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New Risk Assessment Approach for Systemic Insecticides: The Case of Honey Bees and Imidacloprid (Gaucho)

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The procedure to assess the risk posed by systemic insecticides to honey bees follows the European Directives and depends on the determination of the Hazard Quotient (HQ), though this parameter is not adapted to these molecules. This paper describes a new approach to assess more specifically the risk posed by systemic insecticides to honey bees with the example of imidacloprid (Gaucho). This approach is based on the new and existing chemical substances Directive in which levels of exposure (PEC, Predicted Exposure Concentration) and toxicity (PNEC, Predicted No Effect Concentration) are compared. PECs are determined for different categories of honey bees in relation to the amounts of contaminated pollen and nectar they might consume. PNECs are calculated from data on acute, chronic, and sublethal toxicities of imidacloprid to honey bees, to which selected assessment factors are applied. Results highlight a risk for all categories of honey bees, in particular for hive bees. These data are discussed in the light of field observations made on honey bee mortalities and disappearances. New perspectives are given to better determine the risk posed by systemic insecticides to honey bees.

Introduction

In the European Union, formulated pesticides are registered by the European Council Directive (EC-91/414) and the risk posed by these molecules to honey bees is directly assessed by the European and Mediterranean Plant Protection Organization (EPPO) guidelines No. 170 (*1*). These guidelines propose methods for evaluating side effects of agrochemical products on honey bees. The approach is based on a 3 tier assessment scheme comprising early studies in laboratory conditions, followed by semi-field studies, and completed by field studies. According to this Directive, and to the decision making scheme attached to the EPPO guidelines (*2*), moving from tier 1 (laboratory studies) to tier 2 (semi-field studies) depends on a trigger criterion, the Hazard Quotient (HQ = field application rate/oral or contact Lethal Dose (LD_{50})). When the calculated value of HQ is higher than a threshold of 50, further studies are required. This threshold is derived from data which only consider spray applications on honey bees (*3*).

In the case of plants treated by systemic insecticides, honey bees may be at risk via contaminated pollen and nectar (*4*). The contamination of nectar by sprayed systemic insecticides has been long documented (*5*), whereas little information is available on systemic formulations applied in soils and on seeds. Published data deal mainly with aldicarb, a carbamate substance used for the protection of various cultures (*6*). More recently, several authors supplied data on the presence of imidacloprid, a neonicotinoid systemic insecticide, in nectar and pollen of treated plants (*7*).

Generally, systemic insecticides provide the treated plant with a permanent protection from soil invertebrates and sucking insects (*8*). Applied in soils and on seeds, they degrade slowly over time and disperse in all the plant tissues during its growth. Therefore, using the field application rate of active substance as an exposure parameter to assess the risk posed by systemic insecticides to honey bees is not sensible. Unlike sprayed insecticides, which have a short-lasting action on plants, systemic insecticides are persistent. Moreover, these molecules, detected at low concentrations in the pollen and nectar of treated plants, are more likely to affect honey bees by acute, chronic, and sublethal intoxications (*9*) rather than by acute intoxications alone.

In this paper, we propose a new approach to determine the risk posed by systemic insecticides to honey bees. It is based on the European Technical Guidance Directive (TGD) that assesses the impact of new (793/93 and 1488/94/CE legislations) and existing chemical substances (EC-67/54/8 and EEC-93/67 Directives) on ecosystems (*10*). This approach is applied to imidacloprid, which is a good study case because it has been extensively studied and presents a lot of experimental data.

Materials and Methods

A group of experts, namely the Scientific and Technical Committee (CST), was nominated in 2001 by the French Ministry of Agriculture to assess the risk posed by imidacloprid to honey bees. This committee examined all studies, delivered up to July 2004 by the Ministry of Agriculture, on the toxicity of imidacloprid to honey bees (*7*). This paper refers to some of the work achieved by this committee.

For many wildlife species, the standard practice in pesticide regulation (91/414 EEC) is to determine a toxicity exposure ratio (TER) and to compare it to a threshold (a safety factor) that aims at protecting these species. In this paper, we used the PEC/PNEC ratio (predicted environmental concentration/predicted no effect concentration) which aims at protecting ecosystems (*10*). Honey bees (unlike most other species) live in colonies and depend on each other for survival. Such interdependent relationships define the honey bees' colony as a superorganism (*11*). The functioning of a superorganism is similar to that of an ecosystem in the sense that each unit (temporal castes in a colony and species in an ecosystem) is essential to sustain the system as a whole (*12*). Moreover, considering their role as pollinators (*13*), honey bees represent a good model to assess the risk of insecticides to pollinators and to protect many plant species that rely on these organisms, through pollination, to reproduce.

According to the PEC/PNEC approach and with the example of imidacloprid, we determined PECs with the

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TABLE 1. Estimated PECs for Different Categories of Honey Bees to Imidacloprid[a]

categories of bees	sugar[b]	pollen[b]	imidacloprid (20%)	imidacloprid (40%)	imidacloprid (60%)	imidacloprid (80%)	imidacloprid (100%)
worker larvae ($d = 5$)	59	5	41	82	124	165	206
drone larvae ($d = 6.5$)	98	N. A.[c]	63	126	189	252	315
nurses ($d = 10$ for pollen and $d = 8$ for nectar)	272−400	65	219−301	438−602	656−903	875−1204	1094−1505
wax-producing bees ($d = 6$)	108		69	139	208	278	347
winter bees ($d = 90$)	792		509	1017	1526	2034	2543
nectar foragers ($d = 7$)	224−899		144−577	288−1155	431−1732	575−2310	719−2887
pollen foragers ($d = 7$)	73−109		47−70	94−140	140−210	187−280	234−350

[a] Estimated amounts of food (sugar and pollen in mg) and imidacloprid (in pg) consumed per bee over *d* days, with different levels of food contamination (%). [b] Data from ref *21*. [c] N. A.: No available data.

known concentrations of imidacloprid found in the contaminated pollens (sunflower and maize) and nectar (sunflower) consumed by honey bees, and we determined PNECs with the data derived from studies on acute, chronic, and sublethal toxicities of imidacloprid to honeybees.

Criteria for the Validation of Data. To determine the concentration of imidacloprid in pollens and nectar issued from imidacloprid seed-dressed plants and the honey bees' exposure to imidacloprid, the CST validated the data of all the studies that met the following requirements in terms of sampling procedure, chemical analyses, and toxicity testing.

For the sampling procedure, studies needed to describe thoroughly the methods used (sampling location, pesticide treatments history) and gather sufficient samples, both qualitatively and quantitatively. Qualitatively, data obtained from pollens collected directly on anthers of treated flowers, rather than in pollen traps, were kept and validated because the concentration of imidacloprid in pollens of traps is highly variable and depends on the environment (i.e., the amount of plants treated by systemic insecticides) (*14*). Quantitatively, the CST retained the value of a minimum of 10 samples to enable statistics (means and standard deviations). Samples coming from different experiments and locations, but presenting similar protocols, were grouped together to get a minimum of 10 samples.

For the chemical analyses, given the high toxicity of imidacloprid, we validated studies that detected the molecule the most accurately as possible, that is by high performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) (*15*) and using an appropriate limit of quantification (LOQ = 1 μg/kg), limit of detection (LOD < 0.5μg/kg), and sample weight (10 g). The only study that used a radioactivity method coupled with thin-layer chromatography (TLC) and automated multiple development (AMD) techniques (*16*) was validated too because it allowed a clear identification of imidacloprid, unlike less specific methods such as the derivation and gas chromatography (GC) (*17*, *18*).

To determine the toxicity of imidacloprid to honey bees, studies apply standardized tests designed by the OECD guidelines (*19*, *20*). Such tests are developed in laboratory conditions to assess oral (*19*) and topical (*20*) acute toxicities of pesticides (and other chemicals) to adult worker honey bees. These laboratory tests follow the EPPO guidelines No. 170 (*1*) and the recommendations made by the International Commission for Plant−Bee Relationships (ICPBR). While these guidelines propose methods to test oral and topical acute toxicities, there are currently no standardized tests to study chronic and sublethal toxicities of pesticides to honey bees. Nevertheless, a few studies have investigated the impact of imidacloprid on honey bees by chronic and sublethal intoxications in laboratory, semi-field, and field conditions. To assess these studies, we referred to the EPPO guidelines because they present guidelines for semi-field and field experiments (*1*).

PEC Estimates. By definition, a PEC corresponds to the amount of pesticides a honey bee might be exposed to, either by ingestion or contact. In this paper, with the example of imidacloprid and honey bees, we only considered oral exposures because data on topical exposures are scant.

We can estimate honey bees' exposure to both contaminated pollens (sunflower and maize) and nectar (sunflower) with (*i*) the known and validated concentrations of imidacloprid found in contaminated pollens and nectars, and (*ii*) the amount of contaminated pollen and nectar consumed by different categories of honey bees (*21*).

(*i*) The amount of imidacloprid present in the food of honey bees is directly related to the environement. For example, if a hive is located near extensive cultures of maize and sunflower plants treated by imidacloprid, the proportions of pollen and/or nectar that might be contaminated by imidacloprid are expected to be high. Since the relative proportions of contaminated food, versus uncontaminated food, consumed by honey bees are unknown, we considered 5 different levels of contamination ranging from 20% (a low level of contamination) to 100% (the highest level of contamination) (Table 1), although the latter case might rarely occur in natural conditions.

(*ii*) The amount of contaminated food consumed by different categories of honey bees depends on the amount of food the bees require to achieve particular tasks within the colony. Among them, Rortais et al. (*21*) considered the categories that are potentially the most exposed to imidacloprid: those that achieve the most costly tasks in terms of energy and which consume the highest amounts of pollen for their development. Therefore, for the calculation of the PEC, the following categories of honey bees were considered: the worker larvae which consume pollen and nectar for their development over about 5 days; the drone larvae which consume pollen and nectar for their development over about 6.5 days; the nurses which consume pollen over a period of 10 days and nectar and/or honey to maintain the nest temperature at 34 °C over the entire brood attendance period, lasting about 8 days; the wax-producing bees which consume nectar during the period of maximum wax production, lasting about 6 days; the winter bees which consume nectar and honey to maintain the nest temperature at viable

temperature during winter, lasting about 90 days in temperate regions; and the nectar and pollen foragers which consume nectar and/or honey to cover their daily flight expenses. As a forager life span is highly variable (between 1 and 3 weeks), the amount of food consumed by a forager to collect food has been estimated over a minimal period of one week.

Honey Bees' Exposure to Contaminated Sunflower and/or Maize Pollens. The honey bee's exposure to contaminated pollens collected on treated plants is determined by the following equation:

$$\text{PEC}_1\ (\text{pg}) = \text{Validated concentration of imidacloprid found in sunflower and/or maize pollens } (\mu\text{g/kg}) \times \text{Amount of pollens consumed by honey bees (mg)} \times \text{Levels of contamination found in pollen (\%)}$$

Honey Bees' Exposure to Contaminated Sunflower Nectar. The nectar brought back to the colony is consumed by honey bees, either rapidly as it is or later on as honey when it is stored. The relative amounts of nectar and honey consumed by honey bees are unknown. However, the amounts of sugar contained in sunflower honey and nectar are known and are, on average, 80% and 59%, respectively (*22*, *23*). Therefore, the amounts of sunflower nectar and/or honey consumed by honey bees can be determined by their sugar consumption, in relation to their energy requirement (*21*). As a result, for every milligram of sugar required, a honey bee will have to consume 1.25 mg of sunflower honey or 1.69 mg of sunflower nectar. Therefore, a honey bee's exposure to contaminated sunflower nectar can be determined by the following equation:

$$\text{PEC}_2\ (\text{pg}) = \text{Validated concentration of imidacloprid found in sunflower nectar } (\mu\text{g/kg}) \times \text{Amount of sugar consumed by honey bees (mg)} \times 1.69\ \text{Levels of contamination found in sunflower nectar (\%)}$$

Honey Bees' Exposure to Contaminated Sunflower and/or Maize Pollens and to Contaminated Sunflower Nectar. The honey bee's exposure to contaminated sunflower and/or maize pollens and to contaminated sunflower nectar is summarized as follows:

$$\text{PEC (pg)} = \text{PEC}_1\ (\text{pg}) + \text{PEC}_2\ (\text{pg})$$

PNEC Estimates. By definition, a PNEC corresponds to the amount of substances that will have no impact on ecosystems. For numerous substances, the pool of data is usually too limited to predict their effects on ecosystems. In such circumstances, empirically derived assessment factors must be applied. These assessment factors allow the prediction of a concentration below which an unacceptable effect will most likely not occur. The size of these assessment factors incorporates various uncertainties due to extrapolations from single-species laboratory data to a multi-species ecosystem, in particular uncertainties due to intra- and inter-laboratory variations in toxicity data, intra- and inter-species variations, short-term to long-term toxicity extrapolations, and from laboratory data to field impact studies. For the terrestrial compartment, the size of the assessment factors depends on the confidence we have on the representativeness of the toxicity data. For example, the size of these factors is reduced when more data become available at various trophic levels and for several taxonomic groups.

Based on these parameters and in relation to the experimental conditions, the TGD determines various assessment factors. In laboratory conditions, for short-term toxicity tests (LD_{50}) and for one trophic level, a factor 1000 is used, for long-term toxicity tests and for several trophic levels with known NOEC, a factor 100 is applied, and for additional long-term toxicity tests of two or three trophic levels of known NOEC, the factors 50 and 10, respectively, are selected. In field conditions, an assessment factor is determined case by case (*10*).

The approach presented in this paper consisted in finding appropriate PNECs for honey bees derived from PNECs designed for ecosystems. These PNEC values were estimated with the available data obtained from studies on oral acute, chronic, and sublethal toxicities of imidacloprid to honey bees. These values were derived from the lowest validated toxicities (LD_{50}, lowest observed effect concentration (LOEC), or no observed effect concentration (NOEC)) to which assessment factors are applied. This new approach is specifically adapted to honey bees because it allows the assessment of both colonies and individuals. These factors had to be determined case by case, following the standard approach used by the TGD. Every time new data enabled us to reduce extrapolations (chronic toxicity data in relation to acute toxicity data), the assessment factors were generally reduced by a factor 10.

PEC/PNEC Estimates. The hazard posed by new substances to organisms is determined by the PEC/PNEC ratio. When this ratio is over 1, it highlights an intoxication risk for honey bees, whereas when it is below 1, it indicates no risk. According to the TGD (*10*), this ratio is obtained and derived from acute toxicity data, but when a risk is found, the ratio is re-calibrated with new data obtained in more representative conditions. For honey bees, the PECs were determined with all the available scientific data found on honey bees' food consumptions because there were sufficient data, whereas the PNECs required more data. Therefore, following the TGD procedure, PNECs were derived from acute toxicity data. When a risk was highlighted, a new PEC/PNEC ratio was determined with data obtained from chronic toxicity studies. If the new ratio remained over 1, a final PEC/PNEC ratio was then calculated with new data coming from sublethal field toxicity studies. This final ratio is the most representative ratio of the natural conditions of a honey bees' colony.

Results

PEC Estimates. (*i*) In pollen collected directly on the anthers of flowers, the concentrations of imidacloprid found in treated sunflower and maize plants are 3.3 and 3.5 μg/kg, respectively (*24*, *25*), or on average 3.4 μg/kg for both pollen types. The concentration of imidacloprid found in treated sunflower nectar is 1.9 μg/kg (*25*). (*ii*) Based on the estimated amounts of pollen and nectar consumed by honey bees over several days of activity (*21*), the potential amounts of imidacloprid ingested by honey bees were determined (Table 1).

PNEC Estimates. There is currently no test and no toxicity data for larvae. For this category of honey bees, PNECs were derived from the toxicity data obtained in adult workers. Table 2 shows the PNECs determined in adults and derived from acute, chronic, and sublethal toxicity data, to which specific assessment factors were applied.

From acute toxicity data: the lowest validated LD_{50} (48 h) is 3.7 ng of imidacloprid per bee (*26*). According to the TGD (*10*), the assessment factor for acute toxicity data is 1000. However, the toxicity of imidacloprid was determined by several studies which tested models belonging to the same species, and found similar results. As these data present very few uncertainties, an assessment factor of 100 was applied. Therefore, the validated PNEC becomes 3.7/100 = 37 pg/bee.

From Chronic Toxicity Data. In laboratory conditions, the lowest validated value was LD_{50} (10 d) = 0.012 ng/bee (*27*). As this value was obtained from a long-term experiment, it seemed appropriate to apply the same assessment factor as the one used for a long-term NOEC experiment, which is 100 (*10*). However, this factor is used to determine a PNEC for

TABLE 2. PNECs for Oral intoxications of Honey Bees to Imidacloprid

toxicities	experimental conditions (imidacloprid intakes)	doses (ng/bee)	observed variables	assessment factors	PNEC (pg/bee)
acute	laboratory (one determined dose)	LD_{50} (48 h) = 3.7	survival (% mortality)	100	37
chronic	laboratory+ (one determined dose)	LD_{50} (10 d) = 0.012	survival (% mortality)	10	1.2
sublethal	laboratory (one determined dose)	NOEC = 0.94	behavioral dysfunction (knockdown effect)	50	20
	laboratory (several determined doses)	NOEC = 0.2	behavioral dysfunction (proboscis extension reflex)	10	20
	semi-fields (at feeders, several determined doses)	LOEC = 0.075	behavioral dysfunction (feeding)	10	7.5
	fields[a] (at feeders, several determined doses/on plants)	NOEC = 0.25	behavioral dysfunction (dances)	5	50

[a] Feeders contained syrup contaminated by imidacloprid. In field conditions, feeders were placed near hives to reinforce the observed effects of imidacloprid seed-dressed plants on honey bees.

all the taxonomic groups of an ecosystem. To adjust this factor to one taxonomic group (the honey bees), we applied a factor 10. This factor includes all variations found among and within taxonomic groups (inter- and intra-species variations). Therefore, the validated PNEC becomes 0.012/10 = 1.2 pg/bee.

From Sublethal Toxicity Data. In laboratory, semi-field, and field conditions, one or several administered doses might induce behavioral modifications among treated honey bees. When administering a unique oral dose of imidacloprid to honey bees for the testing of the knockdown effect, the lowest validated NOEC was 0.94 ng/bee (*28*). As this value was obtained from a short-term experiment, an assessment factor of 100 should have been applied (TGD). However, this value does not correspond to a LD_{50}; it is a dose that has no impact on honey bees. Moreover, the measured effect is a sublethal effect. Therefore, we applied an assessment factor of 50. The validated PNEC becomes 0.94/50 = 18.8 pg/bee. When administering several oral doses of imidacloprid to honey bees for the testing of the proboscis extension reflex (PER), the lowest validated concentration, after a 10 day experiment, was 0.2 ng/bee (*29*). This value corresponds to a NOEC based on the testing of sublethal effects after a long-term intoxication. Therefore, we applied an assessment factor of 10. The validated PNEC becomes 0.2/10 = 20 pg/bee.

In semi-field conditions, a LOEC (5 d) of 0.075 ng/bee was validated for the testing of the time spent feeding on contaminated syrup (*30*). As this study was conducted in the natural conditions of foragers, an assessment factor of 10 was applied (TGD, *10*). The validated PNEC becomes 0.075/10 = 7.5 pg/bee.

In field conditions, a lowest NOEC (10 d) of 0.25 ng/bee was validated for the testing of dances (*31*). Studies conducted in field conditions present similar conditions to those found in the natural environment of honey bees. Therefore, an assessment factor of 1 should be applied (TGD, *10*), but we selected an assessment factor of 5 because the setting of the feeders is artificial. Therefore, the validated PNEC becomes 0.25/5 = 50 pg/bee.

PEC/PNEC Estimates. According to the previously defined assessment factors, and whatever the level of food contamination is, all the investigated categories of honey bees presented an intoxication risk to imidacloprid (Figure 1). The PEC/PNEC ratio was the highest for winter bees and nurses (between 10 and 100) and the lowest for pollen foragers and larvae (between 1 and 10).

Discussion

The PEC/PNEC derived from the calculation of honey bees' exposure to which appropriate assessment factors were applied show that the risk posed by imidacloprid is alarming for all categories of honey bees. These ratios are all over 1, and greater in adult hive bees than in any other categories of bees. Whatever the validated toxicity data are, the determined PNECs are in a limited range of values (between 1.2 and 50 pg/bee). These estimates are in agreement with observations made in regions of extensive sunflower and maize cultures, which report a decrease in honey production since the launching of imidacloprid on sunflower plants in 1994 (*32*), and several behavioral dysfunctions, foragers disappearances, and great honey bee mortalities in summer, during the blossoming of maize and sunflower plants, and after winter, when all sunflower and maize pollens have been consumed by colonies.

In areas of extensive sunflower and maize cultures treated by imidacloprid, all categories of honey bees, whatever their age is, are at risk of intoxication. In such a situation, honey bees are most likely to bring back food that is contaminated by imidacloprid, and the observed effects might relate to either acute, chronic, or sublethal intoxications, all inducing the death of honey bees.

In areas where sunflower and maize cultures treated by imidacloprid are less abundant, honey bees might be less intoxicated because they might consume a mixture of contaminated and uncontaminated food. In this situation, honey bees are most likely to be intoxicated by sublethal doses, rather than by acute or chronic doses, which might have lethal consequences at the individual and colony levels.

At sublethal doses, pesticides are known to have profound impacts on the colony, in particular on the honey bees' longevity (*34*), the brood production (*35*, *36*), the development of hypopharyngeal glands (*37*), and the egg laying (*38*). Imidacloprid is known to affect the honey bees' cognitive behaviors such as the proboscis extension reflex PER (*33*). Learning and memorization in honey bees' tasks are very important. For example, a forager that is disorientated might get lost and eventually die. In the case of massive foragers' intoxications, the colony is likely to be greatly affected. In an experiment under tunnels, Vandame et al. (*39*) exposed honey bees to deltamethrin at a sublethal dose that is 20-fold lower than the registered dose at which foragers are expected to be exposed to in the environment. They found that 54% of the treated bees were disoriented and took flight toward the sun. The authors concluded that such sublethal effects may be the cause of the symptom called the "disappearance bee disease" by beekeepers who observed colonies' weakening without finding dead bees close to the hives. This hypothesis was formerly raised by other scientists (*40*, *41*).

Imidacloprid can also affect honey bees by chronic intoxications. In the long run, a repeated ingestion of low doses of imidacloprid could cause immunodeficiency and diseases in honey bees. The impairment of the bees' immunity system is a nonspecific mechanism (*42*). For

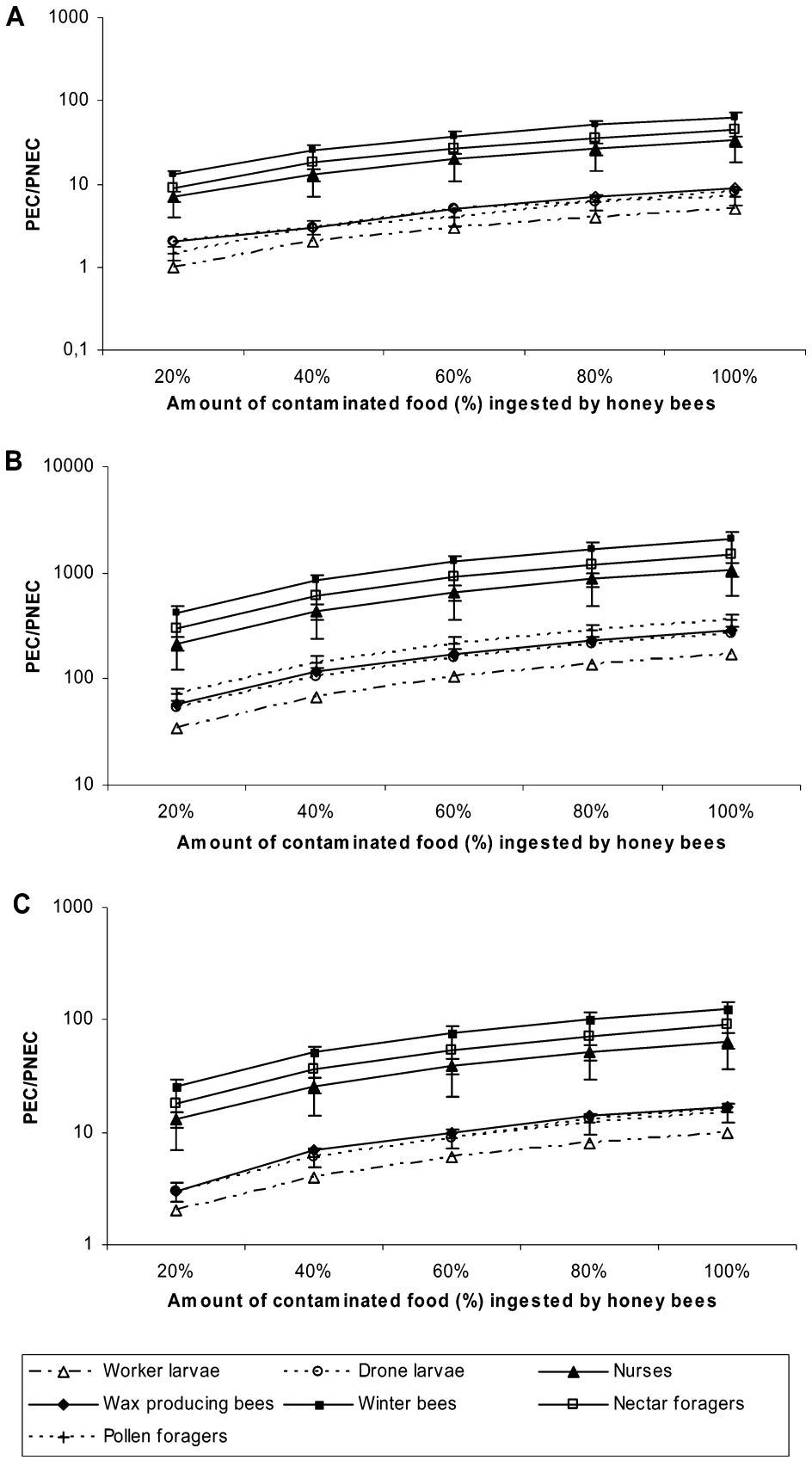

FIGURE 1. Hazard posed by imidacloprid to different categories of honey bees feeding on various proportions of contaminated food: estimated PEC/PNEC ratios derived from (A) acute toxicity data, (B) chronic toxicity data, and (C) sublethal toxicity data obtained in field conditions for foragers and in laboratory conditions for all the other categories of honey bees (a risk is highlighted when ratios are greater than 1).

example, sublethal concentrations of malathion result in higher invasions of treated colonies by the wax moth (*43*).

In some cases, no honey bee troubles were observed by beekeepers, but no scientific study has ever confirmed these

observations. The presence of untreated or very little treated areas near hives, and the presence of compensatory phenomena (increase of brood development, replacement of dead foragers) with no visible harmful consequences for the colony may occur and explain the absence of any observed troubles.

When assessing the risk posed by systemic insecticides, the HQ does not take into account several idiosyncrasic parameters such as persistence in soils, presence in pollen and nectar, and transport in the air. The calculation of the Toxicity Exposure Ratios (TER) (ratio between a toxicological end point and a PEC), regularly used in the risk assessment of pesticides to organisms (mainly vertebrates and nematods), could take into account such crucial parameters. However, for social invertebrates such as honey bees, the use of the new and existing chemical substances approach (herein the PEC/PNEC ratio) should be more appropriate than the use of the TER because it enables the protection of the whole colony. The PEC/PNEC ratio could then be re-calibrated when more data on imidacloprid and on other systemic insecticides are available.

For hive bees (nurses and winter bees), the PNECs could be refined when more data are available on the mechanisms of a colony's regulation (e.g., brood development) in field and semi-field conditions. For larvae, exposures were derived from data obtained on adult toxicities in order to obtain an indicative and comparative value. Given that larvae are more or less sensitive than adults to chemicals (*4*, *44*), more studies need to determine accurately their exposure risk to imidacloprid and to other systemic insecticides.

We could not investigate topical exposures of imidacloprid to honey bees because there are not enough data available on this mode of exposure. However, honey bees' intoxications by topical exposures should not be discarded. For examples, foragers might get contaminated by contaminated dust particles during sowing operations (*45*).

The impact of systemic insecticides on honey bees is not limited to the impact of the parent compound; it also includes exposures to its metabolites. In the case of imidacloprid, some metabolites (e.g., olefin, which is twice more toxic than imidacloprid) are found to be very toxic to honey bees (*9*, *46*, *47*) and some of them are detected at low concentrations (between 0.3 and 1 μg/kg) in rape pollen and nectar (*48*). However, to investigate in further detail the impact of metabolites on honey bees, their concentrations in other types of pollen and nectar must be determined.

Exposures to imidacloprid were estimated by assuming that the molecule is stable in the hive because it is stored in a dark environment. However, the transformation of pollen and nectar into bee bread and honey, respectively, imply the action of several enzymes that might change the stability of imidacloprid. Therefore, the concentration of imidacloprid in the stored food (bee bread and honey) should be measured to test its stability in the hive over time. Honey bees' exposures to contaminated sunflower nectar were determined with data issued from one study (*25*). To confirm and generalize the trend found, it is necessary to conduct more studies (i.e., the concentration of imidacloprid in nectar coming from other varieties of sunflower and from other melliferous plants).

The method and the assessment factors proposed in this paper could be re-calibrated when more data are available. Although the determination of the LD_{50} (48 h) is readily obtained for the calculation of HQ, its representativeness in testing the survival of a honey bee colony is arguable. To assess the risk posed to honey bees, chronic and sublethal toxicity tests must be conducted systematically, especially in the case of systemic insecticides which have a long-lasting action. To achieve these tests, standardized protocols are required and could be elaborated on the grounds of existing experimental studies which have investigated the chronic impacts of systemic insecticides on honey bees (*9*, *49*, *50*), as well as their sublethal effects on the behavior (*41*, *47*, *51*−*53*) and physiology (*38*, *54*, *55*) of these organisms.

Based on the risk assessment method used for terrestrial organisms, this method is original. It includes the assessment of several important parameters such as the following: (*i*) The detection and measurement of the amount of an active ingredient present in the various substrates used by honey bees. These measures are not statutorily requested. (*ii*) The development of various scenarios of honey bees' exposure to the active ingredient. These scenarios better predict the risk posed by systemic insecticides to honey bees because they take into account the biology and particular requirements of a honey bees'colony (*21*). (*iii*) The use of novel and validated methods for the assessment of lethal and sublethal honey bees' intoxications. (*iv*) The use of assessment factors, when experimental designs are tightly related to environmental conditions. This approach is usually applied to assess the risk of chemical substances.

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An incorrect reference was cited in Table 1 in the version published ASAP March 7, 2006; the corrected version was published March 8, 2006.

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