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Hazard/Risk Assessment

Chronic and Acute Effects of Imidacloprid on a Simulated BEEHAVE Honeybee Colony

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Abstract: Honeybees (*Apis mellifera*) are important pollinators for wild plants as well as for crops, but honeybee performance is threatened by several stressors including varroa mites, gaps in foraging supply, and pesticides. The consequences of bee colony longtime exposure to multiple stressors are not well understood. The vast number of possible stressor combinations and necessary study duration require research comprising field, laboratory, and simulation experiments. We simulated long-term exposure of a honeybee colony to the insecticide imidacloprid and to varroa mites carrying the deformed wing virus in landscapes with different temporal gaps in resource availability as single stressors and in combinations. Furthermore, we put a strong emphasis on chronic lethal, acute sublethal, and acute lethal effects of imidacloprid on honeybees. We have chosen conservative published values to parameterize our model (e.g., highest reported imidacloprid contamination). As expected, combinations of stressors had a stronger negative effect on bee performance than each single stressor alone, and effect sizes were larger after 3 years of exposure than after the first year. Imidacloprid-caused reduction in bee performance was almost exclusively due to chronic lethal effects because the thresholds for acute effects were rarely met in simulations. In addition, honeybee colony extinctions were observed by the last day of the first year but more pronounced on the last days of the second and third simulation year. In conclusion, our study highlights the need for more long-term studies on chronic lethal effects of pesticides on honeybees. *Environ Toxicol Chem* 2022;41:2318–2327. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Ecotoxicology; Insecticide; Pesticide risk assessment

INTRODUCTION

Thirty-five percent of global crop production depends on animal pollination (Klein et al., 2007). Honeybees are the most important pollinators for many crops (Klein et al., 2007), making them of key economic importance to agriculture and human welfare. However, honeybee performance is threatened by many potential stressors. These include habitat loss and fragmentation (Horn et al., 2021; Ricketts et al., 2008; Winfree et al., 2009), varroa mites carrying the deformed wing virus (Stokstad, 2007), and exposure to pesticides (Alaux et al., 2010; Stokstad, 2012). Furthermore, honeybees are exposed to a number of these stressors simultaneously (Goulson et al., 2015).

Among pesticides, imidacloprid is the insecticide with the highest acute toxicity to honeybees (Suchail et al., 2000). It was the second most used agrochemical in the world in 2008 (van der Sluijs et al., 2013) and can persist in natural habitats even years after use (Wintermantel et al., 2020). Many effects of imidacloprid on honeybees are well documented: Imidacloprid causes, for example, chronic and acute mortality in honeybees (Dai et al., 2017; Sanchez-Bayo & Goka, 2014; Schmuck et al., 2001; Suchail et al. 2001, 2004) and increases the duration of foraging flights of forager bees (Schneider et al., 2012).

Honeybees are exposed to multiple stressors simultaneously (Goulson et al., 2015), and it is unclear how these stressors interact. Field experiments investigating effects of multiple stressors on bee colonies would require many different combinations of stressors at different intensity levels, making them costly and time-consuming. Moreover, such field studies are difficult to interpret because of high natural variation in bee colonies (e.g., colony size and age, genetic

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diversity) as well as in environmental conditions (e.g., weather, resource availability; Cresswell, 2011; Henry et al., 2015). Furthermore, most field experiments measure aspects of bee performance only over the course of a relatively short exposure phase; thus, potential long-term effects of stressors on bee colonies could go unnoticed (Agatz et al., 2019; Havard et al., 2019; Thorbek et al., 2017). Modeling studies allow sufficient replication of multistressor analyses at low costs. Parameterized with field data, models can simulate many different scenarios and thereby complement field studies. Based on the results of the simulations, one can identify a limited number of very important stressor combinations that are likely to pose the greatest risk for honeybees and should be further tested in future field studies (Havard et al., 2019; Henry et al., 2017).

The BEEHAVE model is a simulation model of a honeybee colony composed of eggs, drone and worker larvae, drone and worker pupae, drones, and worker bees (Becher et al., 2014). The BEEHAVE simulation model consists of a colony model, a mite model, and a foraging model. One time step represents 1 day, apart from the foraging model where the temporal resolution is finer and variable, depending on the duration of foraging trips. The colony model and the mite model are cohort-based, while in the foraging model, forager bees are simulated as super-individuals and are grouped to squadrons of 100 forager bees, with each squadron modeled individually. Overall, BEEHAVE performs well in modeling the dynamics of a honeybee colony (European Food Safety Authority [EFSA], 2015), but to make BEEHAVE suitable for risk-assessment studies, the EFSA (2015) suggests (1) adding a pesticide module, and (2) improving the overly simplistic landscape representation. Since 2015, several extended versions of the BEEHAVE model have been developed that are suitable for studying bee colony sensitivity to increased bee mortality (Thorbek et al., 2017), foraging stress (Horn et al., 2021), and clothianidin exposure (Schmolke et al., 2019). However, imidacloprid is another important pesticide requiring a focused analysis, and the landscape representation has not been extended in a pesticide risk-assessment simulation yet.

The present study extends the BEEHAVE model (Becher et al., 2014) to study the effects of imidacloprid on a honeybee colony. We investigated the role of acute and chronic effects of imidacloprid as a single stressor and in combination with varroa mites carrying the deformed wing virus and landscapes with different temporal gaps in resource availability ("foraging gaps"). To this end, we parameterized imidacloprid effects on bees using data from published literature and parameterized the simulated landscape with nectar data from an agricultural landscape in Poland (Jachuła et al., 2021). A detailed description of our model, following the ODD (Overview, Design concepts, Detail) protocol (Grimm et al., 2006, p. 2020), is available as Supporting Information.

METHODS

Landscape and scales

The BEEHAVE model uses an input that defines the landscape around the hive as a number of flower patches. Each patch is characterized by its distance to the hive in the center of the landscape, the flowering period, and the amount of nectar and pollen provided by this patch during this period. We simulate a stylized landscape because in BEEHAVE the temporal dynamics of the forage availability is much more important than the spatial configuration of fields (Horn et al., 2021). Given a mean flight distance of 1743 ± 95 m of pollen foragers (Steffan-Dewenter & Kuhn, 2003) and assuming that only 95% of the landscape is suitable for foraging, the potential foraging area is calculated as $\pi \times (\text{mean}+2\text{sd})^2 \times 0.95 \approx 1115$ha. To control for effects potentially caused by the spatial configuration of flower patches, the landscape includes 100 flower patches of equal size, all 1743 m away from the colony. The detection probability for each of the 100 flower patches was set to 0.05, resulting per default BEEHAVE behavior (Becher et al., 2014) in a chance of <1% ($[1 - 0.05]^{100} \approx 0.006$) that a searching forager finds none of the 100 flower patches. One time step represents 1 day, apart from the foraging model where the temporal resolution is finer. Simulations are run for 3 years, starting with 10,000 bees, 25 kg honey, and 100 g pollen, from January 1.

We parameterized nectar provision of flower patches with empirical nectar production values from an agricultural landscape from Poland (Jachuła et al., 2021) and approximated pollen provision from these data. For the study of Jachuła et al. (2021), 30 randomly chosen plots with a size of 1 km² located within the Lublin Upland were chosen, and the cover by human-made noncropped habitats (nonforest woody vegetation, road verges, railway embankments, field margins, fallow areas), forests, meadows/pastures, and crops (winter oilseed rape and orchards) was calculated. Mean habitat-level sugar yield was obtained from field data and literature data gathered within the same area. From habitat coverage and habitat-level sugar yield, the landscape-scale nectar provision was approximated. Oilseed rape cover at the plot level varied between 1% and 37%, and oilseed rape provided 32% of the annual landscape-level nectar. Oilseed rape flowering started in late April and peaked in May. Pollen provision of flower patches was estimated from nectar values by using the nectar-to-pollen ratio reported for oilseed rape by Becher et al. (2016; Table 1), assuming that on average pollen and nectar provision are correlated.

Bees forage only under suitable weather conditions, and the number of foraging hours is taken from the BEEHAVE built-in

TABLE 1: Daily nectar and pollen provision of flower patches[a]

Period	Nectar (ml/m²)	Pollen (g/m²)
Mar	0.0005	0.0002
Apr	0.0185	0.0080
May	0.0430	0.0186
Jun	0.0061	0.0026
Jul	0.0119	0.0052
Aug	0.0149	0.0065
Sep	0.0051	0.0022
Oct	0.0002	0.0001

[a]Nectar values taken from Jachuła et al. (2021). Nectar value calculations are given in Supporting Information S1.5; pollen values estimated based on the oilseed rape pollen: nectar ratio (0.13 g/m² pollen:0.3 ml/m² nectar) reported by Becher et al. (2016). From November to February, the landscape provides no resources.

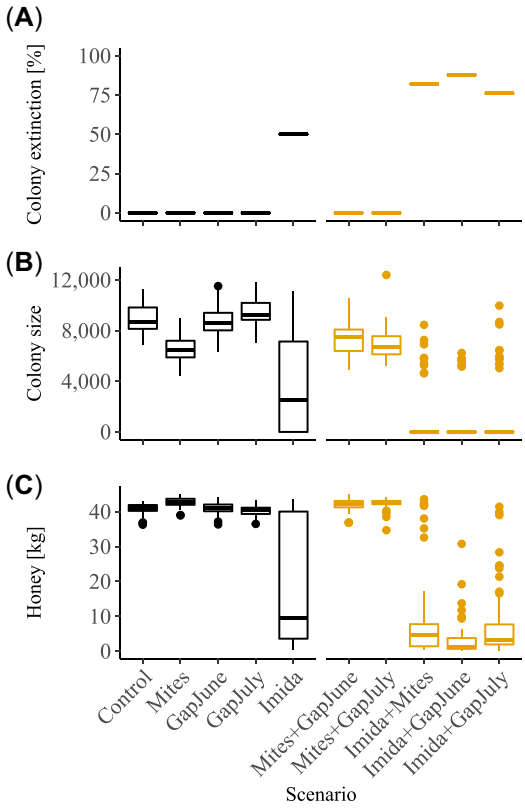

FIGURE 1: Bee colony performance after 3 years, at day 1095, is most strongly affected by imidacloprid but also by mites. Colony extinction (**A**), number of adult bees (**B**), and amount of stored honey (**C**). Single-stressor scenarios in black, double-stressor scenarios (+) in orange. Fifty replicates per scenario. Boxplots show median, 25th, and 75th percentiles (hinges); minimum and maximum values no further than 1.5 × interquartile range from lower and higher hinges, respectively (whiskers), and outliers (symbols). Mites = infected varroa mites; GapJune and GapJuly = 15 days in which the landscape provided no resources, starting from June 1 or July 1, respectively; Imida = imidacloprid, originating from flower patches treated with imidacloprid for 30 days.

weather scenario "Rothamsted 2009–2011," which was parameterized by weather data from Rothamsted, United Kingdom (Becher et al., 2014). We chose the Rothamsted weather setup for two reasons. First, preliminary analyses showed that our landscape and the weather module allowed bees to perform well over the course of 3 years (see also the control results in Figure 1). Second, we had a strong focus on imidacloprid effects on bees and wanted to explore basic relationships rather than to do a simulation that matches the Polish landscape as closely as possible.

Imidacloprid is present in the landscape only during oilseed rape flowering time. Flowering time starts on April 25 and lasts for 30 days, reflecting oilseed rape flowering times reported by Jachuła et al. (2021) and Heimbach et al. (2016). The imidacloprid concentrations in nectar and pollen of all flower patches are 0.81 and 7.6 ng/g, respectively (EFSA, 2012). The concentrations used in our model are from the United States, measured at higher application rates than in the European Union but under comparable meteorologic conditions.

Pollen and honey storage

An important feature of our model is the tracking of imidacloprid concentrations of stored pollen and honey at a daily resolution, which required an extension of the pollen and nectar storage of the original BEEHAVE model (Becher et al., 2014). In the original BEEHAVE model, foragers bring all collected pollen and nectar to the hive but consume honey from storage before unloading the collected resources at the hive (Becher et al., 2014). Pollen and nectar brought to the hive are pooled and stored in the pollen storage and in the honey storage, respectively (Becher et al., 2014). The original BEEHAVE model (Becher et al., 2014) would underestimate potential imidacloprid effects of pollen and nectar containing imidacloprid. This is because in this model all freshly collected pollen is pooled and stored before the bees consume any of it. Therefore, pollen containing imidacloprid is mixed with imidacloprid-free pollen, causing an unrealistic dilution of imidacloprid. In nature, bees consume fresh pollen first (Carroll et al., 2017); therefore, a more appropriate way to store pollen and honey was needed.

Therefore, in our model extension, pollen is stored in age cohorts. Similar to Schmolke et al. (2019), we save pollen collected within the last 8 days in daily cohorts, and all pollen older than 8 days is pooled. Each daily pollen cohort is the sum of all pollen collected on the specific day. Bees consume pollen of the day first; if the pollen demand exceeds the available quantity of the pollen cohort(s), the next oldest pollen cohort is utilized. At the end of each day, after the pollen consumption of bees, each pollen cohort ages by 1 day.

Regarding nectar, freshly collected nectar is distributed by trophallaxis between bees of all age classes within approximately 1 day, and unconsumed nectar is stored in storage cells (Feigenbaum & Naug, 2010; Nixon & Ribbands, 1952). In our model, all nectar collected on the same day is summed up as a honey age cohort, but all honey older than 1 day is pooled. Bees consume the honey of the day first, but if the honey demand exceeds the available quantity of this honey cohort, the pooled honey storage from previous days is utilized. At the end of each day, after the honey consumption of bees, all remaining honey from the day is added to the pooled honey.

Imidacloprid uptake during foraging

During foraging, nectar foragers take up imidacloprid only from the consumed proportion of all collected nectar, and pollen foragers do not take up imidacloprid from collected pollen. In BEEHAVE, nectar is called honey after it is added to the in-hive storage. Foragers consume honey from storage, corresponding to the flight duration (Becher et al., 2014). In our model version, this amount is consumed from the honey age cohort collected on the same day. If this honey age cohort is depleted, foragers consume the pooled honey from storage.

Imidacloprid effects on bees

The acute toxicity of imidacloprid for honeybees was determined in laboratory experiments. In one experiment

(Suchail et al., 2000), groups of 20 adult bees were starved for 2 h and fed with one dose of dimethyl sulfoxide sucrose solutions containing graded doses of (98% pure) imidacloprid (1-[6-chloro-3-pyridylmethyl]-N-nitroimidazolidin-2-ylideneamine). The acute median lethal dose (LD50) after 24 and 48 h was determined with a log-probit analysis as 5.4 and 4.8 ng/bee, respectively. In a similar experiment, Schmuck et al. (2001) obtained acute LD50 (48 h) values ranging between 3.7 and 40.9 ng/bee.

In our model, acute toxicity is calculated from the auxiliary variable *activepesticide* at the end of each day, before metabolization takes place. The *activepesticide* is the amount of imidacloprid active in each bee cohort (=not metabolized so far) at this time. For all cohorts with *activepesticide* ≥5.4 ng/bee (LD50 [24 h]; Suchail et al., 2001), the cohort size is reduced by 50%. The only exception from this rule are forager squadrons. Because the size of each forager squadron is fixed to be 100, each forager squadron with *activepesticide* greater than the respective acute mortality threshold (Table 2) has a 50% mortality risk. Bees metabolize imidacloprid within 24 h (Suchail et al., 2004); thus, in our model the value of *activepesticide* is set to zero after the calculation of acute imidacloprid effects at the end of each day.

Imidacloprid also had sublethal effects on bees in a feeding experiment (Schneider et al., 2012). In the experiment, forager bees were fed with sucrose solution with different imidacloprid concentrations. Imidacloprid doses ≥1.5 ng per 10 µl sucrose solution had negative effects on bee foraging performance within the first 3 h after treatment; doses of 0.15 ng per 10 µl sucrose solution had no negative effects on foraging performance. When bees were observed for another 3-h period 24 h after the treatment, no imidacloprid effects were found.

In our model, based on Schneider et al. (2012), *activepesticide* values ≥1.5 and ≥3 ng/bee, respectively, increase the duration of the same-day foraging trips by 50% and 130% (confusion) and decrease the same-day probability to go foraging again by 33% and 993% (laziness).

Chronic exposure of honeybees of unknown age to imidacloprid concentrations of 0.1 µg/L (0.0012 ng/bee/day) in 50% sucrose for 10 days caused 50% mortality (Suchail et al., 2001). Rondeau et al. (2015) see support for the value of 0.0012 ng/bee/day in an experiment reported by Schmuck (2004) in which one replicate bee colony showed 67% 10-day mortality when exposed to 0.004 ng/bee/day of a metabolite of imidacloprid (6-chloro-nicotinic acid), while Schmuck (2004) argues that the last mentioned replicate was not performed "according to standard experimental practices" and suggests that the age of the honeybees in the experiment of Suchail et al. (2001) was probably inappropriate. However, the findings of Suchail et al. (2001) were not reproduced in three subsequent experiments. In these experiments, bees were exposed to imidacloprid concentrations ranging 4–8 µg/L (no effect after 10 days [Dechaume Moncharmont et al., 2003]), 1.5–96 µg/L (maximum 21% mortality after 10 days for 48 µg/L, control mortality 12% [Decourtye et al., 2003]), and 0.4–4000 µg/L (10% mortality after 10 days for 250 µg/L [Department for Environment, Food and Rural Affairs, 2007]). Consequently, in a meta-analysis (Cresswell, 2011), the findings of Suchail et al. (2001) were called "anomalous." To cover the whole range of reported chronic mortality values, we used the lowest reported values (Suchail et al., 2001) in our main analyses but addressed the importance of the chronic mortality threshold in a sensitivity analysis and discussed the findings under consideration of the other three chronic mortality studies.

In our model, chronic mortality is calculated from the cumulative amount of imidacloprid taken up (*lifetimepesticide*) and the duration of exposure to imidacloprid. If *lifetimepesticide* of a bee cohort divided by the exposure duration in days is greater than or equal to the threshold for chronic mortality effects for longer than 2 days (Table 2), chronic mortality takes effect. For simplicity, chronic mortality is implemented as a reduction in in-hive bee cohort size by 8%. This implementation yields a cumulative chronic mortality that is in good agreement with the empirical cumulative chronic mortality presented by Suchail et al. (2001; Figure 2). Chronic mortality takes place at the end of each day, and *lifetimepesticide* is not reduced by metabolization.

TABLE 2: Thresholds for imidacloprid effects on bees and larvae used in the simulations

Parameter	Effect	Cohorts	Value (ng/bee)
AcuteMortLarvae	Acute 50% lethal	Larvae	4170[a]
AcuteMort	Acute 50% lethal	Foragers, IHBees	5.4[b]
ConfusionLow	Acute (confusion and laziness)	Foragers	1.5[c]
ConfusionHigh	Acute (confusion and laziness)	Foragers	3[c]
ChronicMort	Chronic 50% lethal	Foragers, IHBees	0.0012[b,d]

[a]Dai et al. (2017).
[b]Suchail et al. (2001).
[c]Schneider et al. (2012).
[d]Units are nanograms per bee per day.
Cohorts= affected cohorts: Larvae = drone and worker larvae; IHBees = all adult bees excluding foragers and the queen. Foragers = nectar and pollen foragers. IHBees = in-hive bees.

Analyses

The model was implemented with NetLogo 6.1.0 (Wilensky, 1999), and simulations were run using the nlrx package (Salecker et al., 2019) and R 4.0.2 software (R Foundation for Statistical Computing, 2020). All simulations were run for 3 years with the same conditions throughout. In all analyses, bee performance was measured after 3 years using three well-established indicators of colony health (see Henry et al., 2017; Schmolke et al., 2019): colony survival, colony size, and amount of stored honey. We ran three types of analyses: single- and double-stressor analyses, a chronic mortality sensitivity analysis, and a landscape analysis. All simulations were run with 50 replicates.

We simulated five single-stressor and five double-stressor scenarios. In all imidacloprid scenarios of this analysis, all 100 flower patches were imidacloprid-treated, and both acute and

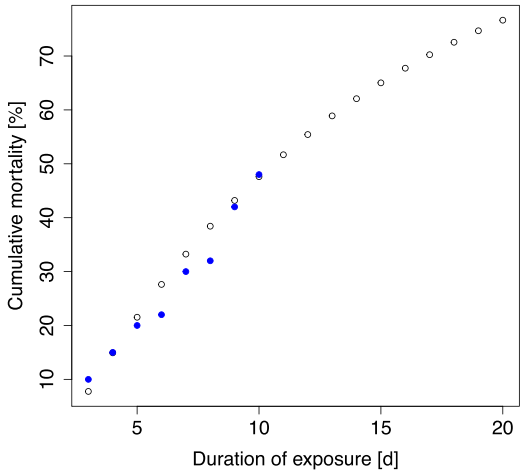

FIGURE 2: Cumulative mortality due to chronic imidacloprid exposure is realistically implemented in the simulation model. The figure shows cumulative chronic mortality of honeybees as a function of exposure duration to imidacloprid for the model (black open symbols) and for experimental data (blue filled symbols). In the model, all bees with higher imidacloprid levels than the threshold value had a daily mortality of 0.08. Experimental data from Suchail et al. (2001, fig. 2A).

chronic effects of imidacloprid on bees were included. Two further factors considered to be important for bee colony losses are varroa mites carrying the deformed wing virus (Stokstad, 2007) and temporal gaps in foraging resources. Regarding mites, the BEEHAVE (Becher et al., 2014) model has the functionality to reduce the number of varroa mites by a varroa treatment, but all analyses were run without varroa treatment.

As a preliminary analysis, we determined the number of mites that caused no colony losses if mites were the only stressors, like in Henry et al. (2017). To this end, the number of infected mites present at day 1 was increased from five in steps of five until one or more colony extinctions in 50 replicates were observed. In all of the following scenarios with mites present, this number of mites ($n = 10$; Supporting Information, Figure S1) was set to be the number of infected varroa mites at day 1. Foraging stress was induced by periods of 15 days' length, in which fields provided no resources. These gaps in resources were present in each simulation year and started either on June 1 or on July 1, like in Horn et al. (2016). In the single-stressor scenarios, simulations were run with only one of the stressors at a time or without stressors (control), whereas in the double-stressor scenarios, we simulated all possible double-stressor combinations except the combination of two gaps in foraging resources.

In the chronic mortality sensitivity analysis, both acute and chronic effects of imidacloprid were included. The threshold for chronic effects of imidacloprid on honeybees was varied between 0.0012 and 0.018 ng/bee/day. In all analyses, 10 infected varroa mites were present at day 1, and flower patches provided no resources for 15 days in June. In the baseline scenario fields contained no imidacloprid; thus, the baseline scenario was similar to the *Mites + GapJune* scenario of the main analysis. In the *baseline + Imida* scenario, all 100 flower patches were imidacloprid-treated, and both acute and chronic effects of imidacloprid were included.

In the landscape analysis, we tested if imidacloprid effects can be mitigated by a proportion of imidacloprid-free flower patches. The percentage of imidacloprid-treated flower patches was decreased from 100% to 0%, in steps of 10%. This complements our previous analyses where, in each single- and double-stressor scenario with imidacloprid, as well as in the chronic mortality sensitivity analysis, all 100 flowering patches were imidacloprid-treated. Imidacloprid levels in imidacloprid-treated flower patches were like in the previous analyses (0.81 ng/g in nectar and 7.6 ng/g in pollen). Again, 10 varroa mites were present at Day 1, and there was a 15-day gap in foraging resources in June.

RESULTS

Due to space constraints, we only present results regarding the effects on the entire colony. Further results that help to better understand how these gross effects emerged are presented in the Supporting Information S1 and summarized in Table 3. In the single- and double-stressor scenarios, after 3 years, only scenarios with imidacloprid and mites caused honeybee colony extinctions (Figure 1). Colony size, in the single-stressor scenarios, was reduced moderately by varroa mites transmitting the deformed wing virus but not by temporal gaps in forage resources and strongly reduced by imidacloprid. In the double-stressor scenarios colony size was most strongly reduced in scenarios with imidacloprid. Honey storage was only reduced in scenarios with imidacloprid. Notably, across all single- and double-stressor scenarios, colony performance reduction was more pronounced after 2 and 3 years than after 1 year (Supporting Information, Figure S2 A365–C365). Ten days after the first exposure to imidacloprid in the single-stressor scenario, colony size was reduced by approximately 20% (Supporting Information, Figure S2 A134).

TABLE 3: Additional findings presented in Supporting Information S1

Chapter	Finding
S1.1	Given 10 disease-transmitting varroa mites at day 1, no colony extinctions were observed within 3 years.
S1.2	Imidacloprid in the single- and double-stressor analyses reduced colony size 10 days after first exposure. Colony extinctions were more pronounced after the second and third years than after the first year.
S1.3	Daily maximum imidacloprid exposure of forager and in-hive bees was approximately 0.11 and 0.05 ng/bee, respectively.
S1.4	Imidacloprid from nectar had a stronger negative impact on bee colony performance than imidacloprid from pollen.
S1.5	Example calculation for the nectar provision of flower patches.

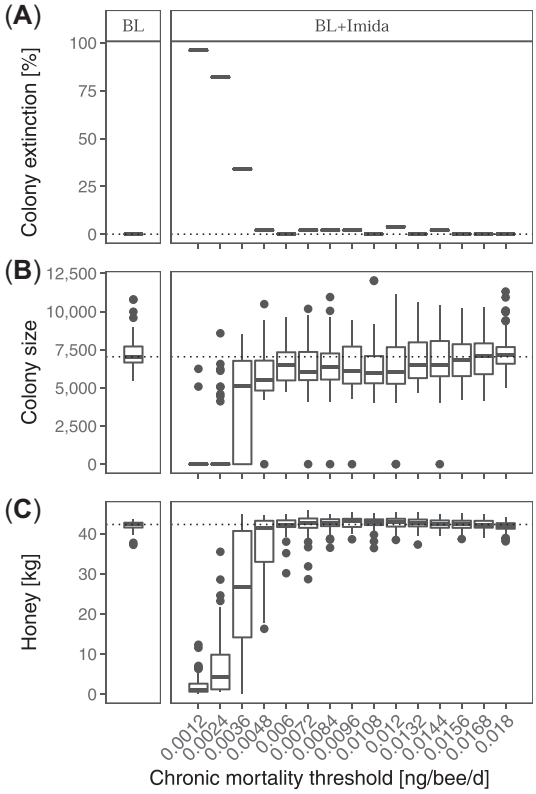

FIGURE 3: Bee colony performance after 3 years, at day 1095, was sensitive to the threshold for chronic lethal effects. Colony extinction (**A**), number of adult bees (**B**), and amount of stored honey (**C**). Imidacloprid had chronic lethal, acute sublethal, and acute lethal effects on bees. Dotted lines show median bee performance in the baseline scenarios. Fifty replicates per scenario. Boxplots show median, 25th, and 75th percentiles (hinges); minimum and maximum values no further than 1.5 × interquartile range from lower and higher hinges, respectively (whiskers), and outliers (symbols). BL = baseline scenario similar to the Mites + GapJune scenario in Figure 2, with 10 infected mites present at day 1; starting from June 1 the landscape provided no resources for 15 days, with no imidacloprid effects. BL + Imida = as BL, but imidacloprid was present during the 30-day flowering period of oilseed rape.

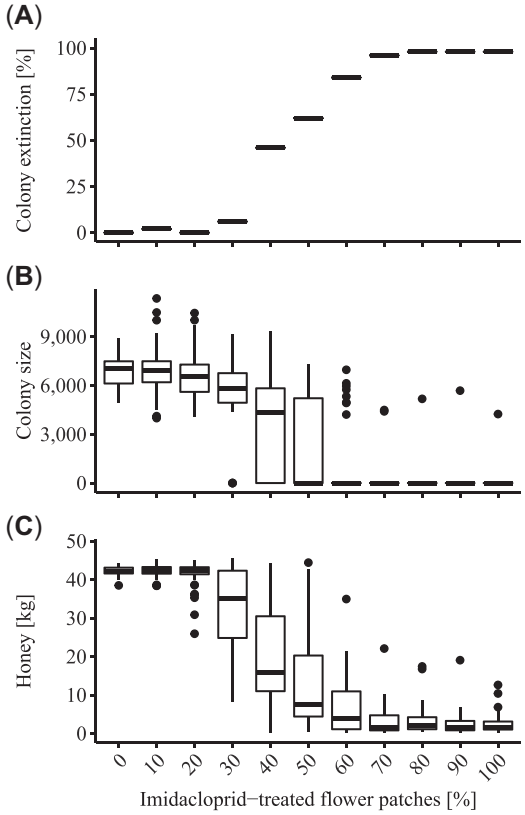

FIGURE 4: Bee colony performance after 3 years, at day 1095, decreased with an increasing proportion of imidacloprid-treated flower patches. Imidacloprid was present during the 30-day flowering period of oilseed rape. Colony extinction (**A**), number of adult bees (**B**), and amount of stored honey (**C**). Five infected mites present at day 1; starting from June 1, the landscape provided no resources for 15 days. Boxplots show median, 25th, and 75th percentiles (hinges); minimum and maximum values no further than 1.5 × interquartile range from lower and higher hinges, respectively (whiskers), and outliers (symbols).

The higher the chronic mortality threshold, the better the bee colony performance (Figure 3). Colony extinctions were observed for thresholds up to 0.0144 ng/bee/day. If the threshold exceeded 0.0048 ng/bee, <5% colony deaths due to repeated exposures over multiple years were observed, and colony size was reduced for some threshold values but never by >18%. Given thresholds ≥0.0156 ng/bee/day, bee colony performance was similar to the baseline scenario without imidacloprid effects.

In the landscape configuration scenario, with an increasing number of imidacloprid-treated flower patches, colony extinctions increased, while colony size and amount of stored honey decreased (Figure 4). Given a landscape with up to 30% imidacloprid-treated flower patches, no more than 10% of bee colonies went extinct, and median colony size as well as the amount of stored honey were comparable to the results given no imidacloprid in the landscape. If all flower patches were imidacloprid-treated, all colonies went extinct.

DISCUSSION

We extended the BEEHAVE simulation model (Becher et al., 2014) to include imidacloprid effects alone or in combination with varroa mites carrying the deformed wing virus and temporal gaps in resource availability. Exposure to US-based maximum imidacloprid levels reported by EFSA (2012) caused 50% of colonies to go extinct within 3 years, and exposure to imidacloprid in combination with another stressor caused approximately 80% colony extinctions. Honeybee colony performance was reduced due to lethal effects from chronic imidacloprid exposure but not due to acute sublethal or acute lethal effects. When only proportions of the 100 flower patches were treated with imidacloprid, colony deaths due to repeated exposures over multiple years were 6% or less for landscapes with up to 30% imidacloprid-treated patches.

In the single- and double-stressor scenarios with imidacloprid, as well as in the sensitivity analysis, all 100 flower patches were imidacloprid-treated during oilseed rape flowering. Imidacloprid levels of oilseed rape nectar (0.81 ng/g) and pollen (7.6 ng/g) were based on maximum values observed in the United States (EFSA, 2012). In a literature review, based on studies from North America ($n = 11$), Europe ($n = 11$), and Asia

($n = 1$), much higher maximum imidacloprid levels were reported for oilseed rape nectar (65.5 ng/g) and pollen (328 ng/g); but median residue nectar levels were 0.5 and 2.6 ng/g, and median residue pollen levels were 0.18 ng/g (Zioga et al., 2020). In France, within 5 years after the European Union memorandum on imidacloprid in 2013, minimum imidacloprid concentrations in positive oilseed rape nectar samples were 0.2 ng/ml, and 91.8% of all positive samples contained <1 ng/ml (maximum 70 ng/ml; Wintermantel et al., 2020). Another field study from France found imidacloprid concentrations in pollen from pollen traps installed at honeybee colonies ranging between 1.1 and 5.7 ng/g (mean = 1.2 ng/g) but did not report if the imidacloprid originated from oilseed rape (Chauzat et al., 2006). Thus, under the assumptions that (1) oilseed rape is the only source of imidacloprid for bees, and (2) imidacloprid application rates in Europe are lower than those in the United States, even before the ban of imidacloprid in the European Union in 2018, average "field-realistic" honeybee exposure to imidacloprid in Europe can be expected to be lower than in our model.

Maximum daily imidacloprid uptake was 0.11 and 0.05 ng/bee for foragers and in-hive bees, respectively, in the single-stressor scenario with imidacloprid. Honeybee workers are reported to metabolize imidacloprid within 24 h (Suchail et al., 2004). In our BEEHAVE model version, this was implemented by setting the variable for imidacloprid exposure relevant for acute imidacloprid effects, *activepesticide*, to zero at the end of each day. Accordingly, the thresholds for acute lethal (5.4 ng/bee, Suchail et al. [2001]) and acute sublethal effects (1.5 and 3 ng/bee; Schneider et al. [2012]) were not reached in the simulations.

In the chronic mortality sensitivity analysis, bees were exposed to imidacloprid in combination with foraging stress and mites carrying the deformed wing virus. If the chronic mortality threshold in the sensitivity analysis was 0.0048 ng/bee/day or higher, colony extinctions were 2% or less, and amounts of stored honey were similar to the baseline scenario without imidacloprid. Considering the low values (0.0012 ng/bee/day, 0.1 μg/L) reported by Suchail et al. (2001) as "anomalous" (Cresswell, 2011) would imply the need to evaluate the effects of the next highest reported minimum effect concentration of 48 μg/L (Decourtye et al., 2003). This value translates into a chronic mortality threshold of approximately 0.6 ng/bee/day. In our simulations, bees were insensitive to imidacloprid if the chronic mortality threshold exceeded 0.0156 ng/bee/day.

In the landscape analysis, only proportions of the flower patches were imidacloprid-treated during oilseed rape flowering to reflect that real bees do not exclusively forage in oilseed rape fields. One field study from Sussex, United Kingdom, found that 90% of foraging took place within 2 km surrounding the beehives and that the proportion of foraging in oilseed rape fields decreased with distance between hives and oilseed rape field margins (Garbuzov et al., 2015). Oilseed rape cover was approximately 3% within 6 km surrounding the hives, and all but one of the oilseed rape field groups were farther than 1 km away from the hives. Based on a waggle dance analysis, one group of oilseed rape fields 0.7 km away from one single hive accounted for 37% of foraging trips, but the average foraging in oilseed rape fields in 2012 was 22% and 26% in April and May, respectively. For the same study, 14% of all collected pollen originated from oilseed rape. In Germany, the origin of pollen grains in spring honey was investigated (Rolke et al., 2016). For the present study, oilseed rape covered approximately 16% and 21% of the study sites (Heimbach et al., 2016), and honey was harvested immediately after oilseed rape flowering. Approximately 70%–80% (mean) of all pollen grains in the honey originated from oilseed rape (Rolke et al., 2016). In our landscape analysis, imidacloprid levels of treated patches again reflected US-based maximum values (EFSA, 2012), but proportions of the flower patches were free from imidacloprid. Given the lowest reported chronic mortality thresholds of 0.0012 ng/bee/day (Suchail et al., 2001), no colony failures were observed in landscapes with 30% or less imidacloprid-treated patches.

Field studies show lower sensitivity of bee colonies to imidacloprid exposure. Cresswell (2011) reports an expected reduction in honeybee performance of 6%–11% during oilseed rape flowering, but we observed a reduction of 20% in colony size 10 days after first imidacloprid exposure in the imidacloprid single-stressor scenario. Bees fed with supplemental pollen containing imidacloprid showed increased winter mortality from 14% (control) to a maximum of 41% (100 ng/g treatment; Dively et al., 2015). Although the imidacloprid doses in the feeding experiment were substantially higher than in our simulations, we observed fewer colony extinctions than in the feeding experiment. As mentioned, in all imidacloprid scenarios of our analyses, with the exception of the landscape analysis, all flower patches were imidacloprid-treated, and bees could not avoid imidacloprid, which may at least partly explain the greater performance reduction in our simulations. In addition, foraging conditions, like forage supply and weather conditions, were different between the field experiments and our simulations. Indeed, in our landscape analysis, imidacloprid effects were mitigated if a fraction of flower patches were not imidacloprid-treated. Given 30% or less imidacloprid-treated flower patches in the landscape, we observed only 6% or less colony extinctions.

The BEEHAVE model, parameterized with one fraction of and evaluated with another fraction of field data from the United States, provided good estimates of bee colony dynamics for the first year, but estimated winter survival and colony performance in the next spring were less reliable (Schmolke et al., 2020). This needs to be considered when interpreting the simulation outcomes data. In our simulations, imidacloprid as a single stressor as well as in combination with another stressor caused colony extinctions after 1 year, but extinctions were more pronounced after 2 and 3 years. Colony size 10 days after first exposure to imidacloprid, as well as at the end of the first year, was 25% below the control colony size. This suggests that colony deaths due to repeated exposures over multiple years were caused, first, by the missing ability of the colony to recover from imidacloprid stress within the same year and, second, by the resulting unfavorable starting conditions in the second and third years. We consider both causes as plausible, but to our understanding only a study comparing simulated bee colony dynamics with field data collected over multiple years could answer this question with great certainty.

We suspect that our model overestimates the chronic effects of imidacloprid because all flower patches were treated with imidacloprid. In nature, a reduction in honeybee colony size 10 days after first exposure to imidacloprid could be less pronounced. Because of high natural variation in honeybee colonies (e.g., colony size and age, genetic diversity) and environmental conditions (e.g., weather, resource availability), imidacloprid-induced declines in colony performance might go unnoticed, especially in studies with too few replicates (Cresswell, 2011). Like in our model, chronic imidacloprid effects might still jeopardize the long-term performance of the bee colony.

Because other pesticides have thresholds for chronic mortality orders of magnitudes lower than for acute mortality (Fiedler, 1987), from a most protective perspective, we consider long-term risk assessment as important for imidacloprid as well as for these other pesticides. Our findings confirm the results of earlier BEEHAVE simulations that stress due to mite infestation (Becher et al., 2014), forage gaps (Horn et al. 2016, 2021), and pesticides (Rumkee et al., 2015) can take 3 years or more before substantial colony losses are observed, unless stress levels are extremely high. This might explain why even large-scale field studies with clothianidin-treated oilseed rape seeds did not find detectable effects within 1 year of exposure or directly after the first winter (Cutler et al., 2014). It has to be kept in mind that the evolutionary "individual" of honeybees is the entire colony, not the individual worker bee (Hölldobler & Wilson, 2009). Stress affecting the performance and survival of worker bees and hence impairment of their functions within the colony therefore corresponds to sub-organismal stress, for example, in nonsocial insects. Honeybee colonies obviously have effective buffer mechanisms, for example, in terms of regulating the number of foraging bees or the activation level among foraging bees. The social organization of honeybees likely evolved as a strategy to cope with unpredictable weather, forage supply, and other stressors. Individual-based models may help to reveal the buffering capacities of social organization (Grimm & Railsback, 2005) and interactions like dynamic task allocations, but this is beyond the scope of the present study and the purpose of the BEEHAVE model. One possible consequence for regulatory risk assessment is to accept proxies for long-term effects, that is, short-term effects that are detectable and that, if combined with other stressors, might lead to colony losses over longer timescales. The widely used bird population model MORPH (Brown & Stillman, 2021; Stillman, 2008) is focusing on such a proxy, winter mortality: Although it is unclear how, precisely, increased winter mortality will affect population dynamics in the long run, it is clear that an increase in winter mortality will make a population more susceptible to other stressors and their combined effects. To detect and quantify such proxies, models like BEEHAVE, in combination with geographically distributed data on, for example, hive weights, are promising tools.

In conclusion, our BEEHAVE model extension illustrated how a 30-day exposure of honeybees to low levels of imidacloprid had a relatively low impact on a bee colony 10 days after first exposure but caused colony extinctions after 1, 2, and 3 years.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5420.

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Data Availability Statement—The model description following the ODD (Overview, Design concepts, Details) protocol is available as Supporting Information S2; the code of the extended BEEHAVE model and the code to replicate all analyses and figures presented within the present study are freely available at https://doi.org/10.5281/zenodo.6567312. This article has earned an Open Data and an Open Materials badge for making publicly available the digitally shareable data necessary to reproduce the reported results. The data are available at https://doi.org/10.5281/zenodo.6567312. Learn more about the Open Practices badges from the Center for Open Science: https://osf.io/tvyxz/wiki.

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