

STATISTICAL ANALYSIS OF HONEYBEE SURVIVAL AFTER CHRONIC EXPOSURE TO INSECTICIDES

FRANÇOIS-XAVIER DECHAUME MONCHARMONT,*† AXEL DECOURTYE,† CHRISTELLE HENNEQUET-HANTIER,‡
ODILE PONS,‡ and MINH-HÀ PHAM-DELÈGUE†

†Laboratoire de Neurobiologie Comparée des Invertébrés, BP23, 91440 Bures-sur-Yvette, France

‡Unité de Biométrie, INRA Centre de Recherches de Jouy-en-Josas, 78352 Jouy-en-Josas Cedex, France

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Abstract—Studies concerning long-term survival of honeybees raise the problem of the statistical analysis of mortality data. In the present study, we used a modeling approach of survival data of caged bees under chronic exposure to two pesticides (imidacloprid and deltamethrin). Our model, based on a Cox proportional hazard model, is not restricted to a specific hazard functional form, such as in parametric approaches, but takes into account multiple covariates. We consider not only the pesticide treatment but also a nuisance variable (variability between replicates). Moreover, considering the occurrence of social interactions, the model integrates the fact that bees do not die independently of each other. We demonstrate the chronic toxicity induced by imidacloprid and deltamethrin. Our results also underline the role of the replicate effect, the density-dependent effect, and their interactions with the treatment effect. None of these parameters can be neglected in the assessment of chronic toxicity of pesticides to the honeybee.

Keywords—Honeybee survival Cox model Chronic toxicity Imidacloprid Deltamethrin

INTRODUCTION

As crop-plant pollinators, honeybees may be exposed to several pesticides that can affect their life expectancy. The European Plant Protection Organisation [1] has set up test guidelines for the assessment of pesticide-related risks to bees. Risk assessment of pesticides for bees is built on a sequential scheme including laboratory, semifield, and field evaluations. The laboratory toxicity tests lead to the calculation of the hazard quotient (ratio between treatment dose and median lethal dose [LD50]). Its value determines the subsequent tests to be carried out: Cage tests, tunnel tests, or field tests [2]. The classical way of estimating the acute toxicity of chemicals is to determine their acute lethal dose, particularly the LD50 [3]. However, the lethal dose estimated during acute toxicity tests appears to be a partial measure of the lethal effect because of the short duration of these tests (1–3 d in most cases). It assumes that only foragers visiting crop are likely to be exposed to the toxic compound. Besides, young hive bees also can be exposed through contaminated stored food. Thus, the possible long-term exposure to a toxic agent by contamination of stored food has been established by studying the transfer into the colony of pesticides sprayed on a crop [4–7]. Therefore, many studies investigating the chronic toxicity to insecticides on honeybees have been carried out at the colony level [8–14]. Although tests on colonies probably reflect the best natural conditions of exposure to pesticides, a high variability (resulting from outdoor conditions, colony size, etc.) can affect the reliability of records. Therefore, we developed experiments involving small groups of caged bees subjected to chronic exposure to pesticides via the oral route. Such caging conditions were previously shown to allow long-term survival [15] and to be suitable for testing the sublethal effects of chemicals [13,16–18].

In chronic toxicity tests, most often only the end result of long-term poisoning (i.e., an increase of cumulative mortality) is analyzed [8,10]. This approach does not consider the evolution of mortality during the course of continuous exposure. The most frequently used statistical methods to analyze survival data in the honeybee can be classified into three categories: Life-table analysis, parametric modeling, and semi-parametric modeling. A very common way of describing survival in the literature has been to compute the life table [19,20]. The distribution of survival times is divided into a certain number of intervals. The analysis relies on the computation, for each interval, of the number and proportion of individuals that entered the respective interval alive, the number and proportion of subjects that died in the respective interval, and the number of cases that were censored in the respective interval. On the basis of these numbers and proportions, several values can be computed: Proportion or cumulative proportion of surviving subjects [21,22], hazard rate [23], and median-survival time (LT50) [24]. These parameters are simple to analyze with nonparametric tests. However, they are inefficient for coping with interacting covariates, and they do not use all the information given by the individual life durations. That is why several authors have preferred to analyze survivorship in the honeybee with a parametric model assuming an exponential distribution [25] or a Weibull distribution [26] for the survival time. To analyze the chemical toxicity during the course of the honeybee life span, Bounias [27] proposed algebraic parameters derived from the Hill equation. He tried to fit the sigmoidal mortality curve with an equation analogous to those used in the description of the enzymatic kinetics. Parametric approaches have widespread applicability and include explanatory covariates, but they are strongly dependent on the validity of the assumption that the survival time has a particular probability distribution. Conversely, the nonparametric approaches are not dependent on a specific distribution function,

* To whom correspondence may be addressed
(dechaume@jouy.inra.fr).

but they do not allow several explanatory covariates to be integrated.

The statistical methods classically used for quantifying chronic toxicity assume that each individual death has no influence on the probability of dying of the congeners. This hypothesis of independence between bees belonging to the same group is not realistic. A covariate that has special importance in the case of honeybees is the density-dependent effect. Indeed, food exchanges, contacts, and pheromonal communication occur among workers [28]. The survival duration of a bee may depend on the survival duration of its nestmates. This fact induces a nonnegligible, time-dependent, population-size effect, and this dependence does not allow a direct comparison of the lifetime empirical distributions. We then developed a Cox proportional hazard model, a statistical method particularly appropriate for survival data analysis [29–31].

The objective of the present study was to present a statistical model for examining survival-curves analysis of honeybees undergoing a chronic exposure to chemicals. This model was applied to the analysis of the effects of two insecticides (imidacloprid and deltamethrin) used at sublethal concentrations.

MATERIALS AND METHODS

Chemicals

We used deltamethrin (98% [w/w] pure; AgrEvo, Gif-sur-Yvette, France) and imidacloprid (99.8% [w/w] pure, Bayer AG, Leverkusen, Germany). These two insecticides were selected because their acute effect on mortality is known [18,32–34] and because their agronomic use is suspected to have induced deleterious effects in field conditions [33,35,36]. The doses were chosen according to the LD50 at 48 h previously established for the tested chemicals [32,37]. These doses were arbitrarily divided by 80 and 160, assuming that the resulting doses would lead to sublethal effects over long-term exposure. Thus, we used two concentrations for each chemical: 15 and 30 $\mu\text{g/L}$ for deltamethrin and 4 and 8 $\mu\text{g/L}$ for imidacloprid. Stock solutions with the proper concentration of each chemical were prepared in acetone and kept at -18°C . Aliquots were used to make each test sucrose solution of specific concentration. The final concentration of acetone in sucrose solutions was equal to 1% (v/v). The test pesticides were compared to an untreated sucrose solution (1% [v/v] acetone).

Insects and experimental setup

Worker bees (*Apis mellifera ligustica*) of known age were produced by placing brood combs from outdoor hives in an incubator at 33°C , then putting the emerging bees in small cardboard cages with 50 ± 2 bees per cage. Bees were reared in an incubator ($33 \pm 2^\circ\text{C}$, $50\% \pm 10\%$ relative humidity). They were provided with sugar food (75% icing sugar and 25% honey mixture [w/w]) and water ad libitum during the first 2 d and with pollen during the first 8 d. After 2 d, the sugar food was replaced by a contaminated sucrose solution (500 g/L). The feeding solutions were renewed every 1 or 2 d. The experiment was replicated twice for each concentration of chemical and three times for controls. All the treatments were applied simultaneously to avoid date or sample effect. The mortality and the consumption of syrup were recorded every 1 or 2 d. Dead bees were removed after each observation. In some cases, the survival time of all the individuals could not be observed, because honeybees either died accidentally or escaped from the cage during handling. Nevertheless, in the latter case, we recorded the time of this censoring event.

Statistical analysis

The survival distribution was estimated directly from the continuous death times. We used the empirical estimator of the lifetime distribution functions proposed by Kaplan and Meier [38] to take into account the censoring time. The Kaplan-Meier survival estimate is a step function, which is constant on the intervals defined by the death times and changes at every distinct death time but does not change at the censoring time (unless a death time happens to be simultaneous with a censoring time). The censoring event only influences the size of the step. If the final observation is an uncensored survival time t , then the Kaplan-Meier survival estimate after t is zero. If the final observation is a censoring time instead of a survival time, then the final value of the Kaplan-Meier estimator at the last uncensored survival time is greater than zero. In this situation, the survival estimate is conventionally represented as continuing at the value calculated at the final censored survival time. However, this estimate generally assumes independence among the individual death events.

The survival analysis was performed with a Cox proportional hazard model [39]. The hazard function or death rate is the instantaneous probability of death for individuals still alive. The Cox model assumes that the individual hazard function depends on a common baseline hazard and the values of the covariates. Given two individuals with particular values for time-independent covariates, the ratio of the estimated hazards over time is supposed to be constant over time. The individual hazard functions are proportional to a common baseline hazard function. We considered not only the treatment but also the nuisance variables as explanatory covariates. The variability among cages under the same treatment was taken into account. Moreover, given that the honeybee is a social insect, it would be too strict an approximation to consider that individuals would die independently from each other. The dependence between workers is taken into account, because the proportion of previously dead insects influences the instantaneous mortality of caged bees. This proportion at time s was computed as the number of total deaths in the cage at time s divided by the number of honeybees still alive in the cage. This covariate induced a nonnegligible, time-dependent, population-size effect. Moreover, interactions between cage and population size were introduced in the model.

The death rate of an individual under treatment i in a cage k at time s had the form

$$\lambda_{ik}(s) = \lambda_0(s) \exp\{t_i + c_{ik} + [\alpha + \beta_i + \gamma_{ik}]X_{ik}(s)\} \quad (1)$$

where λ_0 is the unknown baseline hazard function; t_i is the effect of the treatment i ; c_{ik} is the effect of cage k under treatment i ; $X_{ik}(s)$ is the proportion of dead bees before time s in cage k of treatment i ; α is the main effect of the population size; and β_i and γ_{ik} are the interactions between the proportion of dead bees and, respectively, treatment i and cage k of treatment i . We considered I treatments. For each treatment $i \leq I$, we tested K_i cages of n_{ik} individuals. We considered T_{ikj} to be the instant of the death or censoring of the j -th bee in the cage (i, k) . If T_{ikj} is a death time, then let $\delta_{ikj} = 1$, and if it is a censoring event, then let $\delta_{ikj} = 0$. The set ψ of the model parameters t_i , c_{ik} , α , β_i , and γ_{ik} is estimated by maximizing the Cox partial likelihood:

$$L^c(\psi) = \prod_{i=1}^I \prod_{k=1}^{K_i} \prod_{j=1}^{n_{ik}} \left(\frac{\exp\{t_i + c_{ik} + [\alpha + \beta + \gamma_{ik}]X_{ik}(T_{ikj})\}}{S_n(T_{ikj}, \psi)} \right)^{\delta_{ikj}}$$

(2)

where $Y_{ikj}(s) = 1\{T_{ikj} \geq s\}$ and

$$S_n(s, \psi) = \sum_{i=1}^I \sum_{k=1}^{K_i} \sum_{j=1}^{n_{ik}} Y_{ikj}(s) \exp\{t_i + c_{ik} + [\alpha + \beta_i + \gamma_{ik}]X_{ik}(s)\} \quad (3)$$

We stress the fact that the Cox partial likelihood $L^c(\psi)$, which allows us to estimate the parameters of the model, does not depend on the baseline hazard λ_0 . So, it is not necessary to specify the form of this baseline hazard function, contrary to a completely parametric approach. Maximum likelihood tests are performed for the significance of the parameters. The aim of the modeling process was to determine which combination of potential explanatory variables affects the hazard rate and, more precisely, whether the treatment effect was more important than the variability among cages. The estimators of the parameters are approximately gaussian, because the total number of bees is large enough and the variance of the approximating distributions are estimated from the data. These approximations provide confidence intervals for the true parameter values and for the hazard ratios $\lambda_{ik}(s)/\lambda_0(s)$ from Equation 1 and according to the value of the proportion of dead bees at s . This analysis was done using S-plus® software [40].

RESULTS

Consumption of syrup

We compared the mean consumption per bee and per day using a multivariate analysis of variance including the treatment effect, the time from the beginning of the experiment, and the variability among cages nested in the treatment. No significant difference of consumption was found among treatments on average over the cages ($F_{8,299} = 1.614$, $p = 0.12$). The consumption of syrup was constant during the experiment on average on the cages ($F_{1,299} = 0.582$, $p = 0.45$) and was barely significantly different among cages under the same treatment ($F_{10,299} = 1.92$, $p = 0.042$). The mean consumption of syrup per bee and per day was $20 \pm 0.95 \mu\text{l}$.

Definition of the survival model

We tested the adequacy of the complete model against four nested models:

$$\text{model } M_1: \lambda_{ik}(s) = \lambda_0(s) \exp\{t_i + c_{ik} + [\alpha + \beta_i + \gamma_{ik}]X_{ik}(s)\}$$

$$\text{model } M_2: \lambda_{ik}(s) = \lambda_0(s) \exp\{t_i + c_{ik} + [\alpha + \beta_i]X_{ik}(s)\}$$

$$\text{model } M_3: \lambda_{ik}(s) = \lambda_0(s) \exp\{t_i + c_{ik}\}$$

$$\text{model } M_4: \lambda_{ik}(s) = \lambda_0(s) \exp\{t_i\}$$

$$\text{model } M_5: \lambda_{ik}(s) = \lambda_0(s)$$

The model M_1 is the complete model described by Equation 1; all the covariates and their interactions are taken into account. In the model M_2 , we only consider the treatment effect, the cage effect, the density-dependent effect, and its interaction with the treatment effect. The model M_3 considers the treatment effect and the cage effect without any density-dependent effect. The model M_4 only takes into account the treatment effect, without any consideration of the nuisance variable (cage effect) or density-dependent effect. The last model (M_5) is the null model of interest.

Table 1. Log-likelihood ratio test between the complete model M_1 and the four models nested in the complete model for each chemical^a

Nested model	df	Imidacloprid		Deltamethrin	
		SL	p	SL	p
M_2	4	27.8	10^{-5}	23.8	$<10^{-5}$
M_3	7	31.8	10^{-5}	25.0	$<10^{-5}$
M_4	11	200	10^{-5}	187	$<10^{-5}$
M_5	13	342	10^{-5}	291	$<10^{-5}$

^a The SL represents the log-likelihood ratio as defined in the text, and df represents the degree of freedom of the test. The p value associated with the χ^2 (SL, df) is indicated.

Consider models M_i and M_j (nested in M_i), and let the value of the maximized log-likelihood for each model be (\hat{L}_i) and $\log(\hat{L}_j)$, respectively. We compared the two models by their statistical SL (log-likelihood ratio) $= -2[\log(\hat{L}_j) - \log(\hat{L}_i)]$ that has an asymptotic χ^2 distribution under the null hypothesis that the coefficients of the additional variables from M_j to the complete model M_i are zero. Table 1 presents the values of SL with their degree of freedom and the p value associated to the ratio test for each chemical.

The tests between the complete model M_1 and the four nested models were highly significant in all cases. The complete model was the best model. Therefore, we could not reject any variable of the complete model, and all the interactions were significant. One variable to be taken into account was the density-dependent effect (effect of the ratio of bees still alive). For the two chemicals at the two doses, the density-dependent effect and its interactions with the treatments were significant: The bees from the same cage did not die independently from each other. The variability among cages was also significant, and this nuisance variable was retained as an explanatory covariate. These analyses were completed by diagnostic statistics [30].

For the two chemicals, we plotted the Martingale residuals. These residuals can take value between $-\infty$ and 1, and individuals with a large residual are poorly predicted by the model. In the present study, the residuals were roughly centered around zero, and no widely deviant data were found (Fig. 1). Moreover, to assess the proportional hazards assumption, we also examined the Schoenfeld residuals. These residuals were calculated using the S-plus function *cox.z.ph* [40] for each covariate and all their interactions used in the model corresponding to each chemical. Because the smooth curves were flat and centered around zero, we concluded that the proportionality assumption was reasonable.

Effect of the treatment

The lifetime distributions were plotted in Figure 2. Because all variables and their interactions were significant (Table 1), we explored the treatment effect for each chemical in the complete model M_1 . The cage and the density-dependent effects as well as their interactions were significant, but the treatment effect was more significant. The hazard function of a bee exposed to a chemical was globally multiplied, all other things being equal, by $\exp\{t_i\}$, where t_i is the coefficient associated to the treatment effect. Table 2 gives the coefficient estimates for this treatment effect for each chemical. Imidacloprid and deltamethrin at the two doses increased the hazard of death of caged bees.

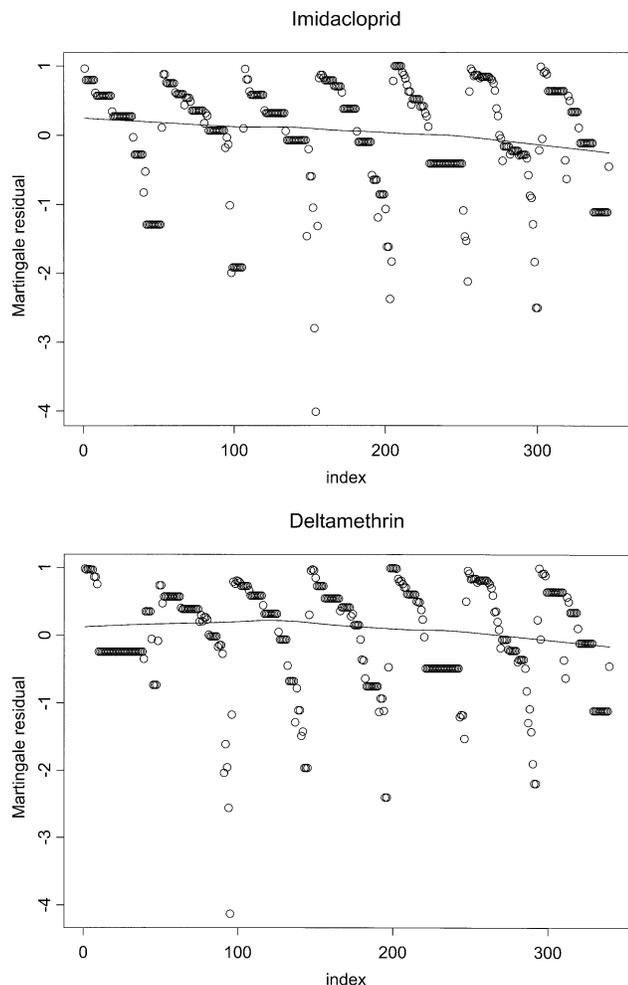


Fig. 1. Martingale residuals plot with loess smooth line for the analysis corresponding to the two chemicals (imidacloprid [top] and deltamethrin [bottom]). These residuals were roughly centered around zero, and no widely deviant observations were noted.

DISCUSSION

Interest of the Cox model

The model proposed in the present study is a semiparametric model. As a nonparametric approach, this model does not need to assume a specific probability distribution, but as a parametric method, it can also take into account the influence of covariates. The model expresses the data in terms of death rate depending on explanatory covariates that include the density-dependent effect. Survival analysis could be conducted by a simple comparison of the Kaplan-Meier curves obtained after the different treatments, and no model assumption would be required. However, such a nonparametric approach would not allow us to take into account the time-dependent covariate that was shown to modify significantly the hazard function in the Cox model. Because a survival function is equivalently defined by a unique hazard function, the nonparametric model may be viewed as equivalent to the submodel M_4 of the Cox model M_1 . As shown in Table 1, the hypothesis of model M_4 should be rejected. A semiparametric approach should be preferred. However, the survival data of caged honeybees cannot be studied via multiple-regression techniques for the following reasons: The nonnormal distribution of survival times, the presence of censoring, and the time-dependent covariates. The

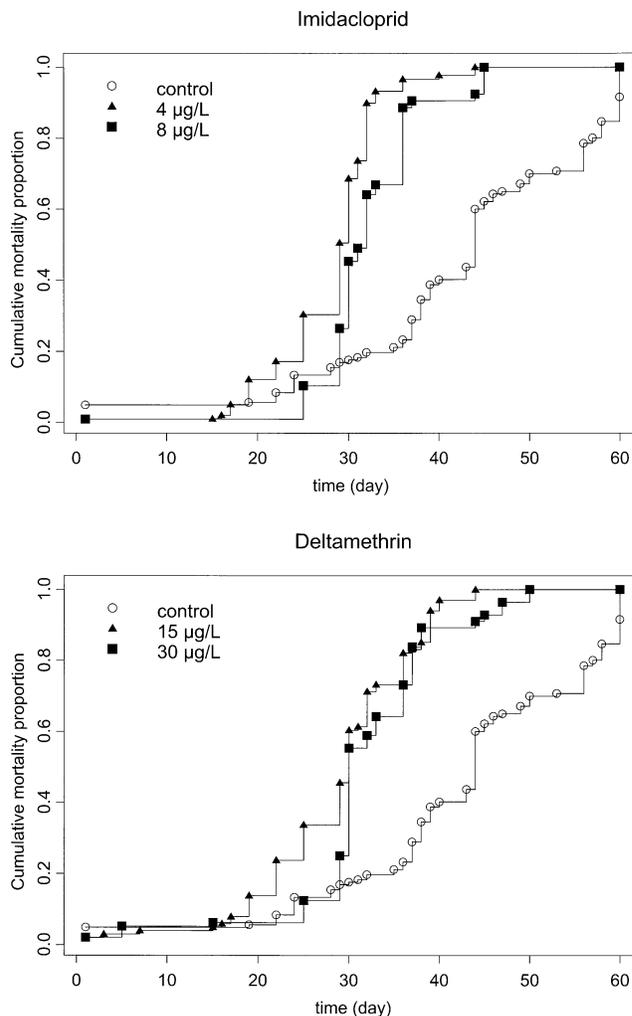


Fig. 2. Kaplan-Meier estimates of the lifetime distribution of bees fed a sucrose control solution, imidacloprid (top), or deltamethrin (bottom). The y-axis represents the Kaplan-Meier estimates of the cumulative mortality proportion. For each chemical, two doses were tested. These lifetime distributions were obtained by pooling all the cages under the same treatment (same chemical and same dose).

survival times being always positive, we could not assume that this type of data has a normal distribution, which assumes that the distribution is symmetric around zero. Therefore, parametric models for the analysis of survival data, like the Weibull analysis, have been used by several authors to study survival times in the honeybee [24,26]. These models are very useful tools, especially in exploratory works, because they can usually be fitted much faster than the Cox models. However, they

Table 2. Coefficient estimates for the treatment effect (t_i) and its standard error (SE) on fitting the complete model M_1 for each chemical^a

Chemicals	Dose	t_i	SE	$\exp\{t_i\}$	p
Imidacloprid	4 µg/L	2.58	0.567	13.2	$<10^{-5}$
	8 µg/L	1.59	0.548	4.9	3.7×10^{-3}
Deltamethrin	15 µg/L	2.23	0.527	9.33	2.3×10^{-5}
	30 µg/L	1.54	0.535	4.64	4.1×10^{-3}

^a The hazard function of a bee exposed to a chemical was globally multiplied by $\exp\{t_i\}$. This table gives the p value of the Wald's test for the significance of the estimated coefficient in model M_1 . For all the treatments, the value $\exp\{t_i\}$ differed significantly from 1.

rely on the assumption of a given parameter of the hazard function. When a less parametric approach, such as the Cox model, is used in the analysis of survival data, a particular probability distribution for the survival times need not be assumed. The hazard function is not restricted to a specific functional form.

The other major feature of the survival data is the censoring. The survival analysis using a Cox model allows us to take into account the partial information contained in the censored data (i.e., the fact that the individual is alive from the beginning of the experiment up to the censoring time). This feature is useful to cope with individuals escaping or dying accidentally, but it also allows us to plan the sacrifice of some individuals for some biological analysis without affecting the result of the experiment. Moreover, the survival of a group of bees depends on several explanatory variables that can be time-dependent (e.g., the number of nestmates already dead in the cage). In most cases, the variability among cages and its interaction with the treatment were significant and could not be discarded. The modeling process also showed that the density-dependent effects and their interaction with the treatment variable significantly affected the form of the hazard function. This result was consistent with that of a previous study investigating the effects of proteinase inhibitors on the survival of bees in a 60-d chronic test [17], in which the individual hazard function was correlated with the number of surviving bees. Nevertheless, it seems relevant to consider the variability among cages not as a fixed effect but as a random effect in a frailty model [41–43]. This random effect may be considered as a nonobserved variable that describes the unexpected risk, or frailty, for the bees of a given cage as compared to another cage with slower mortality under the same treatment. In the present study, the tests of significance for the treatment and the group-size effect into a frailty model gave the same results as those obtained with the model without random effect. Thus, variability among cages must be considered as a fixed effect.

Effects of the pesticide on honeybee survival

A critical point in evaluation of the toxicity of chemicals to caged bees is the actual concentration of the toxic compound received by each bee. In the present experiment, because the mean consumption of syrup per bee and per day was constant during the experiment and did not differ significantly among treatments, and assuming that trophallactic exchanges ensure an equal distribution of food among bees, the exposure to the chemical was considered as homogeneous among bees. In other words, we assumed that the nominal concentration in the contaminated solution was proportional to the dose received per bee. The statistical approach based on the comparison of survival data by a Cox proportional hazard model demonstrated the chronic toxicity of imidacloprid at 4 and 8 $\mu\text{g/L}$. When focusing on the dynamics of survival, we observed a strong increase of mortality with imidacloprid approximately 30 d after the beginning of the observations (Fig. 2). This effect of the pesticide would not have been detected in a short-term experiment. Besides, the classical analysis of toxicity is based on determination of the LD50 estimated after 1 or 2 d of treatment, which is mainly representative of potential effects on foragers. The delayed toxicity of imidacloprid may rely on the differential sensitivity of bees according to age or on the accumulation of the compound in bees. Two arguments favor an age effect as the critical factor. First, imidacloprid has a low lipophilicity ($\log\{P_{ow}\} = 0.57$), which implies that its

fixation in the adipose tissues of animals (i.e., bioaccumulation) is unlikely. Second, our data are consistent with those of previous chronic oral tests on caged honeybees [18]. In a 10-d chronic oral test with honeybees fed syrup contaminated with imidacloprid at 1 and 10 $\mu\text{g/L}$, Suchail et al. [18] observed mortality after 3 d. The worker bees used in that study were captured on honey and pollen combs in the hive corresponding mainly to old bees (20–25 d) [44]. Those bees died after 3 d of exposure (i.e., at the age of 23–28 d). In the present study, the survival time (mean \pm standard error) was 28.3 ± 5.6 d for the treatment with imidacloprid at 4 $\mu\text{g/L}$ and 31.3 ± 4.1 d at 8 $\mu\text{g/L}$, which is in the same range of values as the data given by Suchail et al. [18]. These results are consistent with the hypothesis of an age effect (i.e., variation of the sensitivity to the imidacloprid treatment according to age) rather than of an accumulation of imidacloprid in bees. To confirm this hypothesis, further experiments following the experimental setup and the survival model described in the present study should be conducted by starting the exposure at different ages. The characterization of a differential sensitivity with age is important in the risk assessment of pesticides for honeybees, because it can significantly alter the age distribution within the colony.

The treatment effect was significant for bees exposed to deltamethrin (15 and 30 $\mu\text{g/L}$). An increase of the mortality after 30 d also appeared in deltamethrin-treated bees. Contrary to imidacloprid, the lipophilicity of deltamethrin ($\log\{P_{ow}\} = 4.6$) makes the hypothesis of accumulation likely. Chronic toxicity, such as that caused by deltamethrin, has been previously observed with other pyrethroids in honeybees. During a test period of 10 weeks, permethrin caused an increase of the death rate [12]. Illarionov [45] showed that the toxicity was higher in the case of multiple ingestion of food containing cypermethrin or alphasmethrin than in the case of a single ingestion. Moreover, long-term exposure of bees to cypermethrin showed that this insecticide caused serious damage to treated colonies [16]. Thus, both the present results and those found in the literature demonstrate that pyrethroids, used at low concentrations over a long period of time, might induce mortality.

Inverse concentration–response effects were found, because the lowest concentrations tested of imidacloprid (4 $\mu\text{g/L}$) and deltamethrin (15 $\mu\text{g/L}$) led to the highest level of mortality. For imidacloprid, similar unusual concentration–response effects had been reported in acute toxicity tests [46], in motor activity tests [47], and in cytochrome oxidase staining of the bee brain [48]. Thus, both the present results and those of previous works are in agreement with the conclusion of Calabrese and Baldwin [49]: When studies are properly designed to evaluate chemical toxicity below the traditional toxicological threshold (e.g., LD50), toxic effects at low concentrations can be observed with high frequency. For imidacloprid, Suchail et al. [46] suggested that nonlinear concentration–response effects might account for complex detoxification mechanisms, with low concentrations inducing strong toxic effect because no detoxification mechanism takes place and higher concentrations triggering the induction of detoxifying enzymes, leading to a lower toxicity. The present results suggest that such a detoxification mechanism might be involved in the inverse concentration–response effect with deltamethrin. A recent review [49] established that numerous highly reliable toxicological studies demonstrate the existence of such effects and that they can be generalized to the class of chemicals assessed.

In conclusion, the assessment of long-term toxicity to honeybees from chronic exposure to pesticides by means of a proportional hazard model revealed that the analysis should not neglect the time variation of the mortality through a non-parametric baseline hazard function, the interactions among bees (i.e., the density-dependent effect), and the variability among cages under the same treatment. The originality of the present model is not only to focus on the treatment effect but also to consider explicitly the density-dependent effect and nuisance variables (cage effect). This statistical model seems particularly adapted to the assessment of chronic toxicity to honeybees and, more generally, to any social insect or animal with strong social interactions studied in groups.

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