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Original article

Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees[1]

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Abstract – The hazard posed to honeybees by systemic insecticides is determined by toxicity tests that are designed to study the effects of insecticides applied on the aerial parts of plants, but are not adapted to systemic substances used as soil or seed treatments. Based on the available data found in the literature, this paper proposes modes of honeybees exposure to systemic insecticides by estimating their pollen and nectar consumption. Estimates are given for larvae and for the categories of adults which consume the highest amounts of – pollen, the nurse bees, and – nectar, the wax-producing bees, the brood attending bees, the winter bees, and the foraging bees. As a case study, we illustrate these estimates with the example of imidacloprid because its concentrations in sunflower nectar and in sunflower and maize pollens of seed-dressed plants have been precisely determined, and because its levels of lethal, sublethal, acute, and chronic toxicities have been extensively investigated.

***Apis mellifera* / systemic insecticide / exposure / imidacloprid / nectar / pollen**

1. INTRODUCTION

To be launched on the market, pesticide products need to be granted an authorisation. In this process, tests are required to ensure that these chemicals do not present any harm to pollinators, in particular to honeybees. In Europe, these tests follow the EPPO No. 170 guideline, adopted by the European Plant Protection Organisation (EPPO, 2001). These tests present methods for studying the toxicity and hazard of pesticides to honeybees in laboratory, semi-field (cages or tunnels) and field conditions. This toxicity corresponds to the single dose of insecticide, administered by ingestion or contact that kills half of a treated group of bees in 24 h or 48 h (LD_{50}, expressed in weight of active ingredient per bee).

The toxicity risk of pesticides is commonly estimated with the Hazard Quotient (HQ = application rate/ LD_{50}, EPPO, 2001). This quotient is adapted to pesticides sprayed on plants (i.e. carbamates, organophosphates, organochlorates, pyrethroids), but not to those applied to soil or seeds. Most pesticides sprayed on the surface of the plant have a rapid and residual action of a few hours to a few days, whereas systemic insecticides penetrate into the plant, including melliferous and polleniferous plants, and protect it all through its development from soil invertebrates and in some cases from sucking insects (Elbert et al., 1991). The relevant

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parameter to consider for examining honeybees exposure to the active substance is the contamination of nectar and pollen instead of the application dose to soil or seeds. Therefore, in the case of systemic insecticides, the official HQ should not be used. Moreover, the regulatory testing guidelines do not take into account the potential persistence of these molecules in plants and do not specify which laboratory tests should be performed to estimate the possible lethal or sublethal impacts on bees due to chronic exposures occurring after several days of repetitive ingestion of (or contact with) a given insecticide.

Insecticides sprayed on plants can be toxic to foraging honeybees when they are in contact with treated plants (Koch and Weisser, 1997) and when they fly through the adsorption of contaminated dust particles (Prier et al., 2001). Honeybees can also intoxicate the whole colony by bringing contaminated pollen and nectar back to the hive (Bos and Masson, 1983; Villa et al., 2000). However, the risk of any chemical transfer into the hive is greater with systemic insecticides (Waller et al., 1984). Moreover, in comparison with the majority of the insecticides of the old generation, the toxicities of these new molecules and their metabolites are very high, and although they are detected in pollens and nectars at low concentrations (Schmuck et al., 2001; Bonmatin et al., 2003), their hazard on bees might not be negligible. When honeybees consume small amounts of pesticides, they might exhibit sublethal toxic effects. Such impacts might affect honeybees by disrupting their cognitive capacities (i.e. the learning and orientation abilities) and behaviours (i.e. the collection of food). In such conditions, a forager might not be able to return to the hive and, as it relies on the colony for its survival, might die within a few hours. Therefore, the initial sublethal effect might eventually become lethal to honeybees.

Among systemic insecticides, one neurotoxic molecule, imidacloprid, acting on nicotinic receptors, is widely used. It is commonly used as a seed treatment (formulation Gaucho®) for the protection of maize and sunflower crops.

For the last few years, honeybees have been dying in huge numbers and colonies have been declining dramatically, in particular in regions where large areas of sunflower crops are treated by systemic insecticides (Belzunces and Tasei, 1997). Beekeepers and scientists suspect that these chemicals are responsible for these troubles (Vermandère, 2002; Bonmatin et al., 2003). Considering the essential role of honeybees in honey production and pollination (Williams, 1994), this lack of assessment poses a serious problem that needs to be quickly solved.

The objective of this study was to describe different possible modes of honeybees exposure to systemic insecticides by estimating their individual consumption in contaminated pollen and nectar. To achieve this goal, we used the available data presented in the literature to estimate the total amount of pollen and nectar consumed by different categories of honeybees, and to determine their possible exposure to systemic insecticides when all the food consumed is contaminated by these molecules.

As an example, we considered the case of imidacloprid because its concentrations in pollens of Gaucho® seed-dressed sunflower and maize plants and in nectar of Gaucho® seed-dressed sunflower plants are known, and because its toxicity to honeybees has been determined (Schmuck et al., 2001; Bonmatin et al., 2001, 2002; CST, 2003).

2. AMOUNT OF FOOD BROUGHT BACK TO THE HIVE

Honeybees supply the colony with nectar and pollen collected at varying distances from the hive. Currently, it is considered that 95% of the foraging activity of honeybees extends up to 6 km away from the hive, but honeybees might forage up to 12 km from the hive (von Frisch, 1967; Seeley, 1985; Winston, 1987), which implies that they might visit plants over large areas of several tens of km^2.

2.1. Pollen

Pollen foragers collect pollen on flowers and bring it back to the colony by making and carrying on their posterior legs two pollen pellets. The amount of pollen collected per colony and per year is in the range of a few tens of kilos to about 55 kg (Louveaux, 1968; Seeley, 1985; Winston, 1987). This pollen is composed of a mixture of different plants present in variable numbers, reflecting the floral composition of

Table I. Estimated amounts of sugar (contained in nectar or honey), pollen and imidacloprid consumed by larvae during their development over N days (N = 5 days for workers and N = 6.5 days for drones) and by adults over a period of N days of activity (N = 10 days for nurses, N = 6 days for wax producing bees, N = 8 days for brood attending bees, N = 90 days for winter bees and N = 7 days for foraging bees). The amount of imidacloprid consumed by honeybees is determined by the following equivalence: 1 mg of sugar contained in nectar or honey = 4.75 pg of imidacloprid and 1 mg of pollen = 3.4 pg of imidacloprid in nectar and pollen coming from Gaucho® seed-dressed plants. N.A. = no data available.

Categories of bees		Estimated amounts of food (sugar and pollen) and imidacloprid consumed per bee over N days		
		Sugar (mg)	Pollen (mg)	Imidacloprid (ng)
Larvae	Workers	59.4	5.4	0.3
	Drones	98.2	(N.A.)	0.5
Hive bees	Nurses	–	65	0.2
	Wax-producing bees	108	–	0.5
	Brood attending bees	272–400	–	1.3–1.9
	Winter bees	792	–	3.8
Foraging bees	Nectar foragers	224–898.8	–	1.1–4.3
	Pollen foragers	72.8–109.2	–	0.3–0.5

the environment of the hive. In areas of extensive cultures of polleniferous crops, large amounts of pollens coming from these plants might be brought back to the colony. For example, honeybees can collect 10 to 20 kg of sunflower or maize pollen per year, and sometimes even more (Odoux et al., 2004). During the flowering time of these plants, which lasts between 1 and 1.5 months, sunflower and maize pollens can represent up to 80–90% of the total weight of all pollen types collected by honeybees (Odoux et al., 2004).

2.2. Nectar

Nectar foragers bring nectar back to the colony, which might either be quickly consumed or consumed later after being transformed into honey by water evaporation and by changes in sugar composition. Nectar, depending on its floral origin, contains between 5–80% of sugar and honey contains in average 80% of sugar (Crane, 1975). For sunflower plants, nectar contains on average 40% of sugar (Pham Delègue and Bonjean, 1983). Therefore, if the annual honey requirement for a honeybee colony is about 60–80 kg (Seeley, 1985; Winston, 1987), the total amount of nectar collected by bees each year might be in the range of a few hundreds of kilos per colony.

As we do not know the bees' differential consumption of nectar and honey, we related their sugar consumption depending on whether they consume nectar or honey. With the example of sunflower, when a honeybee requires 1 mg of sugar, it will have to consume either 2.5 mg of fresh sunflower nectar or 1.25 mg of sunflower honey.

3. NECTAR AND POLLEN CONSUMPTION

In the literature, some estimates of honeybees consumption of pollen and nectar are available. These estimates are presented below and summarised in Table I.

During their development, honeybees go through two stages during which they feed: the larval and adult stages. They utilize the proteins contained in pollen to insure their development and growth, and they require the sugar contained in nectar (or honey) to cover their energetic expenses.

Larvae consume royal jelly, produced by nurses, which contains honey and pollen (Haydak, 1943, 1968, 1970; Kunert and Crailsheim, 1988; Malone et al., 2002).

Among adult honeybees, nurses consume pollen during the first 8 to 10 days of their life, to develop their hypopharyngeal and mandibular glands and to produce some of the larval food (Maurizio, 1954; Crailsheim et al., 1992; Hrassnigg and Crailsheim, 1998a). However, under certain circumstances, they might consume pollen until the age of 18 days (Hrassnigg and Crailsheim, 1998b).

Adult honeybees consume nectar to perform various tasks. Among these tasks, some require more energy and sugar than others.

Maximal exposure to systemic insecticides are expected among honeybees that consume the greatest amounts of contaminated pollen and nectar. Large amounts of pollen are consumed by nurses, and to a less extent by larvae, whereas large amounts of nectar are consumed by wax-producing bees, brood attending bees, "winter" bees, and foragers. In this study, we focused only on these categories of honeybees.

3.1. Worker larvae

A worker larva is fed over 5 days and after this feeding stage weighs, on average 150 mg (Jay, 1963). During the first 3 days of its development, it consumes about 30 mg of food (Nelson, 1924), and during the next 2 days, about 120 mg. The latter estimate is based on the results of Bishop (1961), who demonstrated that most of the food consumed by the larva contributes to its gain of weight within these 2 days.

The sugar content of the food of a worker larva is 18% during the first 3 days, and 45%, during the following 2 days (Planta, 1888 cited by Haydak, 1968). Therefore, a worker larva will consume a total of 59.4 mg of sugar in 5 days; that is, 5.4 mg of sugar within the first 3 days and 54 mg of sugar within the last 2 days. It will also consume 5.4 mg of pollen between the 3rd and 5th day of its development (Babendreier et al., 2004).

3.2. Drone larvae

Drone larvae are fed over 6.5 days and after this feeding stage weigh on average 340 mg (Jay, 1963). The development of a drone larva has a similar growth pattern to that of a worker larva (Thrasyvoulou and Benton, 1982). The precise amount of food consumed by a drone larva is not known, but it might be deduced from that of a worker larva.

The sugar content of the food of a drone larva is 9.6% during the first 3 days of its development and 38.5% during the following 2 days (Planta, 1888 cited by Haydak, 1968). Therefore, during the first 5 days of its development, a drone larva will consume a total of 49.1 mg of sugar, 2.9 mg within the first 3 days and 46.2 mg over the next 2 days. During the last 1.5 days, the amount of food consumed by a drone larva is probably the same as the amount consumed earlier, since a drone larva increases its weight by a factor of two during this short period of time. Therefore, a drone larva will consume a total of about 98.2 mg of sugar in 6.5 days. The pollen consumption of drone larvae has never been determined.

3.3. Nurse bees

Within a period of 10 days, the total amount of pollen consumed by a nurse bee is on average 65 mg (Pain and Maugenet, 1966; Crailsheim et al., 1992). However, during this period, honeybees could consume up to 12 mg of pollen within one single day (Pain and Maugenet 1966; Crailsheim et al., 1992).

3.4. Wax-producing bees

The production of wax by a honeybee colony varies greatly, depending on various factors (i.e. blossoming, nectar flow, season, outside temperature, number of young wax-producing bees, gathering of nectar and pollen, etc.) (Hepburn, 1986). However it is generally accepted that the amount of sugar consumed per unit weight of beeswax produced is on average 6:1, notwithstanding racial, seasonal, and colony density variations (Tokuda, 1955; Hepburn et al., 1984). Over the period of maximum wax production, lasting about 6 days, a wax producing bee produces 3 mg of wax per day (Taranov, 1959; Hepburn et al., 1984), requiring 18 mg of sugar per day, or a total of 108 mg of sugar in 6 days.

3.5. Brood attending bees

From April to October, brood attending bees require energy to maintain the brood temperature at about 34 °C (Simpson, 1961; Seeley and Heinrich, 1981; Heinrich, 1985). During this period and in temperate climates temperatures average 15–20 °C, outside the hive. In such conditions, a brood attending bee will consume between 34 mg (at 20 °C) and 50 mg (at 15 °C) of sugar per day (Free and Spencer-Booth, 1958; Simpson, 1961) and a total of 272–400 mg of sugar over the entire brood attendance period, lasting about 8 days.

3.6. Winter bees

In temperate regions, "winter" bees require energy to maintain the nest temperature at 5–8 °C (in the periphery) and 15–20 °C (in the centre) (Winston, 1987). During winter, lasting about 3 months in temperate regions, a honeybee colony composed of about 20,000 of bees will consume on average 20 kg of honey (Farrar, 1952, 1960; Johansson and Johansson, 1969). Therefore, a "winter" honeybee requires about 8.8 mg of sugar per day (equivalent to 11 mg of honey) and a total of about 792 mg of sugar over the entire winter period. This average is a broad estimate which does not take into account any natural variations (periods of low consumption alternated with periods of high consumption in relation to external temperature variations) that might occur during this long period.

3.7. Pollen and nectar foraging bees

Pollen and nectar foragers require about 8–12 mg of sugar per hour of flight (Balderrama et al., 1992). Nectar foragers achieve 10 trips/day on average, of about 30 to 80 min each (Winston, 1987), with a maximum of 150 trips/day (Ribbands, 1953), and pollen foragers achieve 10 trips/day on average, of 10 minutes each (Winston, 1987). If we assume that during 1 h of activity foragers spend 80% of this time flying and 20% foraging, for which the energetic cost is not known, nectar and pollen foragers will spend between 4–10.7 hours/day and 1.3 hours/day, respectively, for flight activities alone. The lifetime of a forager is highly variable and related in particular to its foraging intensity and activity (Winston, 1987), but on average might vary between one and three weeks. To realise their flights, nectar and pollen foragers will consume between 32–128.4 and 10.4–15.6 mg of sugar per day or a total of 224–898.8 and 72.8–109.2 mg of sugar per week, respectively.

During flights, foragers might perform stationary flights, which are energetically very costly (Nachtigall et al., 1989), but the time spent in these flights is not known.

While collecting pollen or nectar, foragers get their body covered by pollen (Parker, 1981). Foragers are also in contact with pollen while making and carrying pellets back to the hive (Louveaux, 1958). Therefore, a topical exposure of foragers to contaminated pollen cannot be excluded, though it is difficult to estimate.

4. THE EXAMPLE OF IMIDACLOPRID

4.1. Pollen and nectar contamination by imidacloprid

4.1.1. Pollen

Pollen contamination by imidacloprid can be determined in two types of pollens: the pollen present in flowers and collected by foragers, and the pollen pellets harvested by beekeepers in pollen traps.

- *Pollen collected on flowers*: the level of contamination of this type of pollen is related to the systemic property of the molecule. In Gaucho® seed-dressed sunflower and maize plants it is about 3.4 µg of imidacloprid per kilo of pollens (Bonmatin et al., 2001; Schmuck et al., 2001).
- *Pollen pellets in traps*: pollen traps are installed at the hive entrance to catch some of the pollen pellets brought back by pollen foragers. For this reason, the pollen sampled in pollen traps is a mixture of different kinds of pollens collected in the foraging area of honeybees. The level of contamination found in pollen pellets varies in relation to the environment of the colony where they are collected (Charvet et al., 2003). If this environment contains many plots of treated plants, the level of contamination found in

the pollen pellets will reach that of the pollens collected in treated flowers. In contrast, if this environment contains few treated plots, the mean concentration of insecticides found in pollen pellets will be lower. For example, the concentrations of imidacloprid found in sunflower and maize pollens collected in the pollen traps of some particular hives were 2.2 and 0.75 µg/kg, respectively, or about 1.5 and 4.5 times less, respectively, than the concentration of imidacloprid found in the same types of pollen collected in flowers (Bonmatin et al., 2001, 2002). Therefore, the level of contamination found in pollen pellets cannot be generalised, whereas that of pollens collected on flowers gives a more accurate estimate of the maximal honeybees exposure to contaminated pollens.

4.1.2. Nectar and honey

To cover their energy requirements, honeybees consume either freshly collected nectar or stored honey.

- *Fresh nectar*: the level of contamination of nectar is directly related to the systemic property of the molecule. For example, in Gaucho® seed-dressed sunflower plants, it is 1.9 µg of imidacloprid per kilo of nectar (Schmuck et al., 2001), or 4.75 pg of imidacloprid per milligram of sugar contained in sunflower nectar.
- *Honey*: the level of contamination of honey by imidacloprid, with a limit of detection that is sufficiently low, has not been determined yet. However, the persistence of this molecule in acid environments (Agritox, 2004) and the low pH value of honey suggest that the imidacloprid contained in fresh nectar might not be degraded in honey at least over several months. Further investigation is required to confirm this assumption.

4.2. Estimated amounts of imidacloprid brought back to the colony through contaminated nectar and pollen

To determine the exact amount of imidacloprid brought back to the colony, it is necessary to know the total amount of contaminated nectar and pollen collected by honeybees. In the case of Gaucho® seed-dressed maize and sunflower plants, the annual quantity of imidacloprid brought back to the hive is 34 µg for every 10 kilos of sunflower or maize pollen and 19 µg for every 10 kilos of sunflower nectar brought back to the hive by honeybees.

However, previous studies tend to demonstrate that foraging bees reduce their visit to syrup feeders when they are contaminated by imidacloprid at concentrations of 3 µg/kg (Colin et al., 2004), 24 µg/kg (Decourtye, 2002), and 100 µg/kg (Kirchner, 1999). This phenomenon might be due to a decrease in the effectiveness of the dances produced by honeybees at the hive to recruit foragers for food collecting (Kirchner, 1999; Decourtye, 2002). Therefore, if honeybees visit treated plants, they might collect and bring back to the hive less nectar than if they visit untreated plants. In such conditions, the amounts of nectar and honey stored in the hive by honeybees should decrease, whereas the amount of pollen stored at the hive might not be affected. However, no experimental study has ever confirmed that this phenomenon occurs with nectar and pollen collected on treated plants.

4.3. Honeybees exposure to imidacloprid

Based on the estimated amounts of pollen and nectar consumed by honeybees over several days of activity, the potential amounts of imidacloprid ingested by honeybees can be determined (Tab. I). As the relative proportions of contaminated and uncontaminated food consumed by honeybees cannot be determined, we considered the case of a food that is 100% contaminated by imidacloprid. Such a case might occur in natural conditions (extensive treated cultures, e.g.), though lower exposure cases might also take place when honeybees consume a mixture of contaminated and uncontaminated food.

4.3.1. Worker larvae

If a worker larva is fed contaminated nectar and pollen, it will consume a total of about 0.3 ng of imidacloprid within the first 5 days of its development as follows: about 0.28 ng of imidacloprid through nectar and about 0.02 ng of imidacloprid through pollen.

4.3.2. Drone larvae

If a drone larva is fed contaminated nectar, it will consume about 0.5 ng of imidacloprid within the first 6.5 days of its development.

The total amount of pollen consumed by drone larvae is not known and, therefore, it is not possible to estimate the oral exposure of a drone larva to contaminated pollen.

4.3.3. Nurse bees

If a nurse bee feeds on contaminated pollen, it will consume up to a maximum of 40.8 pg of imidacloprid within one day of intensive feeding and a total of about 0.2 ng in 10 days.

4.3.4. Wax-producing bees

If a wax-producing bee feeds on contaminated nectar, it will consume 85.5 pg of imidacloprid per day during the period of maximum wax production, lasting about 6 days, or a total of about 0.5 ng in 6 days.

4.3.5. Brood attending bees

If a brood attending bee feeds on contaminated nectar, it will consume between 161.5–237.5 pg of imidacloprid per day or a total of about 1.3–1.9 ng in 8 days of brood attendance.

4.3.6. Winter bees

If a winter bee feeds on contaminated nectar, it will consume 41.8 pg of imidacloprid per day, or a total of about 3.8 ng during winter, lasting about 3 months.

4.3.7. Nectar and pollen foraging bees

If a nectar foraging bee feeds on contaminated nectar, it will consume 152–609.9 pg of imidacloprid per day or a total of about 1–4.3 ng per week of foraging activity.

If a pollen foraging bee feeds on contaminated nectar (for its flight energy requirement), it will consume 49.4–74.1 pg of imidacloprid per day or a total of about 0.3–0.5 ng per week of foraging activity.

5. DISCUSSION AND CONCLUSION

This paper highlights the potential hazard of systemic insecticides to honeybees through contaminated pollen and nectar. This phenomenon has previously been reported but never quantified (Villa et al., 2000). In this study, some estimates are given based on the available data found in the literature on pollen and nectar consumptions of different categories of honeybees. Assuming that this food is contaminated by systemic insecticides, the amount of insecticide consumed by each of these categories of honeybees and their potential exposure to these molecules can be estimated.

In regions of extensive cultures treated by systemic insecticides, honeybees might bring high amounts of contaminated pollen and nectar back to the colony. For example, in the case of sunflower and maize crops, which are attractive plants to honeybees, 10–20 kg of sunflower pollen and 10–20 kg of maize pollen might be stored at the hive every year during the flowering time of these plants (Odoux et al., 2004). Sunflower nectar is also known to be very attractive to honeybees, with honey production averaging at best 80 kg/year, corresponding to some hundreds of kilos of nectar brought back to the colony every year (Vermandère, 2002). However, since 1994, in regions of extensive sunflower cultures, sunflower honey yield has been dramatically declining (Belzunces and Tasei, 1997).

Honeybees might consume several milligrams of pollen (Pain and Maugenet, 1966; Crailsheim et al., 1992; Badendreier et al., 2004) and several tens of milligrams of nectar per day (Farrar, 1952, 1960; Free and Spencer-Booth, 1958; Simpson, 1961; Johansson and Johansson, 1969; Balderrama et al., 1992). Nurses, which require high amounts of protein for the development of their hypopharyngial and mandibular glands and to produce some of the larval food (royal jelly), might consume up to 65 mg of pollen in 10 days (Maurizio, 1954; Crailsheim et al., 1992; Hrassnigg and Crailsheim, 1998). Foragers, wax-producing bees and heat-producing bees, which perform high energetic tasks, require large amounts of sugar contained in nectar. With the example of sunflower plants, nectar foragers might consume 80–321 mg of nectar (equivalent to 32–128.4 mg of sugar) per day and between 560 mg

and 2.25 g of nectar (equivalent to 224–898.8 mg of sugar) in a week of foraging activity.

These estimates suggest that, if honeybee colonies are placed in environments containing melliferous and polleniferous crops treated by systemic insecticides, large amounts of insecticides might be brought back to the hive and thereafter consumed by colonies. These estimates are based on a case of a maximal exposure of honeybees to systemic insecticides; that is honeybees consuming pollen and nectar that are 100% contaminated by systemic insecticides. This relates to the case of colonies placed near extensive cultures treated by systemic insecticides. In regions of less extensive cultures, honeybees might consume a mixture of contaminated and uncontaminated food and therefore less systemic insecticides, but it is impossible to precisely estimate these amounts.

The estimated amounts of nectar and pollen consumed by different categories of honeybees allow the determination of the maximal amounts of insecticides consumed by each of these bees. In this paper, we illustrated these modes of exposure with the example of imidacloprid (formulation Gaucho®), but they might be used to describe the impact of any other systemic insecticides and their metabolites on honeybees, providing that their concentration in pollens and nectars are known. For example, the toxicity of the metabolites of imidacloprid (Suchail et al., 2001; Nauen et al., 2001; Decourtye et al., 2003), suggest that these molecules might also have an impact on honeybees.

In this paper, we focused on oral exposure of honeybees to systemic insecticides, but some possible modes of topical exposure also should be investigated. The data in the literature is insufficient to develop this type of exposure on different categories of bees, although larvae might be topically exposed through contaminated nectar. Larvae under 3 days old float in an excessive amount of food containing nectar (Haydak, 1970). If this nectar is contaminated by systemic insecticides, larvae might be exposed to these molecules by contact. Some new tests have been proposed to estimate the larvae exposure. Among them, field (Oomen et al., 1992) and semi-field (Leyman et al., 1999; Tornier, 1999) tests do not appear appropriate since larval exposure cannot be controlled, whereas laboratory tests (Malone et al., 2002; Brødsgaard et al., 2003) might be used to estimate adequately the larvae exposure.

The different modes of oral exposure presented in this paper, with the example of imidacloprid, might be used to determine the impact of other systemic insecticides on honeybees.

Gaucho® seed-dressed sunflower and maize plants contain on average 3.4 μg of imidacloprid per kilo of pollen. Nurses, which consume the highest amounts of pollen of any other category of honeybees, might be exposed to 0.2 ng of imidacloprid after 10 consecutive feeding days. In Gaucho® seed-dressed sunflower plants, nectar contains 1.9 μg/kg of imidacloprid and nectar foragers, might be exposed to about 0.15–0.61 ng of imidacloprid per day, or to 1.1–4.3 ng in a week of foraging activity.

The lethal toxicity of imidacloprid is in the range of a few picogramms after repetitive ingestions of this insecticide over a minimum period of 8 days (Suchail et al., 2001) to 3.7 ng after a unique ingestion of this insecticide in one or 2 days (Schmuck et al., 2001; Agritox, 2004). Imidacloprid might also induce sublethal effects that might affect bees. In particular, it might modify the learning and orientation abilities of honeybees at concentrations as low as 0.1 ng/bee (Guez et al., 2001) and 1.25 ng/bee (Lambin et al., 2001). However, these results might vary according to honeybees age (Guez et al., 2001), race (Suchail et al., 2000), colony (Suchail et al., 2001), and season (Decourtye et al., 2003).

When comparing the known toxicity doses for imidacloprid to the estimated amounts of imidacloprid consumed by different categories of honeybees, we find out that honeybees are potentially exposed to lethal and sublethal doses. However, it has to be kept on mind that our estimates are based on a case of maximal exposure that might reflect the case of colonies placed in regions of extensive treated cultures. Colonies placed in regions of less extensive treated cultures might, more probably, be exposed to sublethal doses. However, in this last situation, the impact of systemic insecticides on honeybees should not be underestimated since some sublethal effects may induce bee losses in particular if physiological troubles and disorientation of foragers are concerned.

This paper should give some input for the setting of a new risk assessment procedure adapted to these systemic molecules now widely used. In particular, European regulatory guidelines should provide a HQ and its specific threshold, adapted to systemic insecticides, along with test methods taking into account chronic and sublethal effects caused by low doses to honey bee adults and larvae. These new regulatory tests need to assess the toxicity of these molecules and their metabolites. They might be elaborated on the grounds of existing experimental studies which have investigated the chronic impacts of these molecules on honeybees (Stoner et al., 1982; Suchail et al., 2001; Moncharmont et al., 2003), as well as their sublethal effects on honeybees behaviour (Cox and Wilson, 1984; Johansen, 1984; Taylor et al., 1987; Decourtye et al., 2003; Thompson, 2003) and physiology (Bounias et al., 1985; Bendahou et al., 1999; Papaefthimiou and Theophilidis, 2001).

The data provided by these new toxicity tests, combined with the different modes of honeybee exposure developed in this paper, might be used to assess the risk of systemic insecticides according to the approach proposed by the European Commission (technical guidance document on risk assessment in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances, Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances, and Directive 98/8/EC of the European Parliament and Council). In this approach, developed for aquatic organisms, the risk might be estimated by calculating the ratio PEC/PNEC (Predicted Environmental Concentration/Predicted Non Effect Concentration, which is the concentration below which unacceptable effects on organisms will most likely not occur). The values of the PNEC might be refined with the recent results found on the chronic and sublethal toxicity of systemic insecticides associated with an appropriate safety factor. The values of the PEC, usually derived from available measured data and/or from a model of calculation, might be derived from the modes of honeybees exposure presented in this paper, and new risk assessments of systemic insecticides on honey bees can be developed (Halm et al., unpublished data).

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Résumé – Modes d'exposition des abeilles domestiques aux insecticides systémiques : estimation des quantités de pollen et de nectar contaminés consommées par diverses catégories d'abeilles. Les insecticides systémiques, utilisés pour la protection des cultures y compris des plantes mellifères et nectarifères, pénètrent dans la plante. Les abeilles domestiques (*Apis mellifera* L.), par leur consommation de nectar et de pollen, peuvent être intoxiquées par une exposition unique (toxicité aiguë) ou répétée (toxicité chronique) à ces insecticides. Les molécules peuvent induire la mort des abeilles ou provoquer des effets sublétaux sur leur physiologie, leurs capacités cognitives et leur comportement, qui en retour peuvent occasionner des pertes d'abeilles ou affecter le développement de la colonie.

Les tests de toxicité ont été conçus pour étudier des insecticides de faible activité résiduelle appliqués sur les parties aériennes des plantes, tandis que les insecticides systémiques se dégradent lentement et sont hautement toxiques. De nouvelles procédures réglementaires appropriées et des tests spécifiques complémentaires sont donc nécessaires pour estimer les éventuels impacts sublétaux et chroniques de ces insecticides sur les abeilles.

Le but de notre étude était de proposer des modes possibles d'exposition des abeilles aux insecticides systémiques. Basées sur les données disponibles dans la bibliographie, ces expositions sont déterminées en estimant les quantités de nectar et de pollen consommées par les abeilles (Tab. I). Certaines estimations sont proposées pour les larves et pour les catégories d'adultes qui consomment le plus de pollen, les nourrices, et le plus de nectar, les cirières, les nourrices, les butineuses et les abeilles d'hiver. Pour couvrir leurs besoins en protéines, les nourrices ingèrent plusieurs dizaines de mg de pollen en 10 j, et peut-être plus quand la quantité de couvain à nourrir est importante. Pour couvrir leurs besoins énergétiques, en particulier pour exécuter des tâches énergivores, les abeilles ingèrent des dizaines à des centaines de mg de sucre présent dans le nectar ou le miel.

L'imidaclopride est pris comme étude de cas car les concentrations de cet insecticide systémique dans le nectar de tournesols issus de graines enrobées et dans le pollen de tournesols et de maïs issus de graines enrobées ont été déterminées avec précision et les niveaux de toxicité létale, sublétale, aiguë et chronique ont été beaucoup étudiés. Nous avons aussi considéré le cas de pollen et de nectar contaminés à

100 % par l'imidaclopride, c'est-à-dire non mélangé avec du pollen ou du nectar non contaminé.
La concentration en imidaclopride du pollen de tournesols et de maïs traités au Gaucho étant en moyenne de 3,4 µg/kg, la quantité maximum d'imidaclopride consommée par des nourrices sur une période de 10 j serait d'environ 0,2 ng. La concentration en imidaclopride du nectar de tournesols traités au Gaucho étant en moyenne de 1,9 µg/kg, la quantité maximum d'imidaclopride consommée par différentes catégories d'ouvrières adultes pourrait varier entre quelques centaines de pg à quelques ng après plusieurs jours de consommation régulière (Tab. I). Ces résultats, lorsqu'on les compare aux doses minimum d'imidaclopride nécessaires pour induire chez les abeilles des effets létaux et sublétaux, peuvent donner une estimation du risque causé par cet insecticide aux abeilles.

***Apis mellifera* / insecticide systémique / exposition / imidaclopride / nectar / pollen**

Zusammenfassung – Unterschiedliche Exposition von Honigbienen gegenüber systemischen Insektiziden: Abschätzung der aufgenommenen Menge von kontaminiertem Pollen und Honig durch unterschiedliche Bienenkategorien. Systemische Insektizide werden zum Schutz von Nutzpflanzen einschließlich der nektar- und pollenerzeugenden Pflanzen verwendet. Nach Aufbringung auf Boden oder Saat dringen sie in die Pflanzen ein und schützen diese vor bodenbewohnenden oder fliegenden Insekten. Honigbienen, die mit Pollen und Nektar der Pflanzen in Berührung kommen oder diese verzehren, können hierbei einmalig (akute Toxizität) oder wiederholt (chronische Toxizität) den Insektiziden ausgesetzt sein. Dabei können Honigbienen sterben oder es kann sublethale Einflüsse geben, die auf Physiologie, kognitive Fähigkeiten (Lern- oder Orientierungsfähigkeit) und das Verhalten (Nahrungsammeln) wirken. Sublethale Dosen können daher ebenfalls Bienenverluste verursachen oder die Kolonieentwicklung beeinträchtigen.
Die Toxizität systemischer Akarizide wird anhand von standardisierten und zugelassenen Labortests bestimmt. Diese Tests wurden allerdings für die Untersuchung der niedrigen Restaktivität von Insektiziden mit äußerlicher Anwendung entwickelt. Demgegenüber sind die neu entwickelten systemischen Insektizide hochtoxisch und bauen sich nur langsam ab. Es werden daher neue geeignete Regulierungsprozeduren und zusätzliche spezifische Tests benötigt, damit die möglichen sublethalen und chronischen Wirkungen systemischer Insektizide erfasst werden können.
Es wurden die unterschiedlichen Möglichkeiten untersucht, in denen Honigbienen systemischen Insektiziden ausgesetzt werden können. Auf Grundlage vorliegender Literaturdaten wurden die Expositionen durch Abschätzung der von Honigbienen verzehrten Mengen von Nektar und Pollen bestimmt (Tab. I). Für Larven und für die höchsten Mengen von Pollen (die Ammenbienen) oder Honig (wachserzeugende Bienen, brutpflegende Bienen, Winterbienen und Sammlerinnen) verzehrenden Bienenkategorien werden Abschätzungen vorgeschlagen.
Um ihren Proteinbedarf zu stillen, nehmen Ammenbienen in 10 Tagen ein mehrfaches von 10 mg auf, und möglicherweise darüber hinaus, wenn die zu fütternde Brutmenge hoch ist. Zur Deckung ihres Energiebedarfs und besonders um Aufgaben mit hohen Energiekosten auszuüben, nehmen sie 10 bis hunderte mg in Nektar oder Honig enthaltenen Zucker auf.
In einer Fallstudie illustrierten wir diese Abschätzungen am Beispiel von Imidacloprid, einem systemischen Insektizid. Die Konzentrationen von Imidacloprid im Nektar aus saatgebeizten Sonnenblumen und im Pollen von Sonnenblumen und Mais wurden genau bestimmt. Über die Kozentrationslevel von lethaler, sublethal akuter und chronischer Toxizität liegen extensive Studien vor. Wir berücksichtigten zudem den Fall, dass Pollen und Nektar zu 100 % kontaminiert, also nicht mit unkontaminiertem Pollen oder Nektar vermischt waren.
Die Konzentration von Imidacloprid in Pollen von mit Gaucho® saatgebeizten Sonnenblumen- und Maispflanzen betrug im Mittel 3,4 µg/kg. Die über eine Zeit von 10 Tagen von Ammenbienen maximal aufgenommene Menge von Imidacloprid könnte hiernach bei etwa 0,2 ng liegen. Die Konzentration von Imidacloprid im Nektar von saatgebeizten Sonnenblumen betrug im Mittel 1,9 µg/kg. Die maximale Menge der von verschiedenen Bienenkategorien aufgenommenen Menge könnte hiernach nach mehreren Tagen regelmäßiger Aufnahme zwischen einigen hundert Picogramm bis zu einigen wenigen Nanogramm liegen (Tab. I).
Diese Ergebnisse können zu einer Abschätzung des Risikos beitragen, wenn sie mit den Minimaldosierungen verglichen werden, die zur Induzierung lethaler oder sublethaler Wirkungen auf Honigbienen erforderlich sind. Für andere Insektizide als Imidacloprid können diese Abschätzungen ebenfalls genutzt werden, um die Exposition gegenüber den Molekülen zu bestimmen, vorausgesetzt dass ihre Konzentrationen in Pollen und Nektar sowie ihre Toxizität für Honigbienen bekannt sind.

***Apis mellifera* / systemische Insektizide / Exposition / Imidacloprid / Nektar / Pollen**

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