non-independence between foragers, the spatial coarseness of recruitment relative to environmental contamination gradients, the constant temporal fluctuations in both contamination levels and floral reward, and the propensity of foragers to ignore the information of the dance language and

search independently [27] ensure that a colony's thousands of foragers experience a broad distribution of exposure levels [38]. As discussed already, a distribution of doses is not toxicologically equivalent to its central tendency (the exposure of a hypothetical "average bee"), so the distributional nature of exposure must be acknowledge and represented in exposure models [48, 49]. Second, while selective recruitment to resource patches introduces a non-random element to honey bee foraging behavior [26], there is evidence that the initial discovery and continual rediscovery of resource patches is governed by stochastic search behavior [55, 56]. It is impossible to predict exactly how a honey bee colony's foraging force will be distributed across a landscape, which means that the distribution of pesticide doses encountered by foragers is an effectively stochastic phenomenon that should ideally be modeled probabilistically [14]. This problem weakens the predictive potential of exposure models that do not explicitly simulate the stochastic process of patch selection. The EFSA model, for example, conceptually distributes foragers across all foragable patches in proportion to their attractiveness, when in reality a colony would be expected to forage from only a relatively small subset of available patches over any given time interval [32]. This approach is acceptable if the goal is to evaluate the theoretical "average" exposure risk for a colony in a given landscape, but it will likely not yield good predictions of actual exposure under specific scenarios.

It is also worth noting that no existing model of primary exposure explicitly addresses the collection of contaminated water or resin. Water, in particular, may be an importance route of exposure in some scenarios [57] and it deserves to be considered alongside nectar and pollen.

SECONDARY EXPOSURE

Pesticide exposure begins in the field, but processes that occur inside the nest are at least as important in determining the of exposures experienced by individual colony members [14, 20, 23, 58, 59]. The distribution of exposures generated by foraging (i.e. primary exposure) forms the input to secondary exposure, which begins as soon as a contaminated forager returns to the nest.

Biological background

Incoming nectar and pollen can undergo extensive processing and redistribution prior to consumption, which may significantly modify the initial distribution of pesticide concentrations returned to the hive by foragers (Figure 3). The key to elucidating the distribution of pesticide inside the hive is modeling the complex processes of in-hive food transmission. To do this, nectar, pollen, and secreted brood food and royal jelly (hereafter referred to collectively as "jelly") must be discussed separately.

Nectar. A forager returning with nectar transfers her nectar load via trophallaxis to 1 or more "receiver" bees (younger workers tasked with food handling) [60]. A receiver bee, upon accepting a nectar load from a forager, proceeds to store, process, and/or redistribute the nectar according to the needs of the colony. Under typical conditions, the receiver bee initiates a cascade of trophallactic transfers, giving portions of her load to several other bees, which may, in turn, distribute portions of nectar to additional bees [61, 62, 63, 64]. This pattern of food transmission may proceed through many iterations before the nectar is ultimately consumed or deposited in cells for storage [61], and the process is so efficient that labeled sugar syrup gathered by only a few foragers can be detected in many [65] or all [66] colony members within just a few hours of initial collection in the field. Consequently, nectar from a single contaminated floral patch may be ingested by all or most colony members, potentially causing pervasive

intoxication. Such extensive distribution, however, involves thorough mixing with nectar from potentially uncontaminated sources, so a distribution of field concentrations in nectar would become homogenized toward its mean, increasing the likelihood that a pesticide dose consumed by any particular bee will be highly diluted from the concentration of contaminated nectar in the field.

Another important consideration is that honey bees preferentially transfer nectar to nestmates of similar age, resulting in a gradual net flow of incoming food from the older bees (foragers), to the middle-aged bees (receivers, comb builders), and finally to the younger bees that are tasked with feeding the queen and brood [65, 66, 67]. In this way, the younger workers in the colony, along with the queen and brood, may be buffered against toxic exposure arising from contaminated nectar [66]. It must also be noted that foraging honey bees, in addition to receiving nectar/honey from nestmates in the hive, consume some freshly foraged nectar during their return flights from the field [68]. Thus, they are exposed to undiluted pesticide doses against which hive bees are buffered by the diluting effect of nectar transmission. This may serve as a critical safeguard against severe toxic exposure in the colony, since foragers collecting highly contaminated nectar will likely perish in the field before sharing their toxic payload among nestmates. The potentially adaptive nature of forager mortality is especially interesting in light of the fact that homing impairment is a frequently observed symptom of pesticide exposure [69, 70, 71, 72].

Pollen/beebread. In contrast to nectar, incoming pollen is not mixed or shared among nestmates. Instead, a returning pollen forager searches out a storage cell directly and unloads pollen pellets into it [73]. The forager does not process the pollen further, but leaves it to be discovered by pollen-packing bees, which add honey and saliva to the fresh pollen and pack it

tightly into the bottom of the cell (at which point the pollen can be referred to as "beebread") [73]. Successive pollen loads are packed on top of each other, forming a stratified column.

While nectar is consumed by all colony members, pollen is consumed almost exclusively by the nurse bees, young workers whose principal work is the tending of brood and queen. Pollen consumption peaks in bees between 4 and 9 days old and decreases to negligible amounts in bees over 20 days old [74], closely mirroring the age-dependent activity of proteolytic enzymes that enable pollen digestion [75]. Nurse bees convert the nutrients of dietary pollen into protein-rich glandular secretions (jelly) that comprise the primary food of brood and queens and are shared to a lesser extent with adult colony members of all ages [76].

Unlike pesticide-laden nectar loads, which may be thoroughly mixed with other nectar sources prior to consumption, pesticide-laden pollen loads remain segregated in the stratified column of each pollen cell. Any mixing of loads can only occur through individual nurse bees' consuming pollen from more than 1 storage cell or layer during a feeding bout. The extent to which this occurs has never been reported, but even if some mixing occurs by this mechanism, nurse bees feeding on pollen are likely subject to pesticide doses that reflect the distribution of concentrations collected by foragers much more closely than do the extensively homogenized doses arising from nectar/honey transmission.

Jelly. Jelly secreted by nurse bees originates from the hypopharyngeal and mandibular glands of the head, and may be mixed with regurgitated honey and/or pollen, depending on the age and caste of the recipient [21, 77]. The extent to which dietary pesticides can be translocated to the hypopharyngeal and mandibular glands and incorporated into their secretions is largely unknown and no doubt varies with the physicochemical properties of the active ingredient involved. While several studies have documented pesticide residues in jelly [78, 79, 80, 81, 82,

though see 83], it is possible that this contamination arose through the incorporation of contaminated nectar and/or pollen into secreted jelly rather than pesticide translocation to glandular tissue [78, 80].

Grooming. Apart from the transmission of contaminated food, grooming behavior could be a significant pathway of exposure to pesticides carried on the body surface. Honey bees both self-groom and allogroom (groom nestmates). Self-grooming is performed mainly with the legs, but often targets the mouthparts (especially the glossa) [84], while allogrooming is performed with the mandibles [85, 86]. Both forms of grooming, therefore, create the potential for oral exposure. Allogrooming is performed principally by "grooming specialist" bees, a small minority of the worker population [87]. Notably, exposure to particulate matter induces both self-grooming and allogrooming [88], suggesting that grooming may be an especially important route of exposure for microencapsulated pesticides and pesticidal dusts.

Existing models and future steps

Existing models of the in-hive distribution of pesticides have focused on beekeeper-applied acaricides that are introduced directly to the colony [89, 90, 91]. These models have approached the problem of in-hive distribution from the perspective of fugacity, dividing the colony into internally homogeneous compartments (e.g. wax, bees, honey, air) among which a pesticide becomes partitioned according to its physicochemical properties.

Because beekeeper-applied pesticides largely bypass the usual food transmission process, compartment-based fugacity modeling is a reasonable approach to predict their in-hive fate. For pesticides that enter the hive in contaminated nectar and/or pollen, though, the food transmission process is arguably the more important mechanism of in-hive pesticide distribution, at least over short time scales. Moreover, just as in the modeling of primary exposure, it is vital to predict the

distribution of doses experienced by individual bees, not just the aggregate partitioning of pesticide to the "bee compartment".

Unlike honey bee foraging biology, in-hive food transmission has not been studied through mechanistic modeling, and basic work remains to be done before the food transmission dynamics can be incorporated into a pesticide exposure model. There is, however, a wealth of empirical and theoretical studies on in-hive food transmission (reviewed in [92, 93]) that supplies ample material for the design and parameterization of models. The fact that in-hive food transmission involves the complex interaction of many autonomous entities immediately recommends an agent-based modeling (ABM) approach [94] that could take full advantage of the many detailed studies of the behavioral rules of food transmission. Moreover, ABMs are fundamentally designed to track state variables on an individual basis, enabling the distributional modeling of exposure levels experienced by individual bees. The development of an ABM, though, is typically a long and demanding process, especially when the model is intended to support regulatory decision-making [95, 96]. A simpler, though minimally mechanistic, approach would be to simulate in-hive pesticide distribution by Monte Carlo sampling. Given an input distribution of pesticide concentrations in nectar or pollen loads delivered to the hive by foragers, it would be possible to emulate the food transmission process by conducting repeated random draws (representing individual colony members) from the input distribution (or some transformation thereof) in a fashion similar to the probabilistic approach of Macintosh et al. [48].

While we emphasize active food transmission as the key mechanism of in-hive pesticide fate, we acknowledge the importance of complementing food transmission models with models of fugacity and pesticide degradation. This is especially important for pesticide exposure via contaminated nectar/honey. Once ripened, honey can be stored for weeks or months prior to

consumption, during which time passive fugacity and degradation processes would be the main mechanisms affecting the dynamics of nectar-associated pesticides.

The relative importance of grooming as a route of exposure is difficult to estimate. Compared to food transmission, grooming behavior has received little research attention aside from its effects on parasitic mites, and more extensive behavioral studies must precede any attempt at quantitative modeling.

DISCUSSION AND CONCLUSIONS

While we acknowledge the role of simple and conservative exposure models as a component of risk assessment frameworks, effective modeling requires a cycle of mechanistic insight and strategic simplification. Simple models designed for efficient risk assessment must be informed and continually revised by reference to more complex models that aim both to predict pesticide exposure and to understand the fundamental mechanisms that govern it. Thus, while complex mechanistic exposure models may never be practicable as standard risk assessment tools, they are necessary to evaluate the validity of risk assessment models and, just as importantly, to advance the basic study of honey bee toxicology.

Perhaps the most salient shortcoming of existing models (except the Baveco model [46]) is the failure to estimate exposure as a distribution of individual doses rather than a discreet "colony-level" dose. This is true even of the most nuanced conceptual models of honey bee pesticide exposure (e.g. [23]), despite the fact that the individual variability in exposure is empirically evident [38]. What has led to this dubious consensus?

As a eusocial "superorganism", the collective functions of a honey bee colony are insulated from the death or impairment of individual bees by a complex web of compensatory mechanisms and negative feedback loops [59, 97]. Since the endpoints of concern for honey bee risk

assessment are usually colony-level functions like honey production, pollination services, and overwintering survival, there has been a trend in research away from individual-level laboratory assays and toward colony-level studies that aim to observe the net effects of toxic exposure after all the mechanisms of social buffering have played their roles.

An unfortunate consequence of this paradigmatic shift from the individual to the colony is that the legitimate notion of colony-level *effects* has become implicitly conflated with the misconceived notion of colony-level *exposure*. Toxic effects can be understood as perturbations of patterns or processes at either the individual- or colony-level. For example, sublethal neonicotinoid exposure can induce the physiological effect of impaired homing ability on individual foragers (e.g [71]), but if enough foragers suffer homing failure, colony-level functions like food acquisition and brood rearing could be disrupted, potentially leading to a fatal breakdown of colony homeostasis [71]. Individual- and colony-level effects are mechanistically linked, albeit in complex ways, and each is amenable to observation and experimentation. Conversely, toxic exposure is scientifically tractable only when it is understood as the spatiotemporal intersection between a toxic agent and a discrete receptor organism. This makes the concept of "colony-level exposure" highly problematic. If the term "colony" is used to represent a higher-order system defined by patterns, processes, and relationships emerging from interactions between individuals (e.g. information integration, division of labor, genetic structure, collective fitness), then there is no measurable sense in which such a composite of abstractions can be said to intersect in space and time with a toxic agent. If, alternatively, the term "colony" is used simply to represent an aggregation of associated individuals, then "colony-level exposure" is nothing more or less than the set of unique exposure events experienced by the individual members of a colony. Since the first concept of colony-level exposure is not amenable to

empirical methods and the latter concept is indistinguishable from individual-level exposure, it must be concluded that toxic exposure can only be studied as a fundamentally individual-based phenomenon, and that this is equally true of both primary and secondary exposure **(Figure 1)**.

Such an individual-oriented approach to honey bee exposure modeling poses considerable challenges, but without a mechanistic understanding of exposure, the rapidly proliferating studies of toxic effects in the laboratory and in the field will remain insolubly disjunct. There is ample precedent for the development and application of rigorous exposure models in the larger context of ecotoxicology and ecological risk assessment, and while the honey bee poses some unique challenges to exposure modeling, it is among the world's most thoroughly studied organisms, and a wealth of empirical and theoretical literature is available for the construction and parameterization of models. Indeed, many aspects of honey bee biology have already been modeled extensively [54], and the main task of exposure modeling is simply to apply existing knowledge to toxicological scenarios.

The future of honey bee exposure modeling is especially compelling in view of recent advances in mechanistic modeling of pesticide effects, particularly using the versatile BEEHAVE model [98, 99, 100]. The conjunction of mechanistic effects modeling and mechanistic exposure modeling will lead to an unprecedented depth of insight into honey bee toxicology, simultaneously advancing the protection of honey bee health and the basic study of ecotoxicology in a social insect model system.

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Data Availability—As this is a review, the data and equations used in the paper can be found through the referenced publications.

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Figure 1. Relationship between individual-level exposure, individual-level effects, and colony-level effects. A normal distribution of individual exposure levels (A) clustered tightly around the mean (blue dashed line) results in a very small proportion of the colony experiencing doses above the predicted no effect concentration (PNEC) (red dashed line). Bimodal (B) or lognormal (C) distributions having the same mean as the normal distribution result in a much larger proportion of bees experiencing pesticide doses in excess of the level of concern. The distribution of exposures (depicted by red color intensity) experienced by individual bees causes a distribution of individual effects (depicted by opacity), ranging from mild sublethal impairment to death (upside-down bees). These individual effects may translate into effects on colony-level functions.

Figure 2. Comparison of contact and dietary exposure estimates. Bars represent the proportion of foraging habitat that is untreated or treated with an an application rate of 80 g/ha. Scenario A represents equal abundance of contaminated and uncontaminated forage. In Scenarios B and C, the relative abundance is skewed toward either the contaminated forage (Scenario B) or the treated crop (Scenario C). Under contact exposure, axes labeled “Atkins”, “Poquet”, and “Bee-REX” are transformations of the main x-axis (depicting field application rate) using the coefficients by which field application rate is multiplied in each model. The dashed line shows where the exposure predictions of the models fall with respect to the range of environmental contamination levels caused by range of field application rates. Under dietary exposure, the dotted lines represent the predictions of the EFSA model and Baveco models (when the latter is collapsed to its mean) for each scenario, while the dashed lines represent those of the Bee-REX model. Asterisks, with subscripts representing the percentage of foragers exposed to the indicated dose, show the raw distribution of exposure predicted by the Baveco model.

Figure 3. Processing pathways of nectar-associated and pollen-associated pesticide. Pesticide-laden nectar (red) undergoes extensive trophallactic transmission prior to consumption, resulting in widespread but dilute ingestion of nectar-associated pesticides. Pesticide-laden pollen (blue) undergoes no mixing or dilution and is consumed almost exclusively by nurse bees, which may, therefore, receive more extreme (higher and lower) pesticide doses than other colony members. Nurses convert pollen-derived nutrients into glandular secretions (jelly) (purple) that they combine with variable amounts of nectar and raw pollen; these mainly nourish the brood and queen but also supply the dietary protein needed by adult workers and drones.

Table 1. Summary of existing quantitative models of primary exposure. Models are described according to the modes of pesticide application they represent, the modes of exposure they estimate, and their approaches to modeling the critical components of environmental contamination and honey bee foraging behavior.

Model reference	Modes of application	Modes of exposure	Environmental contamination	Honey bee foraging behavior
Atkins (1981)	foliar spray	contact	field application rate	NA
Poquet (2014)	foliar spray	contact	field application rate	NA
USEPA (2012) (Bee-REX)	foliar spray seed treatment soil drench	contact dietary (nectar + pollen)	field application rate	NA
Barmaz (2010, 2012)	foliar spray	dietary (non-specific)	drift gradient	NA
EFSA (2013)	non-specific	dietary (nectar + pollen)	variable contamination	floral attractiveness coefficient
Baveco et al. (2016)	non-specific	dietary (nectar)	treated and untreated areas	variation in nectar concentration and availability; patch selection by energetic optimization

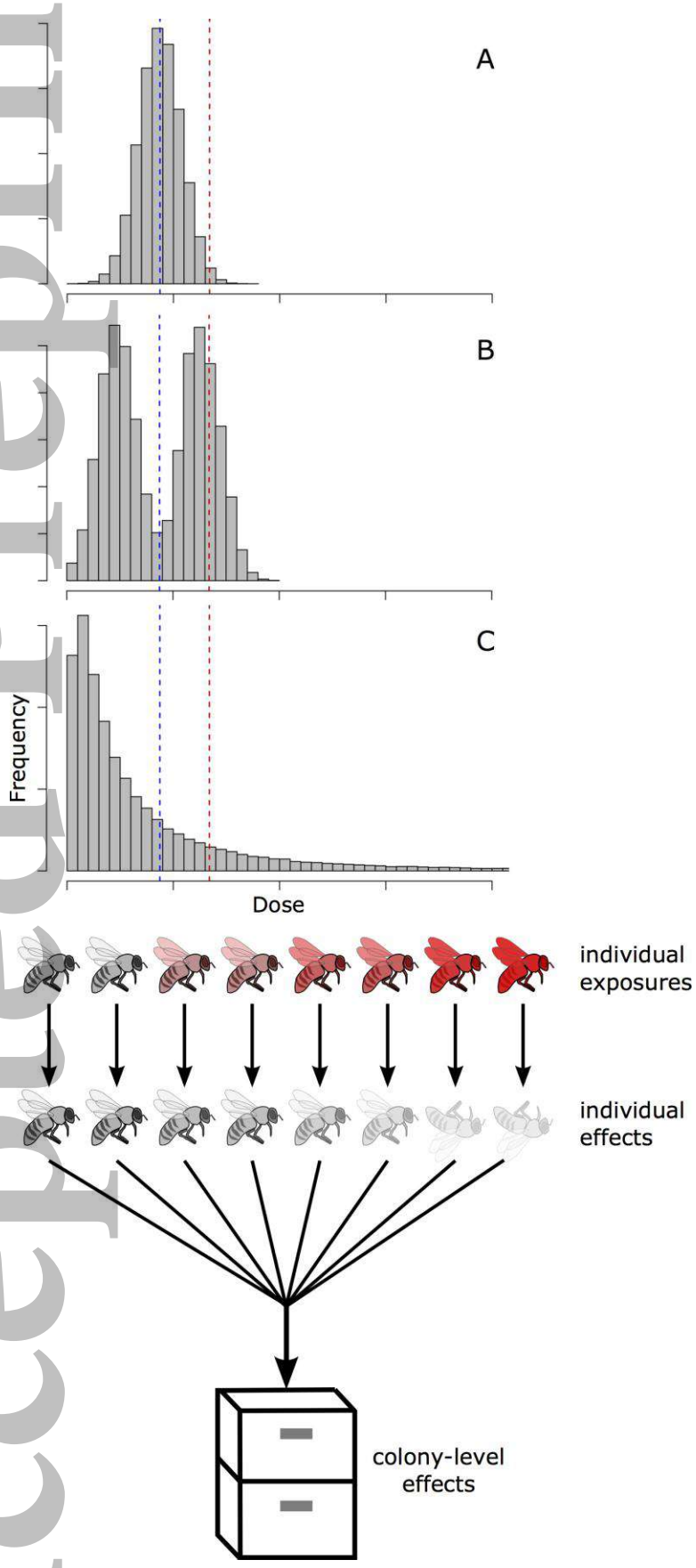

Figure 1

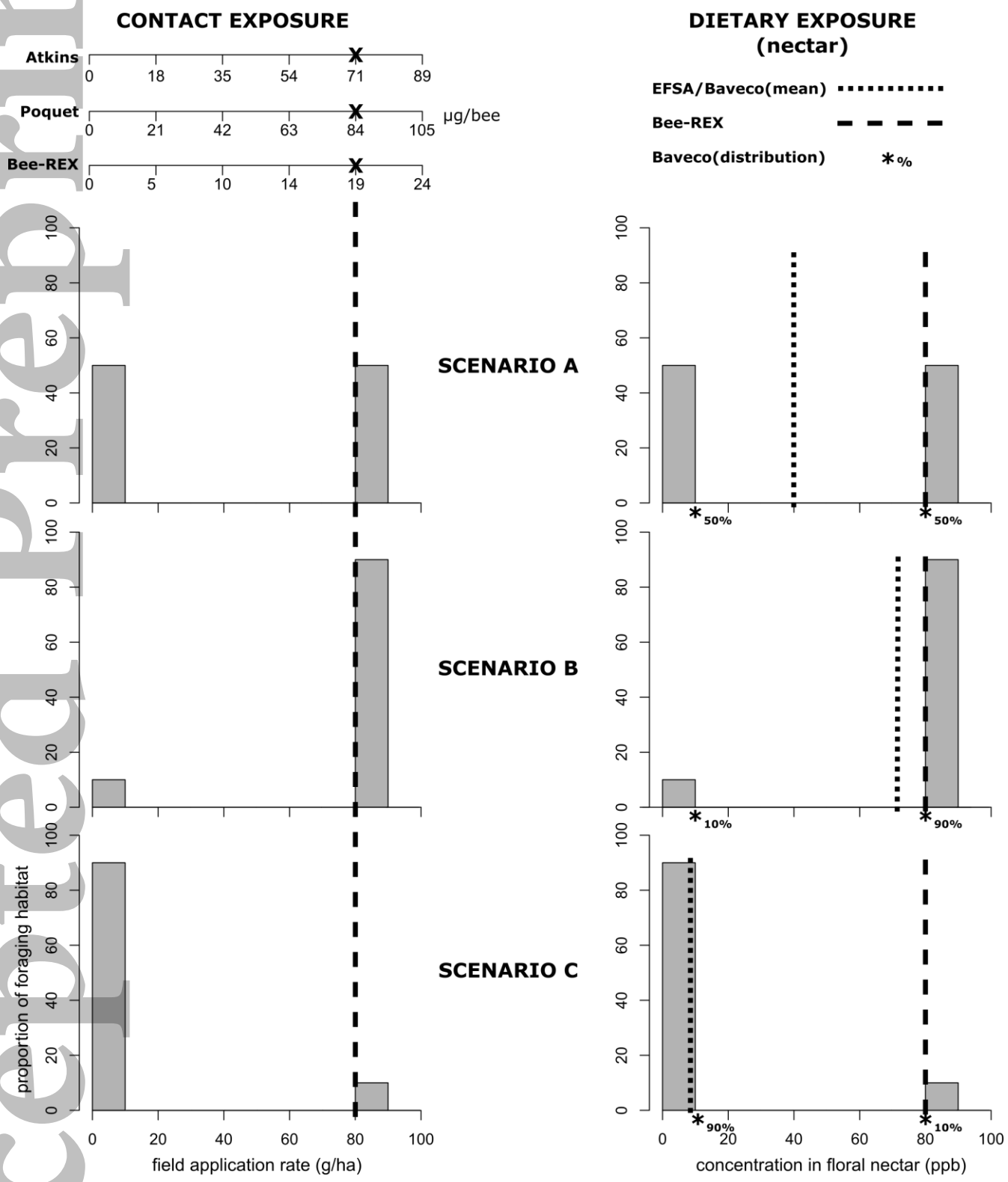

Figure 2

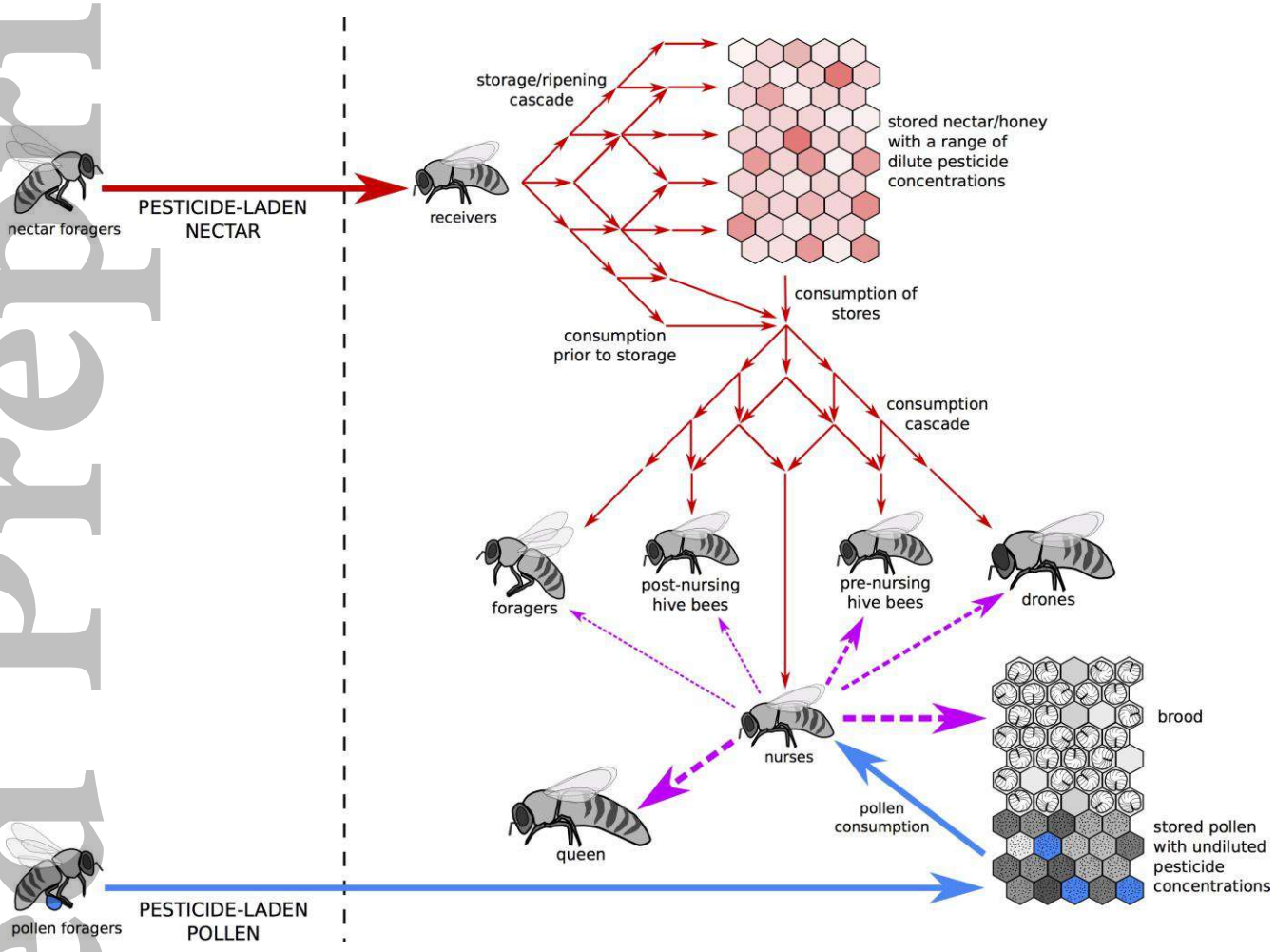

Figure 3