

Quantification of Imidacloprid Uptake in Maize Crops

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The systemic imidacloprid is one of the most used insecticides in the world for field and horticultural crops. This neurotoxicant is often used as seed-dressing, especially for maize, sunflower, and rape. Using a LC/MS/MS technique (LOQ = 1 $\mu\text{g}/\text{kg}$ and LOD = 0.1 $\mu\text{g}/\text{kg}$), the presence of imidacloprid has been measured in maize from field samples at the time of pollen shed, from less than 0.1 $\mu\text{g}/\text{kg}$ up to 33.6 $\mu\text{g}/\text{kg}$. Numerous random samples were collected throughout France from 2000 to 2003. The average levels of imidacloprid measured are 4.1 $\mu\text{g}/\text{kg}$ in stems and leaves, 6.6 $\mu\text{g}/\text{kg}$ in male flowers (panicles), and 2.1 $\mu\text{g}/\text{kg}$ in pollen. These values are similar to those found previously in sunflower and rape. These results permit evaluation of the risk to honeybees by using the PEC/PNEC ratios (probable exposition concentrations/predicted no effect concentration). PEC/PNEC risk ratios were determined and ranged between 500 and 600 for honeybees foraging on maize treated with imidacloprid by seed dressing. Such a high risk factor can be related to one of the main causes of honeybee colony losses.

KEYWORDS: Imidacloprid; maize; corn; pollen; flowers; systemic insecticide; honeybees

INTRODUCTION

Several agrochemical firms have developed new systemic insecticides (e.g., neo-nicotinoids) that can be applied as soil treatment or seed dressing and therefore avoid spraying treatments in the fields. The use of this new generation of insecticides has resulted in a significant reduction of quantities of toxicant in the environment and a decrease of aerial pesticide pollution. Imidacloprid is the most used active ingredient of the neonicotinoid insecticides, and its activity is focused on whole plant protection. It acts against Homopteran insects, such as rice hoppers or aphids, as well as against some other insects such as thrips, whiteflies, termites, turf insects, and some beetles. This compound is most commonly used on rice, maize, sunflowers, rape, potatoes, sugar beets, vegetables, and fruits crops (1, 2).

Imidacloprid interferes with the transmission of stimuli in the insect's nervous system by causing a blockage in the nicotinic neuronal pathway. This pathway is more common in insects than in warm-blooded animals, making the chemical more toxic to insects than to warm-blooded animals (3–5). However, a similar $\alpha 2\beta 4$ subunit of the nicotinic acetylcholine receptor (nAChR), target of this neonicotinoid insecticide, was found in the brain of rats (6). At lethal doses, the binding capacity on the nAChR leads to the opening of Na^+ channels,

resulting in the paralysis and the death of the insect (7). On the contrary, at subchronic levels, imidacloprid action results in the closing of Na^+ channels due to the interaction with a second subunit of the same receptor (8).

Imidacloprid protects roots and shoots after seed germination. The whole plant is also protected during its growth because the systemic imidacloprid is carried by the sap into the various parts of the crop. However, the level of imidacloprid decreases during the growth, and very low levels were expected at the time of flowering. Yet the question of the presence of imidacloprid in the higher part of the plant, such as flowers, nectar, and pollen, is left especially if significant levels can remain as compared to the NOEC (no observed effect concentration) for nontargets insects.

From 1995, beekeepers have observed the death of numerous honeybees and a sharp decrease in honey production in France. Numerous reports confirm this pollinator weakening in France (9, 10), but such problems appeared rapidly to be shared by European countries as well (11). Little is known about other countries where surveys are less effective than in more developed countries. The problem has worsened with the increasing use of the seed-dressing formulation of imidacloprid on sunflower, maize, and rape, in west European countries. From this, imidacloprid has been suspected of having harmful effects on honeybees (9, 12), whereas other factors such as varroa infestations or viruses development had to be studied as well.

Recently, parallel studies on imidacloprid showed that there were several levels of toxicity to honeybees. Acute toxicity is observed at toxicity levels from 3.7 to 40 $\mu\text{g}/\text{kg}$, according to

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a complex mechanism of action, involving at least two receptors subunits targeted by imidacloprid (13, 14). Mortality reaching 50% occurs after a chronic ingestion during 10 days of imidacloprid at levels between 0.1 and 10 $\mu\text{g}/\text{kg}$ (12, 15, 16). This has also been depicted for the several metabolites of imidacloprid including the olefinic, monohydroxy, and dihydroxy derivatives. Finally, sublethal toxicity is observed starting at the level of 1 $\mu\text{g}/\text{kg}$. It appears also that the observed toxicity for honeybees depends on the protocol of exposure and on the characteristics of the honeybees, but the crucial levels are clearly defined in the 1–10 $\mu\text{g}/\text{kg}$ concentration range or below (12, 13, 17).

Another aim of the research was to develop modern analytical methods allowing a high sensitivity at the $\mu\text{g}/\text{kg}$ level (18). Several methods were available but were not suitable for pollen analysis (19–21). For a comprehensive approach of the imidacloprid behavior in fields, the analytical method needed to be efficient for soils, plants, flowers, and pollen from field samples. Therefore, we developed new HPLC/MS/MS methodologies to detect and quantify imidacloprid in such matrixes with a limit of detection (LOD) of 0.1 $\mu\text{g}/\text{kg}$ and a limit of quantification (LOQ) of 1 $\mu\text{g}/\text{kg}$ (22–24). Application of this method was first performed to determine the imidacloprid level in pollen of sunflower to evaluate the corresponding risk for honeybees. This HPLC/MS/MS method permitting quantification of imidacloprid at the $\mu\text{g}/\text{kg}$ level was also adopted by the manufacturer.

Our previous studies (22–26) had shown that imidacloprid is found in the flowers and pollen of imidacloprid treated sunflowers with an average concentration known to induce mortality effects with respect to the levels corresponding to a chronic intoxication. The averaged concentrations of imidacloprid found in flowers and in pollen were respectively at 8 and 3 $\mu\text{g}/\text{kg}$ (25, 26). Note that the use of imidacloprid (formulated as Gaucho) for sunflower crops was temporary suspended in 1999 (for 2 years), then in 2001 (for 2 years), and again in 2004 for an additional 3 years by the French government (27, 28).

Finally, it was also established that, in France, 20–40% of the honeybee harvest during the whole flowering period is constituted of maize pollen when available (10). According to the bio-availability and the consumption of pollen by honeybees, maize pollen could also lead to harmful effects. Thus, the situation for maize crops must be evaluated as well because maize is one of the major cereals in Europe. To date, very little is known about the activity of imidacloprid in fields situations except for its behavior in soils (29–33). The main aim of this study was to analyze various parts of the maize plant, including pollen for the first time, to evaluate the exposition to imidacloprid, through nutrition or by contact, for honeybees and other beneficial pollinators.

MATERIALS AND METHODS

Sampling Procedures. Plants and pollen were collected at the time of tasseling when pollen dehiscence occurs. This time frame was selected to ensure that honeybees would be attracted to the maize in high numbers. Thus, the concentration of imidacloprid measured in maize pollen corresponds to the real levels of exposure during foraging. Sampling was done randomly throughout France, by following a protocol matching the requirements of the Scientific and Technical Committee (CST), at the national level (10). Sampling was performed by a society specialized in field trials on honeybees for pesticide evaluation (Testapi, France). For each sample of plant and pollen, data concerning the field, soil, hygrometry, and weather were recorded on a sampling sheet. Furthermore, the description included the history of the field during the past 4 years (crops, variety, treatments) and of the surrounding areas.

From 8 to 15 beehives were located in the area or immediately on the border of maize fields where plants and pollen were sampled. Plastic traps were installed frontally at the entrance of two of these hives to collect pollen harvested by foraging bees. Because honeybees often forage a large surface around the hives, the composition of trapped pollen was evaluated and recorded. In our field situations, maize pollen constituted about $1/3$ of the total amount of the trapped pollen harvested by honeybees.

Samples. Stems, leaves, panicles, and pollen were collected from maize fields. Samples were taken in the middle of maize fields to avoid edge effects. Panicles pollen were taken from whole plants at tasseling. Trapped pollen were also collected according to the protocol validated by the CST (10). The good health of bee colonies has been checked before the harvest of trapped pollen. For each sample, 10 g (or more) was collected and sealed twice in plastic bags. Samples were often duplicated for verification. All collecting instruments (gloves, scalpels, funnels, ...) were disposable or carefully rinsed after each sampling. Samples were quickly put in a cooler, transported, and stored in a freezer with respect to temperature management in the cold chain. All samples were maintained at -24 °C in the dark until sample analysis was undertaken.

Samples from imidacloprid treated maize were compared to maize grown (i) organically and (ii) on farms using insecticides but not imidacloprid. In the second case and due to the long imidacloprid persistence in soils (32), care was taken to select untreated crops not only for the current crop year but for at least 3 previous years (34).

Analytic Procedures. HPLC grade solvents were used. Imidacloprid, $\text{C}_9\text{H}_{10}\text{ClN}_3\text{O}_2$ molecular mass: 255.7 g/mol. Imidacloprid standard (purity 99.4%) was supplied from Bayer AG (Leverkusen, Germany). Antipyrine (purity >99%) was used as an external standard and was purchased from SIGMA Chemical Corporation (St. Quentin Fallavier, France). Solid-phase extraction was performed using an Isolut 50 mg MFC₁₈-3 mL (IST, UK).

Extractions. Depending on the matrix, two extraction schemes starting from 10 g of material were used as previously described for sunflower (22). One scheme corresponds to stems, leaves, flowers, and flowers pollen, whereas the second corresponds to trapped pollen. Plants and flower pollen were homogenized at room temperature and then extracted using a procedure (method A) that was different from the procedure used for trapped pollen (method B). From the supernatant, 20 μL was injected in HPLC for method A and 25 μL for method B.

Equipment. The LC system was a Perkin-Elmer (Framingham, USA). It was fitted with a C18 Supelcosil ABZ + (150 mm \times 4.6 mm) from Supelco Park, PA. The MS system consisted of a standard atmospheric-pressure-ionization source configured as APCI (22).

Quality Control. GLP were followed to ensure accuracy and quality of the experimentation including all collecting and analytical procedures. This was also done in accordance with the European Directive 96/23/EC (35) regarding a confirmatory method and quality criteria, ensuring results be used at the expertise level for national and European monitoring. Applying these criteria led to a limit of detection of LOD = 0.1 $\mu\text{g}/\text{kg}$ for stems, leaves, and panicles. LOD = 0.3 $\mu\text{g}/\text{kg}$ for pollen. The limit of quantification is LOQ = 1 $\mu\text{g}/\text{kg}$ for all matrixes (22).

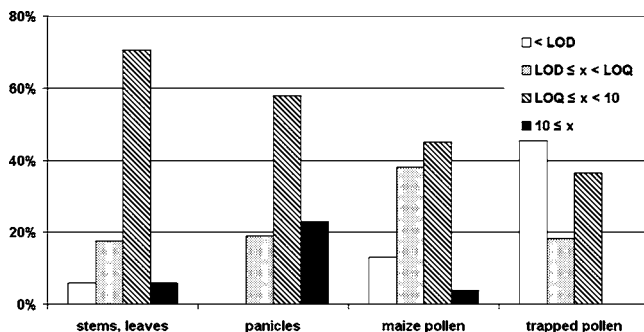
RESULTS

Maize Analysis. Three different parts of maize were sampled from fields treated with the commercial imidacloprid (Gaucho formulation at 1 mg/seed) the year of the sampling: stems and leaves, panicles, and pollen. Thus, it was possible to compare the distribution of imidacloprid in these parts of maize at tasseling. When samples came from organically farmed areas (3 panicles and 2 pollen) or areas not treated with imidacloprid in the last 3 years (3 pollen), imidacloprid was not detected. This procedure allowed us to check the absence of external contamination, the good quality of the sampling procedure, the consistency of the whole analytical process, and the absence of artifact detection.

Table 1. Distribution of the *n* Samples from Maize Fields According to Their Imidacloprid Content (*x*), Classified with Respect to the Analytical Limits

| matrix | <i>n</i> | $x < \text{LOD}^a$ | $\text{LOD} \leq x < \text{LOQ}^b$ | $\text{LOQ} \leq x$ |
|------------------|----------|--------------------|------------------------------------|---------------------|
| stems and leaves | 17 | 1 | 3 | 13 |
| panicles | 48 | 0 | 9 | 39 |
| maize pollen | 47 | 6 | 18 | 23 |
| trapped pollen | 11 | 5 | 2 | 4 |

^a Limit of detection (LOD) = 0.1 $\mu\text{g}/\text{kg}$ for stems, leaves, and panicles; LOD = 0.3 $\mu\text{g}/\text{kg}$ for pollen. ^b Limit of quantification (LOQ) = 1 $\mu\text{g}/\text{kg}$.

**Figure 1.** Distribution (%) of imidacloprid levels in the set of maize samples (Gaucho treated) at the tasseling. LOQ = 1 $\mu\text{g}/\text{kg}$; LOD = 0.1 $\mu\text{g}/\text{kg}$ for stems, leaves and panicles; LOD = 0.3 $\mu\text{g}/\text{kg}$ for pollen. The average levels are 6.6 $\mu\text{g}/\text{kg}$ for stem and leaves, 4.2 $\mu\text{g}/\text{kg}$ for panicles, 2.1 $\mu\text{g}/\text{kg}$ for maize pollen, and 0.6 $\mu\text{g}/\text{kg}$ for trapped pollen.**Table 2.** Imidacloprid Average Concentrations (ξ) with Standard Deviations (σ) and Variance (var.) Found in Each Matrix from Maize Treated with the Commercial Imidacloprid Seed-Dressing (Mean Levels from Maize Fields Are Compared to Results from Sunflowers Fields (23, 26); Samples (*n*) Were Collected at Time of Tasseling (Maize) or Flowering (Sunflower))

| matrix | <i>n</i> | maize | | | sunflower |
|-----------------------------|----------|-----------------------------------|----------|------|-----------------------------------|
| | | ξ ($\mu\text{g}/\text{kg}$) | σ | var. | ξ ($\mu\text{g}/\text{kg}$) |
| stems and leaves | 17 | 4.1 | 0.8 | 0.6 | 4.6 |
| panicles/flowers | 48 | 6.6 | 5.6 | 31.8 | 8.0 |
| plant pollen | 47 | 2.1 | 2.7 | 7.1 | 3.0 |
| trapped pollen ^a | 11 | 0.6 ^b | 1.0 | 1.0 | 3.0 ^c |

^a Trapped pollen are pollen harvested by honeybees and collected at the beehive entry. ^b Trapped pollen on maize fields were 20–40% constituted from maize origin. ^c Trapped pollens on sunflower fields were 90–100% constituted from sunflower origin.

Stem and Leaves. Although pollen analysis was our main objective, we conducted a survey of the imidacloprid content in maize. Stems and leaves mixed together ($n = 17$) were sampled at the tasseling and then analyzed. The results showed that imidacloprid was lower than 0.1 $\mu\text{g}/\text{kg}$ (LOD) in a single sample (Table 1). Concentrations between 0.1 and 1 $\mu\text{g}/\text{kg}$ (LOQ) were found in only three samples. Imidacloprid was found between 1 and 10 $\mu\text{g}/\text{kg}$ in 12 samples (71%), and the level reached more than 10 $\mu\text{g}/\text{kg}$ in the last sample (Figure 1). From this, the average value is 4.1 $\mu\text{g}/\text{kg}$, and it appeared rather constant over the 4 years of sampling (from 2000 to 2003). The standard deviation on this data set is 0.8, and the variance is 0.6 (Table 2).

Flowers (Panicles). A set containing 48 male flowers of maize was collected from areas treated with the commercial formulation of imidacloprid (seed-dressing). In panicles, imidacloprid was always detected (Table 1). Figure 1 shows its distribution according to the concentration ranges. Imidacloprid

was detected but not quantified in 19% of the flowers ($\text{LOD} < x < \text{LOQ}$). A majority of flowers (58%) exhibited concentration between 1 and 10 $\mu\text{g}/\text{kg}$. Finally, 23% of flowers had a concentration above 10 $\mu\text{g}/\text{kg}$ with a maximum of 33.6 $\mu\text{g}/\text{kg}$. Imidacloprid is then always present in maize panicles when the crop has been treated, and the average value is of 6.6 $\mu\text{g}/\text{kg}$. Due to the great variability of the samples in term of soil composition, maize variety, and climate conditions, it was not surprising that the standard deviation, on this data set, reaches 5.6 (Table 2). Obviously, this high value does not mean a lack of analytical accuracy, but shows that field situations can be very different from one area to another.

Pollen from Maize Flowers. For this study, a set of 47 samples of pollen from treated maize was analyzed (Table 1). Here, 87% of the samples contained imidacloprid ($\text{LOD} = 0.3 \mu\text{g}/\text{kg}$). More precisely, 38% of the maize pollen contained a concentration between 0.3 (LOD) and 1 $\mu\text{g}/\text{kg}$ (LOQ), and 45% ranged between 1 and 10 $\mu\text{g}/\text{kg}$ (Figure 1). Only 4% of the maize pollen contained a concentration above 10 $\mu\text{g}/\text{kg}$ with a maximum of 18 $\mu\text{g}/\text{kg}$. Imidacloprid is then present in the flower pollen of treated maize with an average level of 2.1 $\mu\text{g}/\text{kg}$, whereas the standard deviation at 2.7 reflects again heterogeneous cases (Table 2).

Maize Trapped Pollen. A set of 11 trap pollen was sampled from hives placed near treated maize crops. These results show that 45.5% of the pollen was lower than 0.3 $\mu\text{g}/\text{kg}$ (LOD), 18% ranged between 0.3 and 1 $\mu\text{g}/\text{kg}$ (LOQ), and 36% ranged between 1 and 10 $\mu\text{g}/\text{kg}$. It can be mentioned that no sample exhibited concentrations above 10 $\mu\text{g}/\text{kg}$ (Figure 1). Globally, trap pollen contained a significant concentration of imidacloprid in 54% of samples. Here, the average level of the toxicant is 0.6 $\mu\text{g}/\text{kg}$. This value is significantly lower than that of maize pollen directly sampled from flowers (Table 2). Yet microscopic examination of the trap pollen often showed that it was a mixture of maize pollen (treated) and a majority of pollen from other origins (untreated surrounding crops). Consequently, the imidacloprid content was reduced (about 3-fold lower) when compared to pollen collected directly on maize flowers.

DISCUSSION

The use of imidacloprid as seed-dressing induces residual contamination of soils (32, 36, 37). This long persistence in soils has already been depicted and can lead to the recovery of imidacloprid by the next crops. In some cases, such recovery has been determined for sunflower crops during 2 years following the treatment (18, 22). The recovery can be also effective for maize crops (23).

Considering the level of toxicant measured on treated maize (Table 1), the systemic properties of imidacloprid vary according to the variety of maize, the soil composition, and the climate. However, our results show relatively important concentrations of imidacloprid in leaves, flowers, and pollen because averaged levels were respectively determined at 4.1, 6.6, and 2.1 $\mu\text{g}/\text{kg}$. It has to be noted that pollen is of critical importance regarding foraging bees and the food supply of the beehives. Generally, maize pollen represents from 20% to 40% of the protein supply of the beehive. In this respect, it has to be mentioned that only the extreme concentrations in flowers (33.6 $\mu\text{g}/\text{kg}$) and in pollen (18 $\mu\text{g}/\text{kg}$) correspond to the toxicity limit (NOEC: no observed effect concentration) admitted by the chemical manufacturer at 20 $\mu\text{g}/\text{kg}$ (38).

The averaged concentrations of imidacloprid in maize can be compared to those determined in sunflowers, 8 $\mu\text{g}/\text{kg}$ in flowers, 3 $\mu\text{g}/\text{kg}$ in pollen (23, 26), and 1.9 $\mu\text{g}/\text{kg}$ in nectar

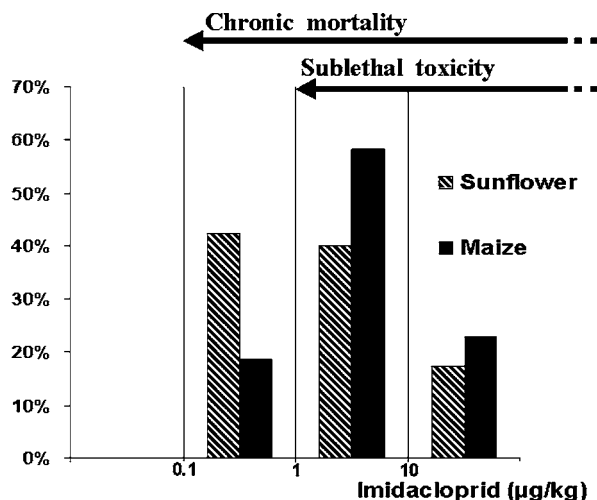


Figure 2. Distribution (%) of imidacloprid levels ($\mu\text{g}/\text{kg}$) in flowers (maize and sunflower) at the time of foraging. An illustration of the domains corresponding to the chronic mortality (lethality following chronic exposure) and the sublethal toxicity is reported according to concentration levels inducing these effects on honeybees (8, 10, 12).

(39), and those determined in rape, 4.4–7.6 $\mu\text{g}/\text{kg}$ in pollen and 0.6–0.8 $\mu\text{g}/\text{kg}$ in nectar (40). A similar situation is thus found between maize and some other major crops, suggesting that the need of efficiency of the active compound is accompanied by such residual residue levels at flowering or tasseling.

In the particular case of maize, pollen samples from traps (beehives) show a 3-fold lower concentration of imidacloprid in comparison to pollen sampled directly from flowers. This is of great importance for evaluation of exposure of the bee colony. The averaged value at 0.6 $\mu\text{g}/\text{kg}$ is clearly explained by the mixture with pollen coming from nontreated crops, which constituted the surrounding area. Actually, the trapped pollen contains between 20% and 40% of pollen from maize origin (10), which is consistent with our measurements. If trapped pollen consisted only of pollen from treated maize, no differences with flower pollen would have been observed, as is the case for sunflowers (Table 2).

Pollen constitutes the only resource of proteins for the beehive. The contamination of pollen can induce both contact and oral-intoxication. The highest risk for honeybees is ingestion of imidacloprid, but during the foraging on flowers, bees are often covered with pollen. This contamination by contact must not be neglected. Moreover, bees and larvae feeding in the beehive are exposed to these levels of toxicant, directly but also with a delay, that is, each time honeybees use the stocked pollen, especially during winter and the spring takeoff of the beehive.

Even if maize contamination does not result in acute toxicity, due to heterogeneous situation in fields, sublethal effects and chronic mortality must be taken into consideration. In these conditions, it was pertinent to evaluate the global average of these concentrations because a lot of parameters induce great variance. Of course, results are heterogeneous because the aim of this study focused on the global situation by using random samples. Figure 2 depicts the situation in maize fields by comparing the level of imidacloprid in pollen and the toxicity levels. Thus, 87% of maize pollen sampled in this study could induce sublethal effects or chronic mortality. The situation appears still worrisome for the trapped pollen for which this proportion is 55%.

This study shows a global situation in France and defines a database for ecological risk calculations, especially for the

evaluation of the risk of exposure for honeybees. As a reference tool, the PEC/PNEC ratio is used to assess this risk (probable exposition concentrations/predicted no effect concentration). It has to be noted that a PEC/PNEC ratio reaching the value of 1 indicates an effective risk; the higher is the ratio, the higher is the risk. Because PNEC was previously calculated by the French expert committee (10), we can extract the PEC counterpart from the present work. As mentioned above, regarding acute toxicity, the data show no evidence of hazard. However, in the cases of sublethal intoxications and chronic mortality, PEC/PNEC values are largely over 1. For instance, considering only a daily individual consumption of 6 mg of pollen, the PEC/PNEC ratio is 20–30 for sublethal effects (on 4 days) and 500–600 for the chronic mortality over 10 days (26, 41). Such conclusions regarding sublethal effects and chronic mortality were recently confirmed, with similar PEC/PNEC values by an extensive risk evaluation of imidacloprid toward honeybees (10). Obviously the situation is worse when the risk calculation includes also the consumption and the storage of contaminated nectar. At this point, it appears that imidacloprid levels measured in maize pollen is one of the major factors contributing to the weakening of bee colonies. Together with the imidacloprid levels found previously in pollen and nectar of other crops, as well as results for other competitor insecticides, our data support the hypothesis that systemic insecticides are largely involved in the depopulation of European honeybees since the mid 1990s. However, in terms of MRL (maximum residue level) and DAI (daily admissible intake), the imidacloprid levels in major crops (maize, sunflower, rape) appeared to fit the actual contamination requirements, unless long-term side effects could be discovered at the $\mu\text{g}/\text{kg}$ level.

Finally, by using sensitive LC/MS/MS methods, the active compound imidacloprid was detected in most of the field samples constituting our large set of maize crops which had been treated with the Gaucho seed-dressing. This confirms the high systemic character of imidacloprid in maize because flowers and pollen were found to be contaminated at the level of a few $\mu\text{g}/\text{kg}$. In this respect, the behavior of the active compound in maize is similar to that in sunflower (22) and rape (40). Heterogeneous concentrations of imidacloprid in the different parts of maize suggest a preferred location of the toxicant in leaves, flowers, and corn. Further studies could provide a more detailed description of levels in the maize parts corresponding to the animal food chain supply.

Imidacloprid in maize pollen is rather heterogeneously distributed. The average level is 2.1 $\mu\text{g}/\text{kg}$, while values ranged from 0.3 to 18 $\mu\text{g}/\text{kg}$. Evaluation of the risk for honeybees, based on the calculation of the PEC/PNEC ratio, leads to values far greater than 1. Assuming that honeybees consume a maximum of 6 mg maize pollen per day, the PEC/PNEC ratio reaches 500–600 (26, 41). The PEC/PNEC ratio is still between 100 and 200 for trap pollen, that is, when maize pollen is the only source of contamination mixed with other pollen free of imidacloprid (a realistic case because maize represents often a third of the pollen source). It has to be noted that heterogeneity in contamination levels leads to heterogeneity of the risk, thus explaining why some field areas may or may not induce chronic mortality of pollinators.

Our data suggest that there is no accumulation of imidacloprid in maize when the field receives the seed-dressing treatment during several consecutive years. This situation differs from that of soils, because we have shown that the level of the toxicant present at the end of the cultivation (after harvesting) slightly increases over the years (34, 42). That is why even if the use

of Gaucho seed dressing has been suspended in France for sunflower since 1999 (27) and for maize in 2004 (43), the behavior and the persistence of imidacloprid (and its metabolites) should be the object of a continuous survey, especially regarding its ecological impact and its probable presence in water resources.

ACKNOWLEDGMENT

We thank F. Westall and A. L. Thomann for their help in this project. Raw data are available on request from the corresponding author.

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Received for review December 8, 2004. Revised manuscript received April 22, 2005. Accepted May 2, 2005. This research was performed for the French Ministry of Agriculture with financial support of the EC 917/2004 program.

JF0479362