Environmental Science & Technology

Article

pubs.acs.org/est

Modeling Effects of Honeybee Behaviors on the Distribution of Pesticide in Nectar within a Hive and Resultant in-Hive Exposure

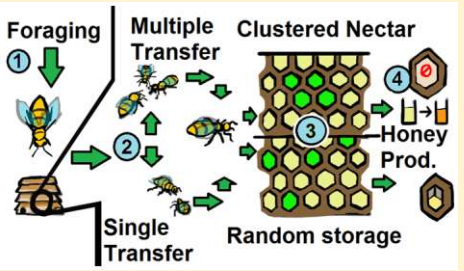
Jack C. O. Rumkee,[†] Matthias A. Becher,[†] Pernille Thorbek,[‡] and Juliet L. Osborne*[,†]

[†]Environment and Sustainability Institute, University of Exeter, Penryn Campus, Penryn, Cornwall TR10 9FE, U.K.

[‡]Environmental Safety, Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, U.K.

Supporting Information

ABSTRACT: Recently, the causes of honeybee colony losses have been intensely studied, showing that there are multiple stressors implicated in colony declines, one stressor being the exposure to pesticides. Measuring exposure of individual bees within a hive to pesticide is at least as difficult as assessing the potential exposure of foraging bees to pesticide. We present a model to explore how heterogeneity of pesticide distribution on a comb in the hive can be driven by worker behaviors. The model contains simplified behaviors to capture the extremes of possible heterogeneity of pesticide location/deposition within the hive to compare with exposure levels estimated by averaging values across the comb. When adults feed on nectar containing the average concentration of all pesticide brought into the hive on that particular day, it is likely representative of the worst-case exposure scenario. However, for larvae, clustering of pesticide in the comb can lead to higher exposure levels than taking an average concentration in some circumstances. The potential for extrapolating the model to risk assessment is discussed.



INTRODUCTION

Pesticides, particularly insecticides, have the potential to impact the honeybee colony if exposure is high enough.[1] The sensitivity of the colony to pesticide stress depends on the scale of the effect and the life-stage being impacted and varies over the year.[2] There has been much discussion of the real world impact of these chemicals, most recently with respect to systemic neonicotinoids,[3] and there is evidence that, at field-realistic doses, the honeybee colony may be able to compensate for pesticide effects.[4−7]

If honeybees forage on a crop that contains pesticide in its pollen or nectar, then foraging bees will come into contact with it.[8] This could cause foragers to fail to return to the colony, either via direct mortality or orientation failure.[9] If they do return to the hive, however, they may bring pesticide into the colony where the younger, in-hive bees and brood will be exposed.[8] It is difficult, but important, to estimate the level of exposure of foraging honeybees.[10,11] It is also important to estimate exposure of bees within the hive,[12,13] both brood and young adults who have not yet left the colony to forage, since it is predicted that losses of these life-stages could have a larger impact on colony health relative to the loss of the older foraging bees.[2] The route of exposure for in-hive bees and brood is likely to be mainly via pesticides in nectar and pollen brought back by foragers.[8] The exposure level will depend on the pesticide concentration in the surrounding forage, metabolism, and dissipation of the pesticide along with the foraging, storage, and feeding behavior of the bees (including processing into brood food by nurse bees).[14,15] We have developed a model that simulates what happens to the nectar when it reaches the colony, specifically focusing on how pesticide in nectar may be distributed, mixed, fed to larvae, and stored in the combs of a colony. There have been many reports of pesticide residues in plants, individual bees, and hive products;[10,16] however, little is known about the intracomb distribution of the pesticide (i.e., how pesticide is spread across the comb cells and how in-hive bees and brood are exposed). For example, if it is contained in nectar stored close to larvae and is therefore more likely to be fed to them, there may be a significant impact on that larval cohort. If it is processed into honey and capped, it is possible that the pesticide will dissipate before the honey is consumed and so will not have an impact.[17] This model will focus on pesticide brought into the hive via nectar,[16] which, depending on the pesticide may present a high level of exposure to the larvae compared to pollen (for example, the neonicotinoid imidacloprid[18]).

This model will assess how the movement of pesticide through the comb via the behavior of the individuals can affect the resultant exposure of those individuals, specifically focusing on the effect that different, extreme behaviors have on the pesticide dose received by larval bees and a generalized adult caste. The purpose of this model is not to predict exposure levels to individuals within the colony but instead to assess the need for the inclusion of the complex, in-hive processes when assessing the risk a pesticide may pose to the hive, or if a conservative estimate of pesticide exposure can be obtained through simpler means, and whether this should be a priority area for research.

Received: August 22, 2016
Revised: May 3, 2017
Accepted: May 9, 2017
Published: May 9, 2017

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

After nectar is brought by the foragers to the hive, it is transferred to one or more receiver bees,[19,20] mixing the nectar loads from multiple foragers. This nectar is then stored in comb cells by the receiver bees, and, while this has been reported to be a random process,[21] there may be patterns of storage based on global factors (such as gravity)[22] or local factors (such as the contents of nearby cells)[23] or potentially based on the concentration of sugar in the nectar[24] (although, see Eyer et al.[25]). The stored nectar, if nectar flow into the colony is abundant, will be concentrated, turned into honey, and capped for later consumption.

In principle a simple way to model the exposure of bees and brood inside the hive to pesticide would be to use the weight of pesticide brought in on a day and divide that into the total nectar volume brought into the hive on that day, giving an average daily pesticide concentration. The dose each bee then receives would then be calculated as the amount of pesticide in the volume of nectar that the bee or larva eats per day. Nectar within the hive is, however, compartmentalized into cells each potentially containing different pesticide concentrations. This heterogeneity of pesticide concentrations, arising from variability in residues in nectar from different sources and the storage and feeding behaviors, could lead to different exposure distributions within the hive.

In order to explore how sensitive the exposure distributions of in-hive bees and brood are to different assumptions about bee behaviors, we used extremes of the behaviors mentioned above. In particular, we wanted to explore under what conditions full mixing of residues in all nectar is worst-case and under what conditions a more detailed description of exposure distribution is needed.

MODEL AND METHODS

We have developed an individual-based model (IBM) implemented in Netlogo 5.2.0,[26] to explore how the distribution of pesticide in the comb is affected by the behavior and decisions of bees. The metabolism and environmental fate of pesticides will also affect the distribution but are not modeled here.

Model Description. The model is described in detail following the ODD protocol (Overview, Design concepts, Details) for the description of individual-based models.[27,28] Selected sections of the ODD are presented here, while the full ODD is available in the Supporting Information SI2.

Purpose. The purpose of this model was to assess how different food storage and feeding behaviors of the honeybee affect the distribution of pesticide concentration in stored nectar and explore how different distributions of pesticides affect the proportion of individuals (brood and adult bees) which will be exposed above a theoretical threshold (set to an arbitrary level here but which could be defined based on a pesticide's toxicity). The model can then be used to assess the complexity required in introducing realistic in-hive pesticide exposure into an existing honeybee colony model (e.g., BEEHAVE[29]). In particular, we set out to compare pesticide distributions as a result of the following contrasting behaviors: (i) comparing multiple transfers between foragers and receivers (M) as opposed to each forager transferring nectar to a sole receiver (S); (ii) comparing when receiver bees store nectar in the comb randomly (R) versus clustering (C); (iii) comparing the effect of capping the nectar cells (as a result of processing to honey) (P) versus no capping (N). We also investigate the impact of differing proportions of foragers bringing pesticide into the colony, a simplified surrogate for pesticide exposure levels in the landscape.

The model is not intended to provide accurate estimates of the absolute values of exposure or toxic effects of pesticide within the hive, rather, it is intended to explore the differences in pesticide distributions in nectar occurring from these simplified behaviors and therefore establish the level of complexity required for a model such as BEEHAVE[12,29] to ensure a conservative assessment of the risk posed by pesticides.

Entities, State Variables and Scales. *Agents/Individuals.* The model contains three classes of agents: The cells of a single, one-sided hive comb, the bees, and the forage patches. The cells of the hive comb are spatial units, implemented as "patches" in NetLogo.

Each cell is characterized by the following state variables: 1) *patch_type*: patch contains nectar or a larva or is empty; 2) *nectar_volume_ul*: the current volume of nectar in the cell, measured in μL; 3) *pesticide_concentration_ugul*: the concentration of pesticide in the cell, measured in $\mu g\ \mu L^{-1}$, if the cell is a nectar cell; and 4) *cell_nectar_concentration_ugul*: the concentration of the sugar, measured in $\mu g\ \mu L^{-1}$ in the nectar contained in the cell;

A single nectar load is assumed to be 14 μL, within the range reported by Huang and Seeley (2003) (14.9 ± 9.8 μL).[30]

The forage patches are characterized by the following variables: 1) *nectar_concentration_ugul*: the concentration of sugar in the patch, measured in $\mu g\ \mu L^{-1}$ and 2) *field_pesticide_concentration_ugul*: the concentration of pesticide in the patch, measured in $\mu g\ \mu L^{-1}$;

Within the class of agents representing the bees, there are four types: 1) foragers; 2) receivers; 3) larvae; and 4) the queen. In the rest of the manuscript, "adults" represent a generalized combination of the foragers and receivers (but not nurse bees), whose feeding requirements are assumed to be the same for simplicity. A nectar load in the model is 14 μL.[30] This is the amount carried by the adult bees and is constant. Pupae are not considered in the model, as they do not receive nectar during pupation.

The forager bees are characterized by the following variables: 1) *pesticide_amount_ug*: the amount of pesticide carried by the forager, measured in μg; 2) *carrying_nectar*?: a Boolean value, true if the forager is still waiting to transfer nectar to a receiver; 3) *carrying_2nd_nectar*?: a Boolean value, true if, when multiple transfer is active, the forager is waiting to transfer the second load of nectar; and 4) *nectar_sugar concentration_ugul*; the concentration of sugar in the nectar load carried by the forager, measured in $\mu g\ \mu L^{-1}$;

Receiver bees are characterized by the following variables: 1) *pesticide_weight_ug*: the amount of pesticide currently carried by the receiver, measured in μg; 2) *destination*: the receiver's cell of choice in which to deposit the carried nectar load; and 3) *nectar sugar_concentration_ugul*: the concentration of sugar in the nectar load carried by the receiver, measured in $\mu g\ \mu L^{-1}$;

Larvae are characterized by the following variables 1) *age*: the age of the individual in days; 2) *pesticide_amount_ug*: the amount of pesticide contained in the larvae, measured in μg; and 3) *cell_choice*: the cell the larvae will be fed from.

The queen is characterized by its location on the comb; the only role of the queen in this model is creating a new brood with a realistic spatial distribution.

The spatial scale of the model is set to represent a typical comb of a National bee hive[31] assuming a frame of 34.1 × 20.3 cm with 4.34 cells per cm^2. The comb consists of a grid of square cells, 80 × 40, giving 3200 cells, a reasonable estimate of the number of worker cells on one side of a frame (Camazine 1991).[21]

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

The model runs in daily time steps with the foraging, receiving, and feeding processes looped to implicitly represent hourly behaviors (e.g., foraging, receiving, storage, and feeding) and others happening once per day (processing).

Units. The model keeps track of pesticide and sugar as both concentrations and mass. When dealing with volumes larger than a single bee's nectar load (such as in a nectar cell or at the forage patch), the substance is stored in the model as a concentration. When being handled by an individual, i.e. in foraging, receiving, storage, and feeding, the substance is stored in the model by the mass of the substance. This facilitates the calculations required when nectar is stored or removed from a large source (cell or forage patch) and allows a practical understanding of the potential exposure of individuals to the substance within the hive (individual dose received and pesticide concentration in nectar stores). For concentrations of pesticides and sugar in the model, we use weight per volume (μg/μL). The mass of a substance is measured in μg, and when discussing the movement of nectar within the hive we use volume (μL). When calculating the concentration of a substance in the cell when a nectar load is added to it, the following equation is therefore used:

$$\begin{aligned}&\text{new concentration in cell } [\mu\text{g}/\mu\text{L}]\\&\quad = [(\text{concentration in cell } [\mu\text{g}/\mu\text{L}]\cdot\text{volume of nectar in cell } [\mu\text{L}])\\&\quad + (\text{weight in nectar load } [\mu\text{g}])]/[(\text{volume of nectar in cell } [\mu\text{L}]\\&\quad + \text{volume of nectar load } [\mu\text{L}])]\end{aligned}$$

Process Overview and Scheduling. Time in the model is first split into days; at the beginning of the day, the "daily update" procedure is called, and at the end of each day nectar is processed. The main procedures of the model (foraging, receiving storage, and feeding) occur once per hour. In the real hive, there will be changes in behaviors throughout the day; however, to maintain simplicity of implementation and analysis, each hour in the model is identically parametrized, although foraging and the resultant storage only occurs for a set number of hours. Within these procedures, when all agents perform an action (e.g., all receivers storing nectar), they are called at random to perform this action. Procedures are performed in the following order each day:

Daily Update. Occurring at the start of each day, daily count variables are reset to 0. Larvae age, and if they are above the age threshold for pupation (by default 6 days), they are removed from the model as, in reality, they pupate and feeding ceases. Eggs are then laid in empty cells to replace the lost larvae, maintaining a constant number of larvae.

Foraging. Each hour while foraging time remains, a defined percentage of foragers are assigned, at random, to one of the two patches (treated with pesticide or nontreated). They are then given a set volume of nectar of the correct sugar and pesticide concentrations for the patch on which they foraged.

Receiving. After each foraging round, receivers take the nectar loads from foragers, chosen randomly from the population of foragers still waiting to transfer nectar. After securing a nectar load the receiver chooses a cell in which to deposit nectar, depending on the scenario either at random or according to the sugar concentration of the nectar (clustering) and deposits the nectar load in the relevant cell.

Feeding. In the real world adult nurse bees feed the larvae; however, as this is the only duty to be performed by nurse bees, in this model, nurse bees are implicit in the behavior of the larvae. Feeding rates in the model do not depend on the source of the nectar, although in a real hive the sugar concentration of the nectar may lead to larvae being fed different volumes,[18] the sugar concentration in this model is arbitrary, and by excluding this resultant differential volume used as food we do not limit ourselves to the scenario in which the pesticide is contained in nectar with a higher sugar concentration. Conversion from weight of nectar to volume of nectar would depend on the sugar concentration of the nectar. The sugar concentration of the nectar in this model is solely used as a label to differentiate between the two nectar sources; the fact that the treated nectar has a higher sugar concentration is arbitrary. It is therefore safe to assume the volume to weight ratio of 360 μL of nectar to 500 mg (0.72 μL/mg) of nectar as used by Schmickl and Crailsheim.[32] This ratio is for honey in their model; however, nothing is lost in this assumption for nectar in this model as feeding rates are not based on the sugar concentration. Every hour in the model (24 times per day), the closest cell to each larva that contains enough nectar for one feed is chosen, implicitly modeling simplified nurse bee behavior representing the empirical observation that nectar and pollen are removed from close to the larvae more frequently,[21] giving the most extreme scenario. The larvae then feed on the nectar from the relevant cell. Each hour, each larva receives 0.81 μL of nectar ($163.5 \cdot 0.72 \cdot 0.0069 - 163.5$ mg required to take one larva to pupation,[33] 0.72 conversion to μL, 0.0069 conversion to hours), assuming 6 days from hatching to pupation, with the conversion of mg to μL as given above. In reality the amount a larva is fed will change based on its age, as well as on the sugar concentration. We have kept the volume of nectar a larva eats constant across each day for simplicity. After the larvae have fed, the adults in the model feed, removing 0.32 μL per day.[18] As nurse bees are only implicit they do not feed, and their exposure is not considered.

One factor that is not included in the model, which may reduce the transfer of pesticide from the nurse bees to the larvae is the metabolism of pesticide by the nurses during the production of the brood food. In the real hive, developing workers are fed royal jelly from the nurses hypopharyngeal glands for 3 days and nectar and pollen on subsequent days. As the nurses collect and process the food for the larvae, any pesticide within the food may be metabolized within the nurses so the content of pesticide within the food the larva receives will be reduced. The extent to which this metabolism takes place is highly dependent on the specific chemistry of the xenobiotic in question and is also not measured in most cases. It will also only reduce the pesticide movement to the larvae. For simplicity, and to maintain the conservative nature of this model, this process has been left out of this model version. We propose that the results from this model remain useful with this simplification as we are not attempting to model the actual levels of exposure of individuals to pesticide, rather, we are exploring how behaviors within the hive could possibly affect exposure to pesticides and, for risk assessment, if these behaviors require consideration in a modeling approach. If the realistic level of exposure of individuals to pesticide was the aim of this modeling exercise, and if there were good empirical data available on the transfer of pesticides via brood food, then this would need to be considered.

Processing. Nectar cells which are more than 95% full are "capped", so they are no longer available to be fed from or deposited in, and the nectar in them is concentrated, representing the transformation to honey. In the model, this processing is simply the reduction of the volume of the nectar by 75%, maintaining the weight of pesticide in the nectar constant (based on the simplified assumption that the nectar contains 80% water,[34] although in reality this is variable dependent on the

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

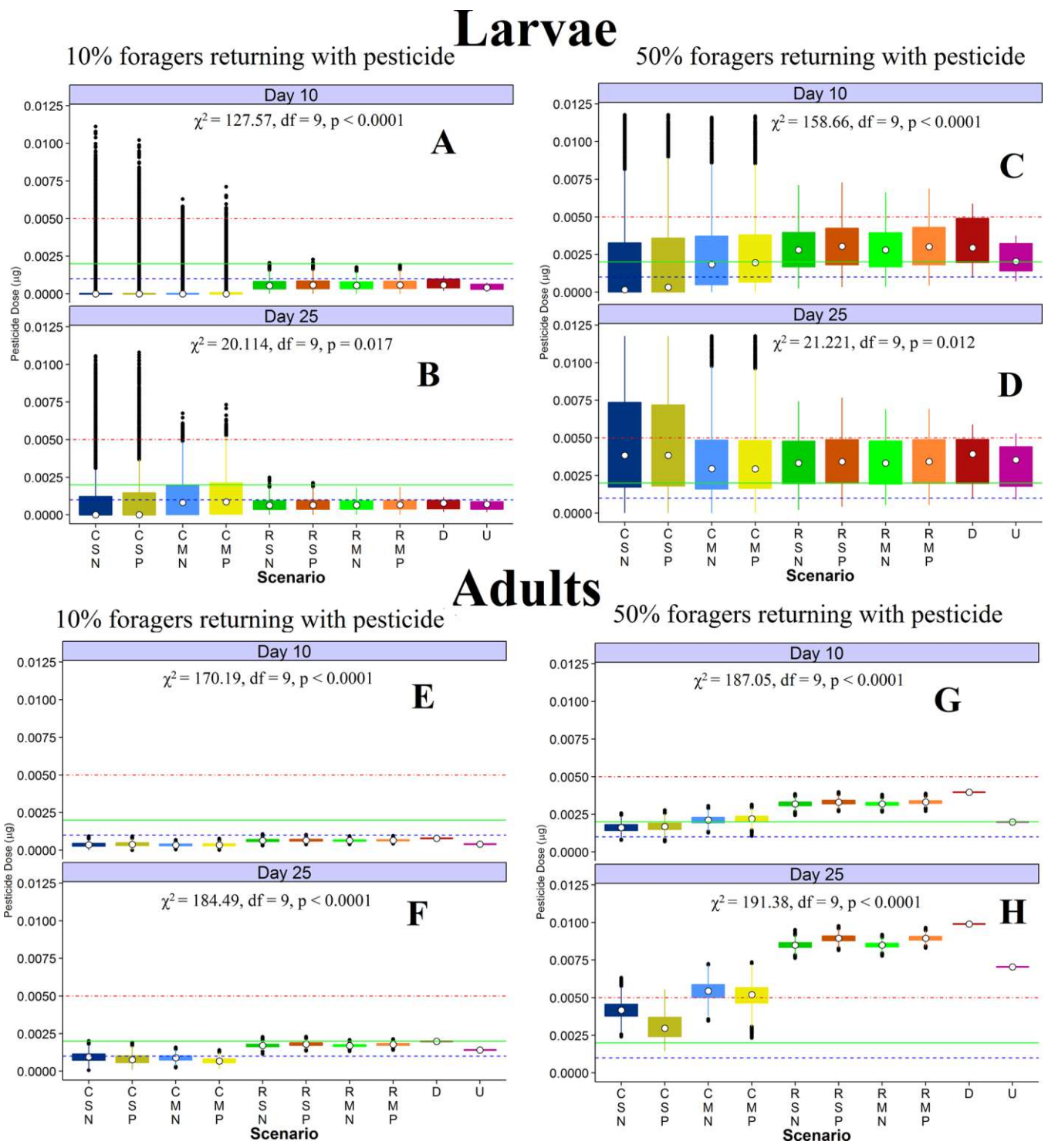


Figure 1. Boxplots showing the dose of pesticide in the larvae (A-D) and adults (E-H) when 10% and 50% of the foragers return with pesticide, on days 10 and 25. White points show the median value of the distribution, considering all individuals across all replications. Scenarios are defined by C – clustered storage, R – random storage, S – single transfer, M – multiple transfer, N – no processing, P – processing, D – daily average, U – uniform average. Boxes show the 25th and 75th percentiles (colors differentiate between the scenarios as in Figure 2), whiskers show the maximum/minimum value within 1.5× the interquartile range, and any other points are shown in black. The blue, green, and red lines show the 1, 2, and 5 ng threshold values used to explore the proportion of individuals receiving a certain pesticide dose (see Figure 2). With respect to the averaged scenarios: for adults, as there is no replacement of individuals, each individual gets the same pesticide dose so there is no variance (E-H). Each larva pupates and is removed from the model and is replaced after 6 days, so this, combined with the effect of the spatial positioning of any pesticide clusters, leads to a distribution of pesticide doses (A-D) in averaged scenarios.

species and climate, and that honey contains 20% water[35]). As the sugar content of the capped nectar is of no consequence in this model and there is no repercussion on the exposure of the bees to the pesticide we consider this extreme simplification of the process reasonable, acting as a placeholder for potential expansion of the model.

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Initialization. At the beginning of the simulation, 150 foragers, 150 receivers, and 400 larvae are created. In a real brood frame, a much larger proportion of the cells could be filled with larvae during the breeding season; however, a single side of a single frame is modeled here providing food for the larvae and adults. Larvae are placed in the comb so there are no more than two cells between each larva, similar to Johnson (2009).[22] Initially 10% of the comb is filled with control (clean) nectar to represent that the frame has been used for brood and food storage for some time prior to a sudden pesticide-containing nectar flow. The concentration of pesticide in the nectar of the forage patch is set arbitrarily to 100 μg pesticide/μL, intentionally high to ensure pesticide reach the in-hive bees. The model was created to test the extremes of the behaviors and not the precise movement of pesticide into the comb and will therefore not provide realistic values of pesticide in the individual bees. Instead an arbitrary value allows us to focus on how the different behaviors alter how the pesticide moves through the hive and the resulting heterogeneity of pesticide residues in nectar, adults, and brood to evaluate which, if any of the extremes, would be the worst-case scenario in terms of risk of exceeding a given toxicity threshold. The sugar concentration of the nectar acts purely as a label as to the source of the nectar, as there is some evidence that nectar could be clustered together based on sugar concentration.[24] This difference in sugar concentration between nectar from the two patches serves only to test receiver bee behavior; in reality the sugar concentration will be highly dependent on species and climate.

In this model, the pesticide does not dissipate and is not metabolized in the individual bees, e.g. during feeding of larvae. Dissipation and metabolism would be highly product specific and could greatly reduce the exposure of individuals to pesticide, by leaving it out from the model we ensure a conservative estimate of the exposure and maintain generality.

Output. The output variables are the cumulative pesticide doses (μg) received by larvae and adults. These outputs were recorded daily. From these, the proportion of both adults and larvae that had received one of two hypothetical theoretical "threshold" doses of pesticide (1 ng and 5 ng) was calculated on each day. In risk assessment this threshold would be set using an endpoint, such as the NOEL or LD_{50} estimated in ecotoxicological studies.[36]

Simulation Scenarios. The design of the simulations was factorial: 3 behaviors, each with 2 levels: (i) the storage of nectar by receivers was random (R) or clustered (C); (ii) foragers transferred to single (S) or multiple (M) receivers; and (iii) the nectar was processed to honey (P) or not (N). So, in total there were 8 combinations of behaviors, giving 8 "behavioral" scenarios. Alongside these, we also included two "averaged" scenarios: (i) The Uniform Average (U) in which the larvae received a pesticide dose calculated from the overall average concentration of pesticide in the entire comb each time they fed, i.e. the total mass of pesticide currently in the comb divided by the total volume of nectar, to show the effect of assuming full mixing of nectar from all sources of food in the hive; (ii) The Daily Average (D) scenario where larvae received a pesticide dose calculated from the daily overall average concentration of pesticide in the nectar brought in on that particular day. Twenty replications of each of these ten scenarios (Table S1) were run, each for 30 days. Each set of simulations was run either with 50% of foragers assigned to the treated food patch or with 10% foragers assigned to the treated food patch, representing foraging in landscapes with different proportions of food patches containing pesticide to show how a range of landscape exposures may affect the heterogeneity of exposure within the hive.

Analysis. Outputs were taken directly into R[37] from Netlogo with the "RNetLogo"[38] library for R and analyzed as follows:

To quantify the heterogeneity and spatial autocorrelation of pesticide in the cells of the frame, two indices (Gini coefficient and Moran's I) were calculated (details in the SI).

The distribution of pesticide doses (μg) received by the larvae and adults was plotted across all ten scenarios to see how pesticide is distributed among the individuals over time. For each scenario, the median dose of pesticide received by both the larvae and the adults was calculated, giving one value for the larvae and one for the adults in each of the 20 replicates. It was confirmed that 20 replicates were sufficient for the stochastic effects to be adequately captured, by plotting the medians of the dose received by the adults and larvae in the 8 behavioral scenarios as the number of replicates increases (Figures S2–S5). To investigate how the output of the model is altered by the initial conditions, simulations were run changing the number of adult bees, larvae, the concentration of the pesticide, and the proportion of foragers returning with pesticide. It was found that the number of either class of individuals did not have a noticeable effect on the output and that the concentration of the pesticide in the nectar and the proportion of foragers returning to the colony with pesticide both have a large effect on the dose received by the individuals in the model (Figures S6–S13). A Kruskal–Wallis test was used to test for significant differences in the median values of pesticide doses received by both the adults and larvae, between the 10 scenarios. In total, 8 tests were run, for the pesticide doses received by the larvae and the adults, both when 50% of foragers return with pesticide and when 10% of foragers return with pesticide on day 10 and day 25 (to examine any change over time). The behavioral and averaged scenarios did not have equal variances, with lower variance in the averaged scenarios (Figure 1), leading to the choice of nonparametric methods. If the Kruskal–Wallis test showed significance, further investigation was carried out with posthoc analysis using the Dunn test with a Bonferroni correction.[39] These pairwise analyses were used to test how, if at all, the 8 behavioral scenarios differ from the averaged scenarios.

Finally, the proportion of larvae and adults that had received a cumulative theoretical threshold dose of pesticide by the end of each day of the simulation was measured and plotted. This was calculated for two hypothetical "threshold" values (1 ng and 5 ng), not intended to represent real world scenarios but chosen solely to further examine the impact of the modeled behavior on potential impact of pesticides within the colony, relevant to theoretical endpoints in risk assessment.

Verification (Test of Model Implementation). The model was tested to ensure it was working correctly by calculating the mass balance of the model. As nectar enters the comb, the total amount of nectar and pesticide are tracked. These are then compared against the total nectar in the comb, nectar lost through feeding, pesticide amount in the larvae, the pesticide concentration of each cell multiplied by its nectar volume in L, and a variable that captures pesticide "loss" from the model, for example, when all cells are full and receivers have no place to store their nectar load.

RESULTS

Heterogeneity and Spatial Autocorrelation. On day one, all scenarios lead to Gini coefficients >0.75 implying that most of the pesticide is contained in a small number of cells (Figure S1).

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX–XXX

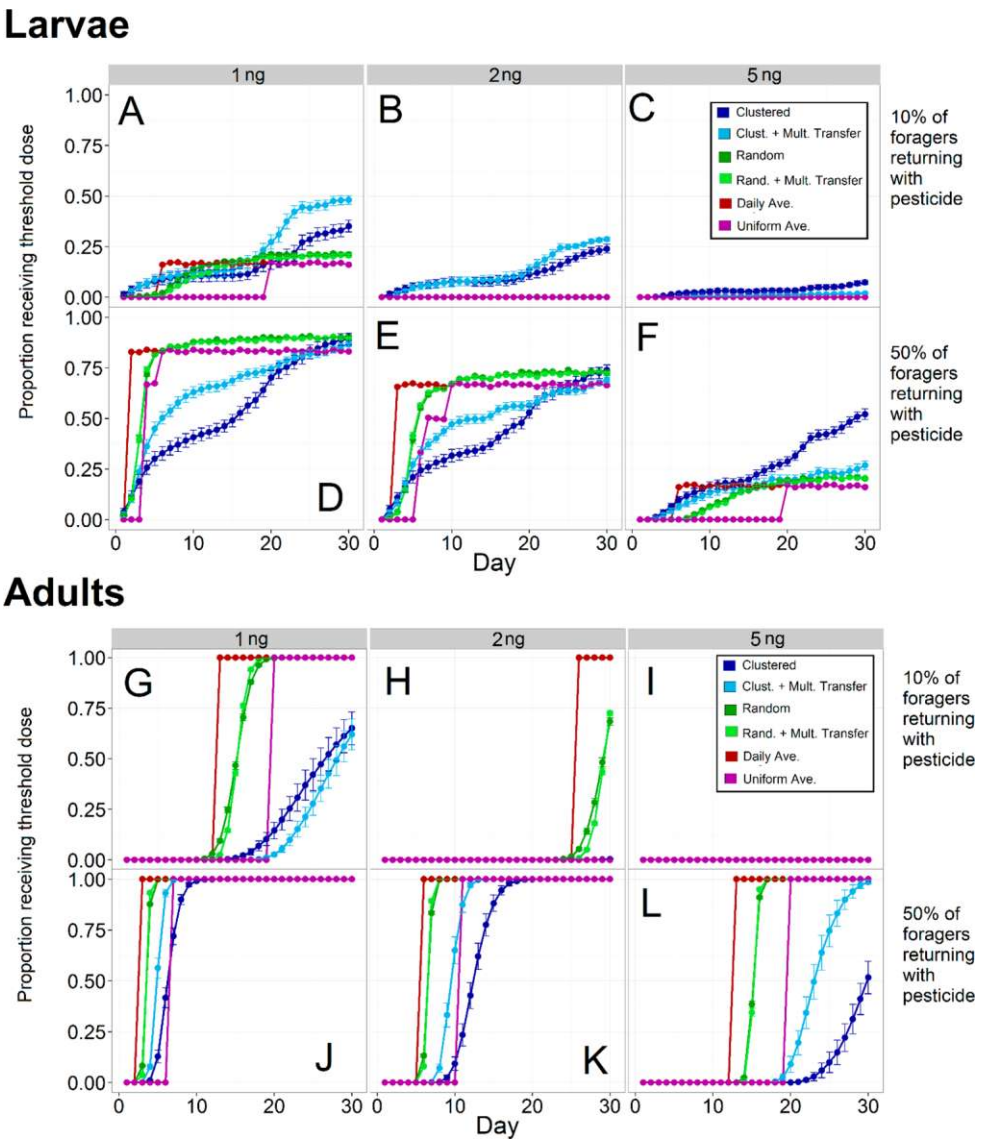


Figure 2. Mean (±standard error) proportion of larvae (A-F) and adults (G-K) that received two "threshold" levels of pesticide over the course of 30 days (means of 20 replicates). The two "averaged" scenarios (daily average pesticide concentration and uniform average pesticide concentration) are shown, along with the four scenarios without nectar processing.

This was lower in scenarios with random storage indicating reduced heterogeneity but remained high with clustered storage.

Moran's I shows that if the receivers are placing nectar randomly, the pesticide is spaced randomly in the comb. As time moves on there is a small increase in Moran's I, as most cells contain pesticide, so there is autocorrelation on the local scale. When the receivers cluster the nectar, Moran's I is higher indicating positive spatial autocorrelation, and this does not appear to change much with time.

Effect of Behavior on Distribution of Pesticide Doses. Kruskal–Wallis tests showed there were significant differences between the scenarios in the median pesticide doses received by larvae and by adults, both when 10% and 50% of the foragers return to the colony with pesticide, on both days 10 and 25 of the simulations i.e. for all eight comparisons, prompting posthoc analyses (presented in Tables S2–S5). Patterns of results are discussed for larvae and adults separately below.

Larvae. When 10% of foragers return with pesticide, the median doses received by larvae were low after 10 and 25 days of the simulations (Figure 1A, B), for all scenarios. As expected they were higher when 50% of foragers return with pesticide (Figure 1C, D). In all comparisons (Figure 1A-D), the *variation* in dose received by larvae was highest for the clustered scenarios.

Results of the pairwise analyses showed similar (although not identical) patterns for both 10% (Table S2) and 50% of foragers (Table S3) returning with pesticide: On day 10, the daily average scenario led to a median pesticide dose higher than all scenarios, other than scenario RMP and was significantly different ($P < 0.001$ in all cases) to the scenarios with clustered storage (which had the lowest medians) and to the uniform average scenario. The median pesticide doses received in clustered scenarios were also significantly lower than the random scenarios. The uniform average scenario varied in its position in ranking of medians. On day 25, there were no significant differences between pesticide doses received in the two averaged scenarios and any of the eight behavioral scenarios. Landscape exposure (10% or 50% of foragers returning with pesticide) appeared to have more effect on average exposure of larvae, than the modeled behavioral scenarios (Figure 1A-D), although this was not statistically compared.

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX–XXX

Adults. Median doses received by adults showed similar patterns (Figure 1E-H). Although the variation in dosage to adults within a scenario was much less than for larval doses, it was still greater as a result of clustering behavior.

For 10% and 50% of foragers returning with pesticide, the patterns in the pairwise analyses results were similar for day 10 and day 25 (Table S4 and Table S5): again the daily average scenario resulted in the highest median dosage to adults, and this was significantly different ($P < 0.001$ in all cases) not only to the scenarios with clustered storage (which had the lowest medians) and to the uniform average scenario but also to the scenarios with random storage and no processing (RSN, RMN). As with the larvae, the clustered scenarios resulted in significantly lower median doses to adults than the random scenarios. The uniform average scenario also often resulted in a significantly lower dose to adults than some of the random scenarios. Overall landscape exposure (10 or 50%) appeared to have greater impact than the different behavior scenarios (Figure 1E-H). For both the adult bees and the larvae, the proportion of foragers returning to the colony with pesticide has a greater impact on the exposure of individuals within the colony than any of the behaviors occurring within the colony (Figure 1A-H). This is not surprising as when 50% of the foragers are exploiting the treated patch, as there are only 2 patches, there is five times as much pesticide entering the colony than when only 10% of the foragers are exploiting the treated patch. As this model seeks mainly to understand the change in exposure as a result of the different in-hive behaviors, the proportion of foragers returning with pesticide is not included as a factor in the statistical analysis. When only a small proportion of foragers are returning to the colony with pesticide in their nectar loads, depending on the storage behavior, there are two potential situations. If the nectar is highly mixed (multiple transfer and random storage), then there will be a low concentration of pesticide in much of the hive nectar. If the nectar is not mixed and clustered into cells solely consisting of the contaminated nectar, then most of the individuals will receive no pesticide, and some will receive nectar with a high concentration. When a larger proportion of foragers returns with pesticide in their nectar, if all the nectar is mixed, there will be a higher concentration throughout the colony stores. If the nectar is not mixed, then there will be a higher abundance of cells containing this maximum pesticide concentration, increasing the likelihood of an individual feeding from it. As the concentration of the pesticide in the forage patch increases, then in all cases the concentration of pesticide in the hive stores increases, but the abundance of cells containing pesticide does not change. This will, however, lead to an overall increase in individuals reaching threshold doses.

Effect of Behavior on the Proportion of Individuals at Risk. *Proportions of Larvae at Risk.* When 10% of the foragers returned to the colony carrying pesticide, until around day 19, in all scenarios, the proportion of larvae receiving the 1 ng theoretical threshold dose remained below 0.25 (Figure 2A). After day 19, scenarios in which receivers clustered nectar had a higher proportion of larvae receiving the 1 ng dose than scenarios with random storage or averaged pesticide concentrations in the food, with the addition of multiple transfer further increasing the proportion (Figure 2A). For the 2 ng (Figure 2B) and the 5 ng threshold (Figure 2C), only the scenarios with clustered storage led to a noticeable proportion of the larvae reaching the threshold with around 25% of larvae reaching the 2 ng threshold and 10% of bees reaching the 5 ng threshold by day 30.

When 50% of the foragers returned to the colony carrying pesticide, scenarios in which the receivers cluster nectar led to the proportion of larvae reaching the 1 ng threshold to rise more slowly than in the other scenarios (Figure 2D) as only larvae close to the pesticide cluster receive any pesticide dose. The addition of multiple transfers alongside clustered placement increases this proportion. This pattern also holds for the proportion of larvae receiving the 2 ng threshold dose (Figure 2E) with the scenarios with clustered nectar storage leading to a slower increase in the proportion of larvae having received the threshold but not leading to a higher proportion than the scenarios with random storage. Additionally, when compared to the 1 ng threshold the overall proportion reaching the 2 ng threshold was lower. When considering the 5 ng threshold (Figure 2F), after day 12, the scenario in which the receivers cluster nectar lead to a higher proportion of larvae reaching the threshold than scenarios with random placement and the two averaging scenarios. When multiple transfers are also occurring alongside clustered storage, the proportion of larvae receiving the 5 ng threshold remains lower and closer to the average scenarios.

Proportions of Adults at Risk. A higher proportion of adults reaches both threshold doses in the scenarios where adults feed from nectar with the daily average pesticide concentration (Figure 2G-L) than any other scenario, regardless of the proportion of foragers returning with pesticide. In the uniform average scenario, regardless of the proportion of foragers returning to the colony with pesticide, it takes longer for 100% of the adults to reach either threshold dose than the daily average or scenarios in which the receivers place nectar randomly. Scenarios in which receivers are clustering nectar lead to a lower proportion of adults reaching the threshold doses than when the receivers are storing randomly. In these scenarios, the pesticide is stored in fewer cells; as the adults pick cells at random, it is less likely that they feed from cells containing pesticide. When only 10% of foragers return to the colony with pesticide, no adults reach the 5 ng threshold (Figure 2I), and only the averaged scenarios and those with random nectar storage led to any adults reaching the 2 ng threshold (Figure 2H).

DISCUSSION

The results from the model presented show that the three behaviors we simulated can lead to significantly different distributions of pesticide doses received by both the larvae and in-hive worker bees (Figures 1, 2). The results also show that, in most cases, assuming each larva or adult feeds on the daily average pesticide concentration (total weight of pesticide brought in on a particular day/total nectar volume brought in) led to higher median doses received by both the larvae and the adult bees (Figure 1, Tables S2−S5), although effects of different behaviors were seen on the *distribution* of those doses among individuals (Figure 1), and on the likelihood and rate at which larvae or adults reach theoretical threshold doses (Figure 2). In particular, the way in which receivers choose to store nectar in the comb (random or not) appears to be much more impactful than whether or not multiple transfer between receivers and foragers takes place, or if some pesticide is removed from the system (capped) in the process of turning the nectar to honey.

The heterogeneity and spatial autocorrelation of pesticide in the cells of the comb (captured by the Gini coefficient and Moran's I respectively, Figure S1) show that on day 1, regardless of the scenario, the pesticide is only contained in a few of the cells. On day 30, those scenarios with random storage show that the pesticide is more evenly distributed across the cells; however,

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

with clustered storage the pesticide remained in fewer cells, which showed some positive autocorrelation. The distribution of pesticide doses received by the individuals (Figure 1) shows, as expected, that when the receiver bees cluster the pesticide-containing nectar, the medians are lower for larvae and adults than when the pesticide-containing nectar is placed randomly. However, for larvae, there is a broader distribution in clustered storage scenarios such that some larvae receive a much higher maximal dose (Figure 1A-D) and more larvae may reach a critical threshold depending on the level of exposure in the landscape (Figure 2A-D). The larvae feed from the cell closest to them with enough nectar to facilitate a single feed (implicitly representing nurse bees). If the pesticide-containing nectar is clustered close to the larvae, those larvae will only be fed on this nectar, leading to the high maximum dose received. In situations where a smaller proportion of the foragers is bringing pesticide into the colony, if there is a cluster of pesticide near the larvae, then some larvae will still be receiving large amounts of pesticide. In Figure 2A and B, this is observable as a higher proportion of the larvae received doses meeting the threshold values in the scenarios with just clustering (CSN) and that with clustering and multiple transfer (CMN) than the daily average scenario. This feeding from the area around the brood leads to this area being emptied and replenished regularly with fresh pesticide-containing nectar, which could influence exposure. A similar phenomenon may occur in the real hive, as empty space is used for storage. Additionally, it is important to remember that, in the real hive, larvae are fed by nurse bees. Through this feeding process, it is likely that in the preparation of the brood food, the pesticide may be metabolized by the nurse and less will reach the larvae, though the extent to which this may occur is highly specific to the chemistry in question. This may mean that the exposure levels in the model are higher than those expected in the real colony; however, as we are interested in the effects of behavior on the distribution of pesticide and the patterns of exposure to individuals, this does not significantly detract from these results and their implications.

In contrast adults feed randomly from the comb in the model, so, even if pesticide-containing nectar is clustered in the comb, over a number of feeds the individual adults will receive a mixture of doses and thus lower maximum doses (Figure 1E-H). In the case of the adult bees, assuming they feed on nectar containing the daily average pesticide concentration gives the most conservative estimate of exposure for all scenarios (Figure 1E-H). Rumkee et al.[2] show that the colony is highly sensitive to the loss of in-hive adult workers, and, as such, it is useful to know that we can assume averaging as the most conservative estimate. The results from this analysis of a generalized adult caste of foragers and receiver bees still provide useful results, as they show the change in exposure on individuals feeding at random within the comb with different in-hive behaviors. For the purposes of this model and the questions it seeks to answer, the differentiation of adult bees into their different jobs by age and resultant nectar consumption adds more complexity than strictly necessary; however, for any predictive models of exposure, this will be necessary.

Based on this model, however, taking the uniform average of total pesticide in the comb across the total nectar volume in the comb does not in most cases lead to a conservative estimate of the individual level exposure for larvae or adults. In practical terms, these results provide an argument that sampling nectar from random cells across the comb to estimate residue levels (equivalent to U) would not give a conservative estimate of risk. Sampling nectar coming into the colony on a daily basis (equivalent to D) (for example sampling honey stomachs from returning foragers) may be more appropriate in the majority of cases.

We have shown that the behaviors of individual bees could influence the movement of pesticide throughout the hive system and should be considered together with the chemical properties of the pesticide in question influencing the movement between compartments (e.g., nectar, wax, bees, etc.). In fact for the same amount of pesticide entering the hive, the behavioral movement of pesticides can have a considerable impact on the resultant exposure of individuals to the pesticide, and, although a daily average is a more conservative estimate of pesticide exposure, the movement of the pesticide through behaviors may need to be considered in some circumstances when attempting to assess realistic exposure. However, it should be noted that while the model was not designed to compare the effects of in-hive behaviors with the effects of external exposure levels, the proportion of foragers bringing contaminated nectar into the hive (set at 10% or 50%) did have considerably more impact on pesticide dosage to larvae and in-hive adults than in-hive behaviors, although this is not surprising given the 5-fold difference in simulated landscape exposure.

The spatial clustering in the model is extreme, with all pesticide-containing cells next to each other. If this extreme clustering of pesticide containing nectar is no worse than full mixing in terms of pesticide exposure, then it follows that less extreme clustering would also be no worse. However, for larvae, we have shown that extreme mixing *can* lead to a higher proportion of larvae receiving some pesticide doses in some circumstances (Figure 2A-D). There is some empirical evidence that clustering of nectars of similar sugar concentrations can occur,[24] although Eyer et al.[25] find clustering of nectar of similar sugar concentrations only occasionally and that this clustering effect is not found after around 3 days. However, as the clustering reported in Eyer et al.[25] is the clustering of nectar by sugar concentration, the resultant pesticide distribution from this clustering behavior would be unknown. The model also only considers a single pesticide in one of only two forage patches; however, in the real landscape there will be many more sources of nectar and, depending on the landscape, a number of sources of pesticides. An abundance of sources of nectar and pesticide is likely to increase the mixing of pesticide within the comb as, even if receivers sort nectar by sugar concentration, there may be nectar sources with similar sugar concentrations and yet varying pesticide concentrations and vice versa. Along with multiple transfers, nectar will likely be mixed within the hive by in-hive workers removing nectar from one cell and moving it into another, further reducing the heterogeneity of pesticide concentration across the comb cells. The model results imply that assuming the larvae are fed pesticide with an averaged pesticide concentration or from nectar that is well mixed is not, in all cases, the worst-case scenario will depend on the levels of pesticide in the landscape. As the model is intended to be extreme, more detailed investigation would be needed to assess exactly what level of pesticide clustering is realistic and the complexity of in-hive pesticide distribution necessary to obtain a worst-case exposure estimate for the larvae. When considering the exposure of the larvae to pesticides, the model results highlight the importance of knowing the prevalence of the specific pesticides in the landscape. If there is little pesticide in the landscape (here simulated by only 10% of foragers returning with pesticide), and if the pesticide in question is highly toxic to

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

the larvae (here simulated as a 1 ng threshold, Figure 2A), then the clustering of nectar in the colony may have a significant effect on the resultant impact of the pesticide on both individuals and therefore potentially on the colony.[2] Similarly, if the pesticide is prevalent (e.g., present in 50% of the forage sources), then Figure 2C and D imply that assuming an average dose is fed to the larvae is worst-case if the threshold dose required for an effect is low, as all larvae are likely to reach the threshold; but this is not the case for less toxic pesticides with higher thresholds (here simulated as 5 ng).

The European Food Safety Authority (EFSA) recently reviewed the BEEHAVE model[29] and highlighted the need for a pesticide module.[12] If necessary the model presented here could be incorporated into such a module, for the situations in which assuming an average, fully mixed pesticide concentration is not the most conservative estimate for exposure via nectar (e.g., Figure 2A: high toxicity pesticide affecting the larvae). If this were to occur, and the model was intended for use as a predictive, risk-assessment tool, the behaviors of the individuals within the colony, simplified for the purposes of this study, would need to be made more explicit. This would include the explicit inclusion of the nurse caste and the creation of brood food. For a more complete picture and the calculation of actual exposure levels, a similar approach to the model is presented here to explore the flow of pesticides into the model via pollen. However, in order to model this in a more realistic way, a detailed experimental study of in-hive behavior would be necessary. We suggest that the behavioral movement of pesticides could be a valuable route for empirical research, as we have shown that in the case of honeybees it can lead to a significant change in the exposure of individuals within the colony to pesticides, and it is likely that this will be the case in other areas of ecotoxicology. However, for risk assessments, using the average pesticide concentration of nectar brought in on a given day is protective under most circumstances.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b04206.

Appendix 1, figure showing the values of the Gini coefficient and Moran's I and tables describing the scenarios and abbreviations and presenting the full results of the pairwise analyses; Appendix 2, further details of the model, including the remainder of the ODD protocol and a table showing parameters used in the model with references (PDF)

Model file (ZIP)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +44 1326 259474. E-mail: j.l.osborne@exeter.ac.uk.

ORCID

Juliet L. Osborne: 0000-0002-9937-172X

Notes

The authors declare the following competing financial interest(s): Pernille Thorbek is employed by Syngenta, and this work is part of a Ph.D. studentship at the University of Exeter, jointly funded by the BBSRC and Syngenta.

■ ACKNOWLEDGMENTS

J.R. was funded to do this work on an Industrial CASE Ph.D. studentship funded by the Biology and Biotechnology Sciences Research Council of the UK (BBSRC) and Syngenta. J.O. and M.B. were supported by BBSRC project BB/K014463/1. P.T. works for Syngenta.

■ REFERENCES

(1) Johnson, R. M. Honey Bee Toxicology. *Annu. Rev. Entomol.* **2015**, *60* (1), 415−434.

(2) Rumkee, J. C. O.; Becher, M. A.; Thorbek, P.; Kennedy, P. J.; Osborne, J. L. Predicting honeybee colony failure: using the BEEHAVE model to simulate colony responses to pesticides. *Environ. Sci. Technol.* **2015**, *49* (21), 12879−12887.

(3) Eisenstein, M. Pesticides: Seeking answers amid a toxic debate. *Nature* **2015**, *521* (7552), S52−5.

(4) Rundlöf, M.; Andersson, G. K. S.; Bommarco, R.; Fries, I.; Hederström, V.; Herbertsson, L.; Jonsson, O.; Klatt, B. K.; Pedersen, T. R.; Yourstone, J. Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature* **2015**, *521* (7550), 77−80.

(5) Genersch, E.; von der Ohe, W.; Kaatz, H.; Schroeder, A.; Otten, C.; Büchler, R.; Berg, S.; Ritter, W.; Mühlen, W.; Gisder, S.; et al. The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* **2010**, *41* (3), 332−352.

(6) Godfray, H. C. J.; Blacquière, T.; Field, L. M.; Hails, R. S.; Potts, S. G.; Raine, N. E.; Vanbergen, A. J.; McLean, A. R. A restatement of recent advances in the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. *Proc. R. Soc. London, Ser. B* **2015**, *282* (1818), 20151821.

(7) Henry, M.; Cerrutti, N.; Aupinel, P.; Decourtye, A.; Gayrard, M.; Odoux, J.-F.; Pissard, A.; Ru ger, C.; Bretagnolle, V. Reconciling laboratory and field assessments of neonicotinoid toxicity to honeybees. *Proc. R. Soc. London, Ser. B* **2015**, *282* (1819), 20152110−20152110.

(8) Krupke, C. H.; Hunt, G. J.; Eitzer, B. D.; Andino, G.; Given, K. Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One* **2012**, *7* (1), e29268.

(9) Henry, M.; Beguin, M.; Requier, F.; Rollin, O.; Odoux, J. F.; Aupinel, P.; Aptel, J.; Tchamitchian, S.; Decourtye, A. A Common Pesticide Decreases Foraging Success and Survival in Honey Bees. *Science (Washington, DC, U. S.)* **2012**, *336* (6079), 348−350.

(10) Godfray, H. C. J.; Blacquiere, T.; Field, L. M.; Hails, R. S.; Petrokofsky, G.; Potts, S. G.; Raine, N. E.; Vanbergen, A. J.; McLean, A. R. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. *Proc. R. Soc. London, Ser. B* **2014**, *281* (1786), 20140558.

(11) Carreck, N. L.; Ratnieks, F. L. W. The dose makes the poison: have "field realistic" rates of exposure of bees to neonicotinoid insecticides been overestimated in laboratory studies? *J. Apic. Res.* **2014**, *53* (5), 607−614.

(12) EFSA Panel on Plant Protection Products and their Residues (PPR). Statement on the suitability of the BEEHAVE model for its potential use in a regulatory context and for the risk assessment of multiple stressors in honeybees at the landscape level. *EFSA J.* **2015**, *13* (6), 4125.

(13) Hörig, K.; Maus, C.; Nikolakis, A.; Ratte, H.-T.; Roß-Nickoll, M.; Schmitt, W.; Preuss, T. G. The advantage of a toxicokinetic model of the honey bee colony in the context of the risk assessment of plant protection products. *Julius-Kühn-Archiv* **2015**, *0* (450), 51.

(14) Tremolada, P.; Bernardinelli, I.; Colombo, M.; Spreafico, M.; Vighi, M. Coumaphos distribution in the hive ecosystem: Case study for modeling applications. *Ecotoxicology* **2004**, *13* (6), 589−601.

(15) Bonzini, S.; Tremolada, P.; Bernardinelli, I.; Colombo, M.; Vighi, M. Predicting pesticide fate in the hive (part 1): experimentally determined Tau-fluvalinate residues in bees, honey and wax. *Apidologie* **2011**, *42* (3), 378−390.

(16) Sanchez-Bayo, F.; Goka, K. Pesticide Residues and Bees - A Risk Assessment. *PLoS One* **2014**, *9* (4), e94482.

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(17) Jacobsen, R. E.; Fantke, P.; Trapp, S. Analysing half-lives for pesticide dissipation in plants. *SAR QSAR Environ. Res.* **2015**, *26* (4), 325−342.
(18) Rortais, A.; Arnold, G.; Halm, M. P.; Touffet-Briens, F. Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* **2005**, *36* (1), 71−83.
(19) Hart, a. G.; Ratnieks, F. L. W. Why do honey-bee (Apis mellifera) foragers transfer nectar to several receivers? Information improvement through multiple sampling in a biological system. *Behav. Ecol. Sociobiol.* **2001**, *49* (4), 244−250.
(20) Kirchner, W. H.; Lindauer, M. The causes of the tremble dance of the honeybee, Apis mellifera. *Behav. Ecol. Sociobiol.* **1994**, *35* (5), 303−308.
(21) Camazine, S. Self-organizing pattern formation on the combs of honey bee colonies. *Behav. Ecol. Sociobiol.* **1991**, *28* (1), 61.
(22) Johnson, B. R. Pattern formation on the combs of honeybees: increasing fitness by coupling self-organization with templates. *Proc. R. Soc. London, Ser. B* **2009**, *276* (1655), 255−261.
(23) Montovan, K. J.; Karst, N.; Jones, L. E.; Seeley, T. D. Local behavioral rules sustain the cell allocation pattern in the combs of honey bee colonies (Apis mellifera). *J. Theor. Biol.* **2013**, *336*, 75−86.
(24) Greco, M. K.; Lang, J.; Gallmann, P.; Priest, N.; Feil, E.; Crailsheim, K. Sugar concentration influences decision making in Apis mellifera L. workers during early-stage honey storage behaviour. *Open J. Anim. Sci.* **2013**, *3* (3), 210−218.
(25) Eyer, M.; Greco, M. K.; Lang, J.; Neumann, P.; Dietemann, V. No spatial patterns for early nectar storage in honey bee colonies. *Insectes Soc.* **2016**, *63* (1), 51−59.
(26) Wilensky, U. *Netlogo*; Center for Connected Learning and Computer-Based Modeling: Evanston, IL, 1999.
(27) Grimm, V.; Berger, U.; Bastiansen, F.; Eliassen, S.; Ginot, V.; Giske, J.; Goss-Custard, J.; Grand, T.; Heinz, S. K.; Huse, G.; et al. A standard protocol for describing individual-based and agent-based models. *Ecol. Modell.* **2006**, *198* (1−2), 115−126.
(28) Grimm, V.; Berger, U.; DeAngelis, D. L.; Polhill, J. G.; Giske, J.; Railsback, S. F. The ODD protocol: A review and first update. *Ecol. Modell.* **2010**, *221* (23), 2760−2768.
(29) Becher, M. A.; Grimm, V.; Thorbek, P.; Horn, J.; Kennedy, P. J.; Osborne, J. L. BEEHAVE: a systems model of honeybee colony dynamics and foraging to explore multifactorial causes of colony failure. *J. Appl. Ecol.* **2014**, *51* (2), 470−482.
(30) Huang, M. H.; Seeley, T. D. Multiple unloadings by nectar foragers in honey bees: a matter of information improvement or crop fullness? *Insectes Soc.* **2003**, *50* (4), 330−339.
(31) British Standard Bee Hive Frame Dimensions. http://www.dave-cushman.net/bee/bsframedimensions.html (accessed May 11, 2017).
(32) Schmickl, T.; Crailsheim, K. HoPoMo: A model of honeybee intracolonial population dynamics and resource management. *Ecol. Modell.* **2007**, *204* (1−2), 219−245.
(33) Harbo, J. R. Effect of brood rearing on honey consumption and the survival of worker honey bees. *J. Apic. Res.* **1993**, *32* (1), 11−17.
(34) Potts, S. G.; Vulliamy, B.; Roberts, S.; O'Toole, C.; Dafni, A.; Ne'eman, G.; Willmer, P. G. Nectar resource diversity organises flower-visitor community structure. *Entomol. Exp. Appl.* **2004**, *113* (2), 103−107.
(35) Frankel, S.; Robinson, G. E.; Berenbaum, M. R. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. *J. Apic. Res.* **1998**, *37* (1), 27−31.
(36) Campbell, P. J.; Hoy, S. P. ED points and NOELs: how they are used by UK pesticide regulators. *Ecotoxicology* **1996**, *5* (3), 139−144.
(37) R Core Team. *R: A language and environment for statistical computing*. 2013, R Foundation for Statistical Computing: Vienna, Austria.
(38) Thiele, J. C. R Marries NetLogo: Introduction to the RNetLogo Package. *Journal of Statistical Software* **2014**, *58* (2), 1−41.
(39) Dunn, O. J. Multiple Comparisons Using Rank Sums. *Technometrics* **1964**, *6* (3), 241−252.

DOI: 10.1021/acs.est.6b04206
Environ. Sci. Technol. XXXX, XXX, XXX−XXX