23

Side-Effects of Pesticides on the Pollinator *Bombus*: An Overview

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1. Introduction

For the pollination of crops, agriculture relies largely on managed colonies of the honeybee *Apis mellifera* (Gallai et al., 2009). Unfortunately, recent crashes of colonies have been reported worldwide, also better known as "Colony Collapse Disorder" (CCD) (Mullin et al., 2010). In this context several authors pointed out that factors such as parasites and pesticides or a combination of these factors might be responsible for a decline in honeybee health (Van Engelsdorp et al., 2009). As a response multiple studies were conducted to assess pesticide residues in the field. The results were dramatic. For example, a study of apiaries in North American orchards recovered 121 agrochemicals in honeybees, pollen and the wax (Mullin et al., 2010). However the impact of our agricultural landscape is not limited to honeybee colonies. Indeed, also other pollinators suffer. Since 40 years non-*Apis* species such as bumblebees are decreasing in abundance (Goulson et al., 2008). Bumblebees, important for the pollination of many wild flowers, are crucial for the terrestrial ecosystem (Goulson, 2010). In addition, these pollinators as *Bombus terrestris*, *Bombus impatiens* and *Bombus ignitus* are also commercially reared for the pollination of agricultural and horticultural crops (Velthuis & van Doorn, 2006). Therefore, side-effects of pesticides need to be assessed for conservation and economic reasons. However, our current knowledge of pesticide toxicity on pollinating insects is fragmented for bumblebees since it is still mostly restricted to *A. mellifera*. One explanation to this can be found in bumblebees belonging to a less familiar group in the area of environmental protection. To date only a few pesticides have been tested on their compatibility with bumblebees prior to their commercial release, while for honeybees oral and acute toxicity tests are required for pesticide registration. Newer generation pesticides, which are thought to be less harmful to humans and the environment than the older pesticides such as synthetic organophosphate, carbamate and pyrethroid insecticides, are on the current marketplace. Nonetheless, even sublethal effects of pesticides may have significant impact on bees and pollination in addition to the more easily observable mortality.

This chapter provides for the first time an extensive overview of the side-effects of pesticides also called as "Plant Protection Products" (PPPs) on bumblebees. In a first and second part we will discuss the testing strategies so far employed to evaluate pesticide compatibility on bumblebees. Here attention will be given to the different "tier" levels, the various biological endpoints of effect, and the impact of the route of exposure. Then in a third part, an

overview will be given on the compatibility data that are currently available for the different groups of chemical and biological pesticides such as insecticides, acaricides, fungicides. A fourth part will compare the pesticide sensitivity between both pollinators for the different groups of PPPs. Finally, based on our increasing knowledge on the insect body we will make suggestions to improve some existing tests in order to work more standardized which would allow comparison between different PPPs in future.

2. Risk assessment at different "tier" levels with individual workers and micro-colonies in the laboratory to full colonies in the field

When assessing the toxicity of pesticides the first question one should address is: Can exposure to the pesticide occur? In the field, possible routes of exposure for bumblebees are by direct contact after a spray or orally via the consumption of contaminated food. However, evaluating the effect of a single pesticide or residue on an organism under field conditions is complex. However, in the case potential side-effects cannot be excluded, the risks need to be assessed in a stepwise approach with different "tier" levels (Figure 1).

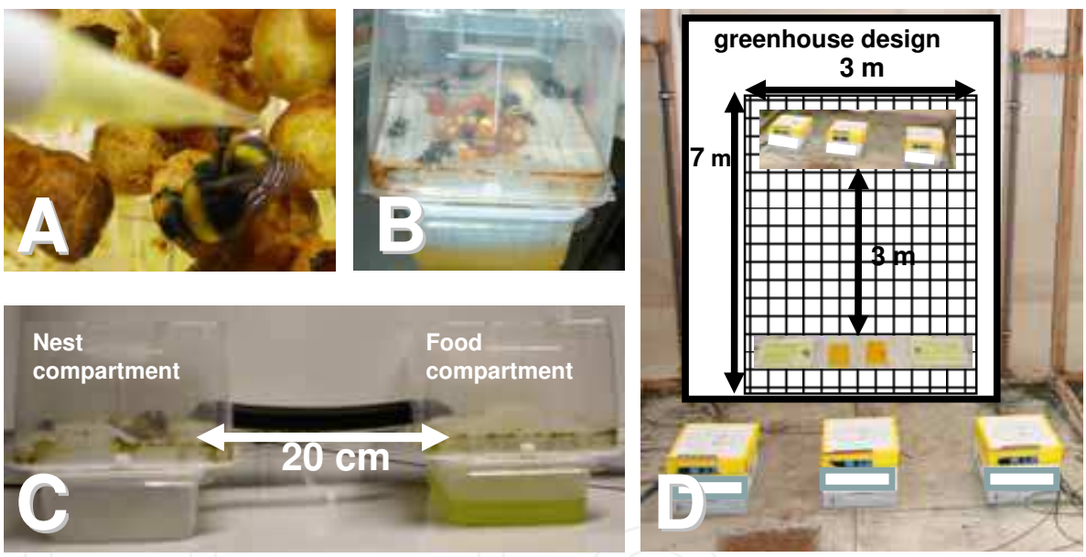

Fig. 1. Schematic overview of the different "tier" levels with (A) individual workers in the laboratory ("tier 1"), (B) micro-colonies in the laboratory without foraging ("tier 2"), (C) micro-colonies in the laboratory including foraging behavior ("tier 2"), and (D) full colonies in small greenhouse compartments ("tier 3"). The inset of D gives the greenhouse design (3 x 7 m) with the bumblebee colonies placed at 3 m from the food (own photographs).

At "tier 1" level, individual bumblebee workers are exposed to a worst case scenario in a laboratory insect toxicity test. To assess direct contact toxicity due to a spray application several experimental setups have been used (see for review Thompson, 2001; van der Steen, 2001). Currently, pesticides are dissolved in acetone and worker bumblebees are anesthetized with carbon dioxide up to 7 s prior to application of specific doses/concentrations to the bumblebee workers. For pesticide application dishes containing individual bumblebee workers are placed under a Potter spray tower (Scott-Dupree et al., 2009; Gradish et al., 2010). After treatment workers are transferred to cups with wax paper

where they are then provided with fresh sugar water. Then 48-72 h post-treatment acute toxicity is evaluated and the median lethal dose/concentration (LC_{50} or LC_{50}) is calculated. For this test at least 30 individual bumblebees need to be exposed. Also for the assessment of the acute toxicity via oral exposure, several protocols have been developed over the years (see for review Thompson, 2001). Bumblebees were first starved for 2-3 h and then fed with a 10 µl mixture of the pesticide dissolved in 50% sucrose which they had to consume within 2 h (see for review van der Steen, 2001). Hereafter the bumblebees were provided with regular sugar water and the LD_{50} was determined after 24-72 h. In the controls, an acceptable mortality level of ≤10% was set. The same method has also been recently used by Wu et al. (2010). These researchers assessed the oral toxicity in the laboratory with individual workers of the three bumblebee species *B. ignitus*, *Bombus hypocrita* and *Bombus patagiatus* and the diverse pesticides that are frequently used in Chinese greenhouses. In general, acute toxicity studies in the laboratory are easy to perform, but here attention should be given to the age of the individual workers used as susceptibility might change with the worker age. Some studies conduct their risk assessment with callow workers (<24 h), while others use bumblebees between 9-10 days or do not give any information on how the workers were selected.

A criticism on the aforementioned laboratory risk assessment tests with individual bumblebee workers over 72 h, is that side-effects of pesticides might take a longer time (>72 h) before becoming visible under practical conditions and that bumblebee workers show a social organization with the building of a nest (brood) and with foraging behavior to gather food from outside to inside the nest. It is therefore recommended to conduct an extended laboratory test as a second step of the risk assessment ("tier 2"). In order to cover all potential side-effects, bumblebees are exposed as in the insect laboratory test with individual workers ("tier 1") to PPPs concentrations recovered in the field, to concentrations as recommended for use or to the maximum field recommended concentration (MFRC). To date several studies evaluated potential postponed effects up to 11 weeks following exposure to insecticides, acaricides and fungicides by use of micro-colonies (Besard et al., 2010, 2011; Gradish et al., 2010; Mommaerts et al., 2006a,b, 2008, 2009, 2010a,b). Micro-colonies are artificial nests made of 3 to 5 workers of the same age, however a number of 5 workers is to be recommended for long chronic exposure assessments (Figure 1B). The wide application of this method in risk assessment studies with bumblebees can be explained by the low cost, the easy in use, the possibility to work standardized and with multiple replicates resulting in statistical power and thus in reproducible data. For the direct contact toxicity all the workers of the nest are treated by contact with a 50 µl drop of an aqueous solution made of the pesticide and tap water, on the dorsal thorax. These data give already strong indications on the compatibility of the pesticide with bumblebees, but other routes of exposure also occur. In the past, systemic compounds like neonicotinoids have been recovered in pollen. Also more recently, large studies in Europe and North-America showed the presence of PPP residues in pollen collected by honeybees (Skerl et al., 2009; Mullin et al., 2010; Wu et al., 2011). To simulate an oral chronic exposure via contaminated food, the bumblebee workers in the micro-colonies can be fed continuously with treated food (sugar water and/or pollen) over a period up to 11 weeks, or they can be fed for a period of 30 days after which they are then provided for 30 days with untreated food. For the sugar water treatment a solution is made of commercial sugar water (50%) or artificial home-made sugar water and the pesticide. Contaminated pollen paste is prepared by spraying pollen until saturation with an aqueous solution of the pesticide, prepared in tap water (Besard et al.,

2010; Mommaerts et al., 2006a,b, 2008, 2009, 2010a). However, the pesticide can also be dosed at exact amounts to pollen grains, which are then mixed with sugar syrup, and finally offered as a homogenous food source to the bumblebee workers. A final route of exposure is via residues left on plant surfaces. To simulate this situation, Wu et al. (2010) sprayed solutions of the pesticide (as prepared in water) on paper which was then air-dried before exposure to the bumblebees. To assess such effect upon exposure to biological insecticides, Hokkanen et al. (2004) developed two different methods. First, by treatment of the flowers until drip-off, and secondly via a "maximum challenge test". In the latter test bumblebee workers walk through a Petri Dish containing the growing and sporulating fungus. Considering the worker mortality, the aim of the extended laboratory tests is to classify PPPs. Unfortunately, criteria for a classification of substances are up until today not available for bumblebees. However, the side-effects' classification for arthropods and beneficial organisms by the "International Organization for Biological Control of Noxious Animals and Plants" (IOBC) is useful: "class 1": <25% effect, non-toxic; "class 2": 25-50% effect, weakly toxic; "class 3": 50-75% effect, moderately toxic; and "class 4": >75% effect, highly toxic. There is still no validation of this classification at present. For example when a product causes a loss of <25%, it is considered as not toxic. However, Goulson (2010) argued that the effect of a loss on the colony is directly depending on the colony size. We therefore suggest that in future these classification classes should be defined in relation to the range of the colony size.

Besides worker mortality (i.e. lethal side-effects), risk assessment studies also need to cover potential sublethal side-effects on bumblebee reproduction, larval development and the foraging ability of adults. These parameters are of crucial importance to guarantee the crop pollination. At first colonies containing adult workers and brood were fed on a treated 50% sugar solution during 24 h. Then, the brood (consisting of egg cups, open cups containing larval and pupal stages) was evaluated by observations at 3 times per week and this over a period of 3 weeks (see van der Steen, 2001). However, collecting data on effects on brood is difficult and thus de Wael et al. (1995) developed a method where the brood was daily checked and by photographing the brood from a fixed point. Although this was already an improvement a better protocol was developed by Gretenkord & Dresscher (1996). Here a more detailed evaluation was possible as eggs were removed from the colony and incubated until hatching where after the number of larvae was standardized to 10. For exposure, the larvae were placed in small boxes containing 3 workers that fed treated pollen during 24 h. Then, the amounts of pollen consumed by the larvae and the numbers of larvae developing into an adult were determined. Also these sublethal endpoints can be assessed with micro-colonies (Mommaerts et al., 2006a,b, 2010a; Gradish et al., 2010), but this will be discussed in more detail under 2.1. Moreover, in "tier 2" also laboratory trials including side-effects on the foraging behavior can be included. For example, Mommaerts et al. (2010b) recently reported on a "foraging bioassay" which made use of micro-colonies. As depicted in figure 1C, a box containing a micro-colony was connected by a tube of 20 cm in length with an empty nest containing the food (pollen and sugar water). This experimental setup allows the evaluation of interferences with the orientation capacity of the adult bumblebee workers. However, also other endpoints important for the foraging process can become affected after pesticide exposure. Hereto flight cages are a good tool. Morandin et al. (2005) connected colonies to flight cages (1.2 m x 1.2 m x 1 m) wherein artificial flowers were placed to evaluate the impact of an insecticide on the flower handling time and on the foraging speed.

Finally, in a last step, the PPPs are to be tested under semi-field and field conditions ("tier

3"). The aim of such complex studies is to get more insight in the risks for bumblebee colonies under more practical, field-related conditions. However, up until today the numbers of such studies are limited (see for review van der Steen, 2001). Gretenkord & Drescher (1996) was the first to describe a protocol for semi-field testing. According to his method a colony of at least 100 workers was placed in a cool box in the ground. Then this box was connected to a gauze tent (3 m x 2 m x 4 m) containing flowering *Phacelia tanacetifolia* plants. At a foraging intensity of 10 workers the connection tube is closed, the colony is standardized (containing one queen, 10 foragers, 5 nurses, 4-6 egg cups, and brood that is consisting of one cup with larval stages of 1-2 days, 3-4 days, and 5-6 days old and with 10-15 pupae), and the plants are sprayed. Bumblebees are exposed during 2-3 weeks and thereafter lethal and sublethal side-effects are assessed during 2 weeks in the laboratory. Similarly, Sechser & Reber (1996) placed free flying colonies in a tent (5-9 m^2) that was sprayed with the recommended concentration of the pesticides, and in addition colonies were fed with sugar water supplemented with the pesticide. Here effects were evaluated on all stages after 6 weeks. Moreover, next to tents, semi-field tests have also been conducted in small greenhouse compartments (3 m x 2 m) with a crop area of 2 m^2 (Tasei et al., 1993). However, the main problem with the use of crops in small compartments is that the size of the colony is not proportionate to the crop size, resulting in not enough pollen and nectar for the colony. To circumvent the use of plants, as depicted in figure 1D, Mommaerts et al. (2010b) provided bumblebee colonies with commercial pollen and treated sugar water at a distance of 3 m from their nest in greenhouse risk assessment experiments.
For field testing, a first protocol was described by Schaefer & Mühlen (1996). They placed six bumblebee colonies in a 2400 m^2 field with flowering *Phacelia* plants. Here worker mortality, colony activity and colony development were evaluated by collecting dead workers, activity observations on 5 x 1 m^2 for 1 min and by counting adults, dead larvae and photographing the brood. Also here the IOBC classification for side-effects in arthropods and beneficial organisms has been used to classify substances, but again it should be remarked that no validation has been done so far. According to this classification system for (semi-)field testing the following three classes can be distinguished: "class N": harmless or slightly harmful, 0-50%; "class M": moderately harmful, 51-75%; and "class T": harmful, >75%. Besides a lack of a proper classification system, it is still unclear how long bumblebees should be exposed. Some studies provide bumblebees during 5 weeks with treated food followed by a period of 5 weeks of uncontaminated food, while in other studies bumblebees were exposed during their entire life-span. Consequently, comparison between the determined risks resulting from the different assessment tests with the same pesticides is difficult.

2.1 Different biological endpoints for the assessment of side-effects

At present, risk assessments for PPPs follow regulatory guidelines which are for Europe defined by the European Council Directive 91/414. The aim of these guidelines is to protect honeybees and other pollinators. Here only side-effects on adult and larvae of honeybees are considered, while exposure in the field to other pollinators cannot be excluded. For example bumblebees might be exposed to pesticides in greenhouses through spraying via residues left on plants or by consuming contaminated nectar and pollen. Following exposure, the most obvious effect is worker mortality, but pesticides may also cause sublethal effects. Moreover, due to the increasing development of chemicals with different modes of action there is a demand to define valuable endpoints of effects. At present the increasing

economic importance of bumblebees in agriculture results in a growing body of literature on side-effects of pesticides of which on overview is given below.

2.1.1 Lethal effects

For a long time risk assessment studies with bees only considered the LD_{50} or LC_{50} of pesticides. Most likely this approach is probably based on honeybee risk assessments where at first the risk was calculated by the hazard quotient which is the application rate divided by the LD_{50} as calculated after 72 h of exposure (i.e. "tier 1"). To date for acute worker mortality, insect death (i.e. lethal endpoint) which is easy to observe, is not adequate enough. Indeed, the lethal dose is only a partial assessment of the risk for loss of survival as the test runs only for 3 days. Therefore Gradish et al. (2010) scored workers as dead when they did not move upon touching. This criterion considers also the effect of slower acting pesticides such as for the pesticides abamectine and metaflumizone that causes paralysis of the insect, resulting in feeding cessation and death. In conclusion, to date acute toxicity (via oral and contact exposure) is evaluated on the level of individual insects, whereas studies evaluating the long term side-effects (i.e. chronic exposure) make use of micro-colonies of bumblebees. It is to be noticed that the latter experimental setup has the wide advantage to consider potential pesticide transfer between bumblebees which might occur upon contact.

2.1.2 Sublethal effects

Considering the growing interest to determine potential sublethal effects following pesticide exposure, several methods have been reported to identify and characterize these for beneficial arthropods. A first comprehensive review on this research topic was published by Desneux et al. (2007). Here the authors mainly focused on effects on honeybees and natural enemies. However, the use of bumblebees in agriculture demands for examinations of sublethal side-effects as pollination must be guaranteed. For bumblebees, the reported sublethal effects of pesticides include effects on adults and on brood with fecundity and abnormal larval development resulting in reduced offspring. More details are discussed hereunder.

2.2 Exposure to different developmental stages

2.2.1 Exposure to adult workers

Following pesticide exposure, adults can directly be affected. At first adult longevity was shown affected after exposure to lethal and/or sublethal concentrations. For example Gradish et al. (2010) observed a shortened life-span when adult workers were fed on imidacloprid-treated pollen by scoring the number of dead workers.

So far pesticide exposure occurs in long-term studies by feeding the bees with contaminated food. For bumblebees food consumption is crucial as workers need sugar water for energy and pollen for ovary development (Heinrich, 1979). Based on this often also a second endpoint has been evaluated, namely, worker biomass. To determine worker biomass, some studies determined the weight of collected dead workers, while others used newly emerged workers which were cooled before weighed (Gradish et al., 2010; Wu et al., 2010).

Moreover, considering the importance of food for ovary development the moment of first oviposition has been used as a third endpoint. Care is needed as a reduction of the fecundity (oviposition) can be the result of a reduced food uptake or of a physiological effect of the pesticide. For example for diflubenzuron (IGR), Mommaerts et al. (2006a) showed

transovarial transport and accumulation in the eggs after pollen consumption by adults resulting in egg mortality. Next to a reduction, pesticides can also induce a stimulatory effect on the oviposition. Topical contact of adult workers with a sublethal concentration of kinoprene (IGR) resulted in a significant increase of both ovarian length and the numbers of eggs present in the ovaries (Mommaerts et al., 2006b).
Finally, pesticides are known to induce behavioral changes on adults (Thompson, 2003). To date several studies demonstrated that ingestion of small amounts of pesticides (e.g. imidacloprid, deltamethrin) by adult honeybees (Colin et al., 2001; Decourtye et al., 2003) interferes with their learning and orientation capacity. Similarly, sublethal concentrations of imidacloprid affected bumblebee behavior as Mommaerts et al. (2010b) demonstrated with use of the "foraging bioassay", thus when adult bumblebees (*B. terrestris*) needed to gather their food, that adult bumblebees had difficulties to find back the way to their nest resulting in a severe reduction of the offspring. For foragers orientation and memory are essential to find food. Assessment of these side-effects occurs in honeybees by use of the proboscis extension response (PER) (Decourtye & Pham-Delègue, 2002; Decourtye et al., 2004a,b; El Hassani et al., 2008). However, for bumblebees PER has been conducted with *Bombus occidentalis* but not in the context of risk assessments (Riveros & Gronenberg, 2009). Therefore future studies might include this method to broaden the endpoints which might become affected when adult bumblebees are exposed to pesticides.

2.2.2 Exposure to eggs, larvae and pupa

Bumblebee foragers gather pollen and nectar which is transported to the hive. Pesticides can also be brought to the hive via this route. Thus, in the field bumblebee brood can become indirectly exposed to pesticides sprayed on crops when the brood (larvae) is fed with contaminated pollen/nectar. For the assessment of these side-effects micro-colonies have been used successfully. In micro-colonies, comprising of 3 to 5 callow workers, one worker becomes dominant and starts to lay eggs after one week, while the other workers assist her in rearing the brood. Eggs laid in micro-colonies are not fertilized and will develop over 4 larval stages and 1 pupal stage into male adults (drones) after 4 weeks. Effects on brood are scored as the numbers of larvae that are removed from the brood. This criterion is based on the typical behavior of bumblebee workers to remove larval stages with abnormalities or dead larvae from the respective brood clump (Mommaerts et al., 2006a,b, 2009, 2010a; Gradish et al., 2010). Moreover, this endpoint was further refined in accordance with the mechanism of action of pesticides under investigation. For example, in case of the IGRs as developed to interfere with the developmental processes in insects, the different stages of the removed larvae were determined based on their head width (Mommaerts et al., 2006a,b).
For effects on the reproduction, the number of offspring (drones) produced was already used by multiple studies as endpoint (Mommaerts et al., 2006a,b, 2008, 2009, 2010a,b; Besard et al., 2010, 2011). Here drones were measured on a weekly basis and this during a period up to 11 weeks; the drones were removed from the micro-colonies after scoring.
Considering behavioral effects, Morandin et al. (2005) showed that spinosad (insecticide) when administered during the entire larval stage affected other parameters crucial for the foraging capacity of adult workers. Hereto the authors connected a bumblebee (*B. impatiens*) colony with a flight cage, containing two different types of artificial flowers. A "simple flower" consisted of an Eppendorf tube without caps, while a "complex flower" an Eppendorf tube with the caps attached leaving an opening of 7 mm. With this experimental setup data were collected on the time period needed to access the first artificial flower, the

handling time, and the foraging rate. However, there exists a debate to date whether sublethal effects must be investigated, particularly at lower tier level, because potential side-effects are expected to become visible in experiments at higher tier level. It should be noted that there is not enough information to make a firm conclusion in this matter.
Next to indirect exposure via food, only a few studies examined the effect of a direct contamination of the brood by contact. For example Mommaerts et al. (2008) treated third- and fourth-instar larvae by dermal contact with a suspension of a biological insecticide in water to assess the larval toxicity of the compound. Also van der Steen (2005) evaluated side-effects on bumblebee brood. Hereto all adult workers were removed from the colony prior to spraying.

3. Different classes of pesticides

3.1 Chemical pesticides

3.1.1 Insecticides

To date risks assessment studies conducting the side-effects of conventional insecticides are mostly limited to acute toxicity studies. A summary of all available data on effects of the older insecticides including the pyrethroids, the carbamates and the organophosphates is given in table 1. Interestingly, van der Steen (1994) found that the acute toxicity (oral and contact) for dimethoate was correlated with the size of the bumblebee. In addition, for the pyrethroid deltamethrin also sublethal effects have been described. At double the recommended rate, Gretenkord & Drescher (1993) reported a repellent effect. Similarly, Tasei et al. (1994) showed an increase of 40-100% in sucrose uptake when *B. terrestris* were treated by dermal contact with 0.08-0.16 mg/kg, whereas a higher dose of 0.1-0.2 mg/kg caused a 47-59% decrease of sucrose uptake. Overall, when considering conventional insecticides it is remarkable that none of all compounds included (n=59) was considered as non-toxic (Figure 2).
Neonicotinoids are systemic insecticides which interfere with the insect nervous system by binding on the nicotinic acetylcholine receptor. The most studied compound within this group is imidacloprid. Exposure to bumblebees (*B. terrestris* or *B. impatiens*) caused acute worker mortality after contact/oral exposure (Incerti et al., 2003; Marletto et al., 2003; Scott-Dupree et al., 2009; Gradish et al., 2010; Mommaerts et al., 2010b). Also effects such as bumblebee trembling, reduced brood production, pollen consumption, vitality, and impaired foraging behavior have been observed after exposure to imidacloprid (Tasei et al., 2000; Gels et al., 2002; Incerti et al., 2003; Morandin & Winston, 2003; Gradish et al., 2010). However Tasei et al. (2001) concluded by use of a greenhouse test that imidacloprid when applied as a seed coating at the registered dose did not affect *B. terrestris* foraging and homing behavior. Although imidacloprid received much attention in risk assessments, this group of neonicotinoids also contains other compounds. Recently, Mommaerts et al. (2010b) reported that the neonicotinoids with a nitro group (imidacloprid and thiamethoxam) caused the greatest side-effects. Here it should also be remarked that not only the mother product but also metabolites were shown to affect bee survival. For example clothianidin, derived from thiamethoxam, was highly toxic after contact on *B. impatiens* workers. In contrast, acetamiprid and thiacloprid both belonging to the group of the cyano-neonicotinoids, were less toxic. In total only 17% of the 6 compounds considered were safe (Figure 2).
IGRs are classified as more selective due to their interference with insect-specific targets however only 47% of the compounds tested has been found non-toxic (Figure 2). Within the IGRs, three different groups can be distinguished: chitin synthesis inhibitors (CSIs), juvenile

hormone analogs (JHAs), and ecdysteroid agonists or also called molting-accelerating compounds (MACs).

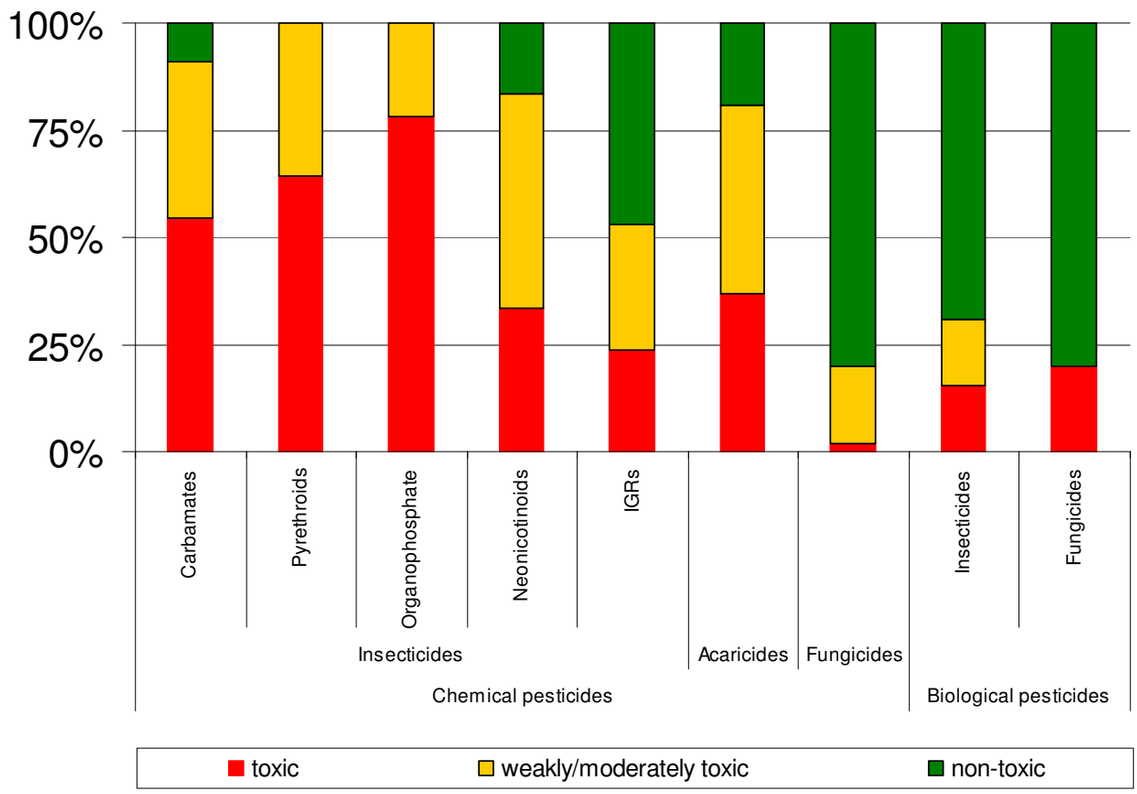

Fig. 2. Overview of the toxicity of chemical and biological pesticides towards bumblebees (*Bombus terrestris*). For each pesticide group the bars represent the percentage of compounds which are non-toxic (green), weakly/moderately toxic (yellow-orange) and toxic (red). The division in toxicity levels is based on the recommendations made by the side-effect list when available, or on the obtained toxicity with micro-colonies ("tier 2"). The numbers of compounds considered per group are n=11 for carbamates, n=14 for pyrethroids, n=32 for organophosphates, n=6 for neonicotinoids, n=17 for IGRs, n=13 for biological insecticides, n=27 for acaricides, n=66 for chemical fungicides, and n=5 for biological fungicides. For details with references, see table 1, 2 and 3.

CSIs are mainly larvicides and act through the inhibition of chitin formation. So far no mortality was reported by CSIs against adult bumblebee workers (de Wael et al., 1995; Tasei, 2001; Mommaerts et al., 2006a; Scott-Dupree et al., 2009). However, severe effects have been observed on reproduction. Dermal contact exposure to the MFRC of diflubenzuron (288 mg/l) and teflubenzuron (150 mg/l) caused a total inhibition of adult formation (Mommaerts et al., 2006a). Also for diflubenzuron transovarial transport was confirmed. The NOEC for this compound was 100-10,000 times lower than the MFRC. Consequently, it is not recommended to use these compounds in combination with bumblebees. Other CSIs tested comprise novaluron, flucycloxuron, flufenoxuron, lufenuron, buprofezin and cyromazine (Mommaerts et al., 2006a; Scott-Dupree et al., 2009). Here the route of exposure will determine the effect with the strongest effects seen when CSIs were administrated via

the pollen. Overall, the MFRC of all CSIs were also detrimental to larval growth as significantly more larvae of the first and second instar were removed due to an abnormally formed cuticle.

The JHAs with a function resembling the juvenile hormone (JH), are contact and stomach poisons. In insects, JH is responsible for the regulation of the metamorphosis and the synthesis of vitellogenin. For *B. terrestris* toxicity tests by use of micro-colonies showed that JHAs (pyriproxyfen, fenoxycarb and kinoprene) did not cause acute/chronic worker mortality by oral/contact exposure (Mommaerts et al., 2006b). Similarly, no effect on the reproduction was reported when *B. terrestris* workers were exposed during 11 weeks to the MFRC of these compounds. In contrast, pollen exposure to pyriproxyfen (25 mg/l) and kinoprene (650 mg/l) resulted in a significantly higher numbers of removed third- and fourth-instar larvae, implying a lethal blockage of the development before metamorphosis (Mommaerts et al., 2006b). Interestingly, for the latter compound a low concentration of 0.0650 mg/l had a stimulatory effect on brood production, resulting in longer ovaries that contained more eggs than in control dominant workers.

The MACs are active after contact and ingestion when they bind on the receptor site of the insect molting hormone 20-hydroxyecdysone, the ecdysone receptor. For the bumblebee *B. terrestris* the MFRC of tebufenozide and methoxyfenozide did not affect worker survival, worker reproduction and larval development (Mommaerts et al., 2006b). In conclusion, the extended laboratory tests with micro-colonies indicated that these MCAs are compatible with the use of bumblebees.

Finally, within the class of the chemical insecticides metaflumizone, chlorantraniliprole and a natural plant derivate Matrine (Kingbo) have also been tested. These insecticides are currently used in the greenhouse vegetable production. For metaflumizone 0.1-1 g/l caused direct contact toxicity, whereas chorantraniliprole was harmless (Gradish et al., 2010). Also both insecticides at the recommended rate did not affect reproduction in *B. impatiens* micro-colonies (Gradish et al., 2010). The natural plant derivate Matrine was only evaluated for its impact on worker survival. After contact exposure to dry residues Wu et al. (2010) observed a significant effect on worker mortality when application doses used in the greenhouse were tested (1/5000, v/v). For oral toxicity it was interesting that the LD_{50} for *B. hypocrita* (0.0019 µg per bee) was significantly higher than for the other bumblebee species (*B. ignitus* and *B. patagiatus*) (Wu et al., 2010).

3.1.2 Acaricides

Studies evaluating the impact of acaricides are limited. Recently Besard et al. (2010) published a first extensive evaluation of 23 acaricides (traditional and novel ones) on *B. terrestris* by using the laboratory micro-colony design. Also here effects are different according to the route of exposure with the strongest effects observed after oral exposure via the drinking of treated sugar water. According to Besard et al. (2010) abamectin, bifenazate, bifenthrin and etoxazole were not compatible with *B. terrestris*. At a concentration of 18 mg/l (i.e. MFRC) abamectine caused 100% worker loss. Similarly, Gradish et al. (2010) reported for *B. impatiens* 80-100% worker mortality after contact to 0.1-1.0 g/l while oral exposure via pollen caused several sublethal effects such as reduced colony lifespan and delay of oviposition. Overall, of the 27 compounds tested only 19% was non-toxic (see figure 2). For more detailed information concerning the different acaricides so far tested see table 2.

3.1.3 Fungicides

Risk assessments including fungicides are limited resulting in only fragmented data (see table 3). Overall, it can be concluded that at the recommended rates the fungicides tested (myclobutanil, potassium bicarbonate, difenoconazole and copper abietate) did not cause a negative effect on *B. impatiens* worker survival and reproduction. Also the side-effect list (see Biobest side-effect list: http://www.biobest.be, and Koppert side-effect list: http://neveneffecten.koppert.nl/), comprising data of more than 50 active ingredients of applied fungicides, recommends that bumblebee hives do not need to be removed before product application, however except for carbendazim, cyprodinil+fludioxonil, dimethomorph, fosetyl-aluminium, penconazole, pyrazofos and tebuconazole. Here it is recommended to remove the hives prior to application and this until 24 h after. On this list only one active ingredient, namely zineb (Zerlate), is indicated as not compatible. Consequently, of the 66 compounds included 66% is classified as non-toxic (Figure 2).

3.1.4 Weed crop control products and plant growth/health regulators

To our knowledge no data is available at present on the compatibility with bumblebees of herbicides, plant growth regulatory hormones (e.g. straw shorteners) and plant health stimulating compounds, such as chemicals that induce systemically acquired resistance (SAR) in the treated crops.

3.2 Biological pesticides

3.2.1 Bio-insecticides

The group of the biological insecticides includes 13 different compounds of which 69% is considered as safe (Figure 1).

Beauveria bassiana GHA and *Metarhizium anisopliae* caused side-effects on *B. terrestris* (Hokkanen et al., 2004; Mommaerts et al., 2009). In the laboratory contact exposure to 2.5 x 10^{10} CFU/l (i.e. MFRC) of *B. bassiana* GHA resulted in 92% worker mortality after 11 weeks, while oral administration did not affect worker survival. In addition, also sublethal effects on the reproduction and changes in the foraging behavior have been observed with *B. bassiana* GHA (Mommaerts et al., 2008, 2009).

For the MFRC of *Cydia pomonella* granulovirus no detrimental effects have been observed after contact and oral exposure (Mommaerts et al., 2009).

In the laboratory with the micro-colony design no worker mortality was seen after contact and oral exposure via eating pollen to the MFRC of *Bacillus thuringiensis* kurstaki and *B. thuringiensis* aizawai (Mommaerts et al., 2010a). In contrast, oral exposure via sugar water treated with *B. thuringiensis* aizawai caused a 100% loss, but this effect disappeared when the concentration was 10 times diluted. Similar effects were also reported on *B. occidentalis* and *B. terrestris* by Morandin & Winston (2003) and Babendreier et al. (2008) when pure Cry proteins (Cry1Ab and Cry1Ac) were taken up via pollen and sugar water. Concerning the sublethal effects on reproduction var. kurstaki was harmless, while var. aizawai administered at 0.01% via the pollen reduced reproduction by 31%. Both strains did not induce behavioral changes.

For the naturalyte spinosad, consisting of spinosyn A and D derived from the fermentation of the bacterium *Saccharopolyspora spinosa*, acute oral and contact toxicity tests demonstrated its toxicity for bumblebees (Mayes et al., 2003). However, according to Morandin et al. (2005) colony losses only occurred when bumblebees (*B. impatiens*) were exposed to an unrealistically high dose of 8.0 mg/kg. Nonetheless, at realistic field concentrations (0.2-0.8

mg/kg) sublethal effects were observed. For example larval exposure to 0.8 mg/kg via the diet (pollen) resulted in adults foraging slower on artificial complex flowers, whereas such effects were not visible at lower concentrations. Similarly, Besard et al. (2011) demonstrated for *B. terrestris* that oral feeding with the MFRC (400 mg/l) of spinosad caused 75% worker mortality after 72 h. Here bumblebee workers showed tremors causing paralysis and finally insect death. Moreover, at 0.4 mg/l spinosad was harmless. In contrast, the novel spinosyn spinetoram was less toxic as the MFRC (25 mg/l) resulted only in 55% worker mortality. No sublethal effects were scored at 0.025 mg/l. In addition, a wet and dry residue test also confirmed the higher toxicity of spinosad over spinetoram.

3.2.2 Bio-fungicides

In total the MFRC of 5 different microbiological fungicides have been tested with the micro-colony design (see table 3). All were classified as harmless via the different routes of exposure, except *Bacillus subtilis* QST713 (Figure 2). Here the MFRC (7.5 x 10^9 CFU/l) resulted in a severe total loss of adult *B. terrestris* workers ("class 4" for extended laboratory testing) after contact and oral exposure to treated sugar water (Mommaerts et al., 2009).

4. Sensitivity for pesticide side-effects: does there exist a correlation between honeybees and bumblebees?

To determine the sensitivity for pesticide side-effects between closely related pollinators as *B. terrestris* and *A. mellifera* we will first compare the overall toxicity of the different classes of PPPs. Then, for the chemical insecticides we will investigate if a correlation exists on product level between bumblebee and honeybee toxicity by use of a regression analysis with available LD_{50}-24h. Finally, for the insecticides such as the IGRs whereof no LD_{50} could be found the side-effects on bumblebees were compared with those on honeybees.

As mentioned above the toxicity of different PPPs for bumblebees is given in figure 2. At present toxicity data of all PPPs are available for honeybees. An overview of the relative toxicity based on the LD_{50}-48h after contact and oral exposure on honeybees (*A. mellifera*) for the different classes of PPPs (the same selection of PPPs as for figure 2) is given in figure 3. Comparison of both figures 2 and 3 clearly demonstrates a similar trend in sensitivity between bumblebee and honeybee toxicity. For example the overall toxicity of older chemical insecticides (including the carbamates, pyrethroids and organophosphates) is comparable and ranges between high to moderate except for one product (oxamyl) which was safe for bumblebees and highly toxic for honeybees. A similar trend can also be seen for the newer chemical insecticides (IGRs and neonicotinoids), the biological insecticides and the chemical fungicides, although it should be remarked that honeybees were more sensitive than bumblebees. Furthermore, an equal toxicity was observed for the different products belonging to the class of the acaricides and biological fungicides. Based on these results we argue that bumblebee toxicity, can be used as a first indication for honeybee toxicity but care is needed when different endpoints can be affected because honeybees and bumblebees are very distinct (colony live, behavior,…). Nonetheless, this would imply that a first toxicity screening can be done by using bumblebees which are easier to work with as compared to honeybees.

As mentioned above, the toxicity of the chemical insecticide class is comparable between bumblebees and honeybees. However, figure 4 shows a regression analysis with the available LD_{50}s for the different PPPs belonging to the carbamates, pyrethroids,

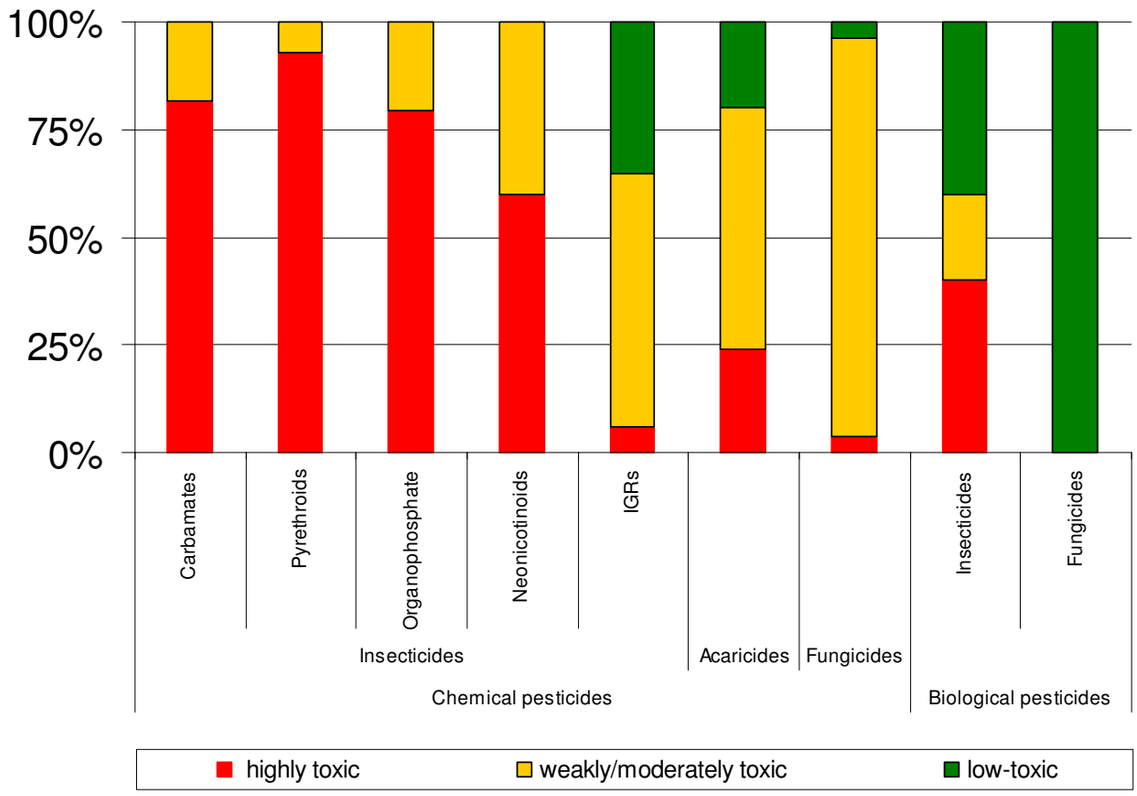

Fig. 3. Overview of the honeybee (*Apis mellifera*) toxicity of chemical pesticides and biological pesticides as available for bumblebees (*Bombus terrestris*). For each pesticide group the bars represent the percentage of compounds which are non-toxic (green), weakly/moderately toxic (yellow-orange) and toxic (red). The toxicity levels are based on the LD_{50}-48h obtained after contact and oral exposure (see http://sitem.herts.ac.uk/aeru/footprint/en/index.htm). The numbers of compounds considered per group are n=11 for carbamates, n=14 for pyrethroids, n=29 for organophosphates, n=5 for neonicotinoids, n=17 for IGRs, n=5 for biological insecticides, n=25 for acaricides, n=62 for chemical fungicides, and n=3 for biological fungicides.

organophosphates, and neonicotinoids. Here the LD_{50}s obtained after 24 h exposure were used and this for 17 insecticides. When the values were expressed as µg/g, then these were recalculated to µg/bee based on the weights as published by Thompson (2001) (with 0.10 g for *A. mellifera* and 0.21 g for *B. terrestris*). The poor linear regression (R=0.36) between the toxicities of the different compounds confirms that extrapolation of toxicity data between these two pollinators is not possible. In case of the carbamates, the LD_{50}s of 4 compounds were obtained. Here it was shown that for 75% of the products (carbaryl, methomyl and propoxur) *B. terrestris* was up to 10 times less sensitive than honeybees. Only the LD_{50} for ethiofencarb was lower (more sensitive) for *B. terrestris* (0.205 µg/bee) than for *A. mellifera* (6.85 µg/bee). For the group of pyrethroids, *A. mellifera* was more sensitive for all 5 products. For the organophosphates, the *B. terrestris* sensitivity was variable. Out of the 7 organophosphates, there were 4 products (acephate, chlorpyrifos, demeton-S-methyl and dimethoate) for which *A. mellifera* showed a higher sensitivity than *B. terrestris*. Equal sensitivity for both pollinators was seen for oxy-demeton-methyl and paraxon while *B.*

terrestris was 10 times more sensitive for chlorpyrifos-methyl. For the neonicotinoids *A. mellifera* was most sensitive to imidacloprid. This is in agreement with Hardstone & Scott (2010) who concluded that *A. mellifera* was among the most sensitive for imidacloprid. In contrast, the sensitivity for acetamiprid was equal between both pollinators.

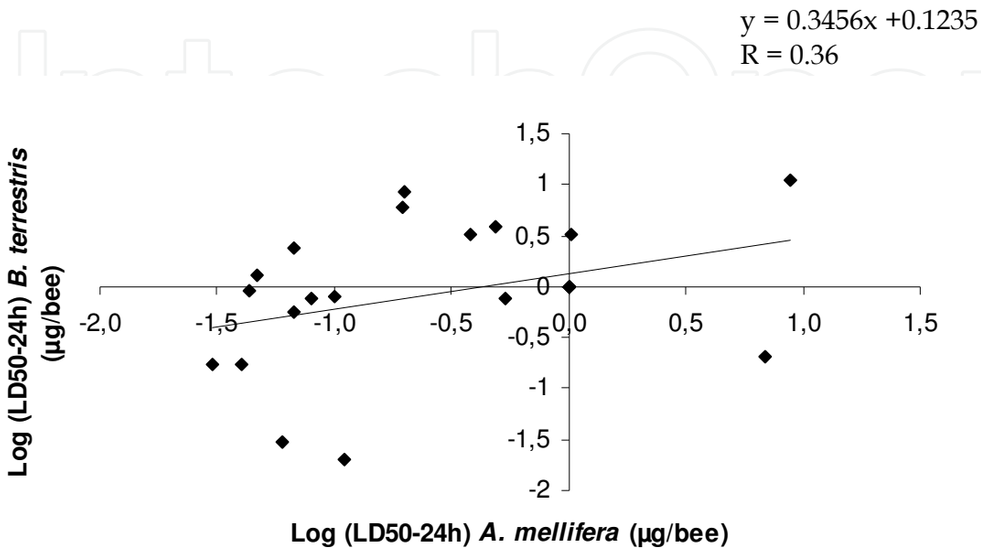

Fig. 4. Sensitivity of pesticide side-effects on bumblebees (*Bombus terrestris*) versus honeybees (*Apis mellifera*). Linear regression analysis was conducted with LD_{50}-24h values after contact and oral exposure for 17 different insecticides (carbamates, pyrethroids, organophophates and neonicotinoids). Data are presented as a mean log (LD_{50}-24h) and originate from Thompson (2001), van der Steen et al. (2008) and Hardstone & Scott (2010).

Although no linear regression could be drawn, this analysis gives a first idea of bumblebee versus honeybee sensitivity for pesticides. It needs to be remarked that the power of this analysis is limited because an LD_{50} was not available for each insecticide. However, based on available data for different IGRs, MACs are safe for both bumblebees and honeybees (Thompson et al., 2005; Mommaerts et al., 2006b), whereas no correlation can be found for the other two classes (CSI and JHA). Indeed, for diflubenzuron (CSIs) the LD_{50}-24 h on larvae showed that *B. terrestris* larvae are more sensitive than *A. mellifera* (LD_{50}-72 h) (Tasei, 2001). For the same compound also Mommaerts et al. (2006a) reported a total loss of *B. terrestris* reproduction, while Thompson et al. (2005) found only short-term effects on *A. mellifera* colonies. In contrast, for the JHA fenoxycarb, *B. terrestris* larvae were less susceptible (LD-24h: >0.650 µg/larvae) than *A. mellifera* larvae (LD_{50}-48h: 0.013 µg/larvae) (Tasei, 2001). Similarly, exposure of micro-colonies to fenoxycarb at its MFRC did not result in negative effects on reproduction (Mommaerts et al., 2006b), while *A. mellifera* colonies started the season slower and queen mating and egg laying were affected after (oral) exposure (Thompson et al., 2005). Based on this information and in order to have a total idea of the pollinator sensitivity towards pesticides, it is recommended that future studies should also evaluate the sensitivity of pesticides on other developmental life-stages. Finally, the pesticide side-effects sensitivity between honeybees and bumblebees is not only different for chemical insecticides. Indeed, for spinosad a biological insecticide comparison showed that

honeybees (LD_{50}-48h: 0.16µg/bee) were 100 times more sensitive than bumblebees (LD_{50}-48h: 19.4 µg/bee) (Halsall & Grey, 1998; Aldershof, 1999).
From the above mentioned results, it is clear that risk assessment bioassays need to evaluate side-effects on species level. The reason for this difference is not only due to a difference in sensitivity, but as already argued by Thompson & Hunt (1999) due to a difference in exposure profile. In this context they identified the following factors: namely the foraging active period, the species of crops visited, and the time of spraying (time on the day and time in the season). For example insecticides belonging to the class of the pyrethroids are applied in the early morning or late evening when they are more toxic and thus perform a higher risk for bumblebees. Similarly, risk assessment measures in honeybees are not useful for bumblebee losses which occur by pesticide applications in March-April, the moment of the year when bumblebee queens emerge and forage to find a nest place in order to start a colony (Thompson & Hunt, 1999).

5. Conclusions and future perspectives

This review gives on overview of the available toxicity data of PPPs on bumblebee species used for the pollination of crops. However, when looking at the obtained data set it is clear that the information is more fragmented in comparison with honeybees. Although in the past efforts have been made to assess risks by developing a variety of methods, we propose to conduct them in a tier approach in order to assess risks in a more complete way. The different levels are: (1) laboratory tests on individual insects ("tier 1"), (2) extended laboratory tests with micro-colonies which include the evaluation of pesticides on key processes such as worker survival, reproduction and behavior ("tier 2"), and (3) semi-field and/or field tests ("tier 3"). Unfortunately, to date most studies do not include semi-field and/or field tests, while it is crucial to make a link between the observed toxicity in the laboratory and the risks under field conditions in order to fully assess the risks. For example laboratory tests ("tier 1 and 2") do not consider pesticide degradation which might occur under field conditions. In addition, the goal of each tier is to classify the PPPs according to their compatibility with bumblebees. However, this point has been overlooked as no guidelines exist for bumblebees and thus these of the IOBC are used without any validation. Proper guidelines are therefore urgently needed which resemble the consequences at colony level by, for example, taking into account the consequences of worker loss according to the size of the colony.
In this review also a wide variety of effects (lethal and sublethal effects) have been reported following pesticide exposure. For lethal effects (worker mortality) the methods used are well defined. However, comparison between pesticide toxicities remains difficult. Therefore we suggest that in the future the already available lethal toxicity tests are more standardized by using a fixed exposure time and worker age and by determining the size of the worker as the length of the bumblebee body is variable. For sublethal effects on adult workers, different endpoints have already been evaluated such as worker life-span, worker biomass, start of oviposition, and this with adequately developed methods. In contrast, sublethal effects on the bumblebee brood have been assessed but these bioassays need to be further improved. Indeed, in honeybees a brood test was recently developed where brood is kept in individual cells without the presence of adults. For bumblebees, the development of such method would benefit from the existing one where side-effects are evaluated by collection of the larvae removed from their cocoon. Moreover, such new test would allow to work

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects				Compatibility	Route	Ref.
				contact	oral sugar water	Oral pollen		Reproduction		Adult/ Behavior			
							contact	oral sugar water	oral pollen				
Insecticides													
Carbamates													
aldicarb	NI	NI	RR								not compatible	s	Biobest
bendiocarb	NI	NI	RR								not compatible	s	Biobest
carbaryl	NI	NI	dose-range		LD50-24h: 3.92 µg/bee; LD50-72h: 3.84 µg/bee								de Wael et al. (1995)
	B. impatiens	field					reduced brood biomass			non irrigated: reduction of colony vitality; reduced worker biomass			Gels et al. (2002)
	NI	NI	RR								remove colonies before product application, retention time 36h	s	Biobest
carbofuran	NI	NI	RR								not compatible	s	Biobest
carbosulfan	NI	NI	RR								not compatible	s	Biobest
ethiofencarb					LD50-24h: 0.205 µg/bee; LD50-48h: 0.78 µg/bee; LD50-72h: 0.158 µg/bee								van der Steen et al. (2008)
	NI	NI	RR								remove colonies before product application, retention time of 48h	s	Biobest
methomyl	*B. terrestris*				LD50-24h: 1.42 µg/bee; LD50-48h: 0.57 µg/bee; LD50-72h: 0.44 µg/bee;								van der Steen et al. (2008)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality		Sublethal effects				Compatibility	Route	Ref.
												Thompson (2001)
	B. lapidarius			LD50-24h: 3.2 µg/bee; LD50-72h: 2.6 µg/bee								Thompson & Hunt (1999)
				LD50-48h: 2.78 µg/bee; LD50-48h: 2.4 µg/bee; LD50-72h: 2.18 µg/bee								
	NI	NI	RR							remove colonies before product application, retention time of 72h	s; i	Biobest
methiocarb	NI	NI	RR							not compatible	s	Biobest
oxamyl	NI	NI	RR							not compatible	s	Biobest
pirimicarb	*B. terrestris*			LD50-24h: 8.5 µg/bee								Gretenkord & Dresher (1993)
		cage test				no effect at 900 g/ha						
propoxur				LD50-24h: 3.19 µg/bee; LD50-48h: 2.017 µg/bee; LD50-72h: 1.6 µg/bee								van der steen et al. (2008)
			RR		10-30%							van der steen et al. (2008)
		cage test				no effect at 2400 ml/ha						
	NI	NI	RR							not compatible	s	Biobest
Pyrethroids												
acrinathrin	NI	NI	RR							remove cololies before product application, retention time of 72h	s	Biobest
alphacypermethrin	*B. terrestris*			LD50-24h: 0.17µg/bee	LD50-24h: 0.52 µg /bee							Thompson & Hunt (1999); Thompson (2001)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality		Sublethal effects				Compatibility	Route	Ref.
				LD50-72h: 0.15 µg/bee	LD50-72h: 0.36 µg/bee							Thompson & Hunt (1999); Thompson (2001)
	NI	NI	RR							not compatible	s	Biobest
bioresmethrin	NI	NI	RR							remove cololies before product application, retention time of 48h	s	Biobest
cyfluthrin				LD50-24h: 0.56 µg/bee	LD50-24h: 0.13 µg/bee							van der Steen et al. (2008)
	NI	NI	RR							not compatible	s	Biobest
cypermethrin	NI	NI	RR							not compatible	s	Biobest
deltamethrin	*B. terrestris*			LD50-48h: 0.9 µg/bee	LD50-24h: 0.6 µg/bee							Thompson (2001)
	B. impatiens	individual bees treated with a spray potter tower	dose-range	LC50-48h: 690 mg/1								Scott-Dupree et al. (2009)
	NI	NI	RR							remove colonies before product appliction, retention time of 72h	s	Biobest
esfenvalerate	NI	NI	RR							not compatible	s	Biobest
fenpropathrin	NI	NI	RR							not compatible	s	Biobest
fenvalerate	NI	NI	RR							not compatible	s	Biobest
flucythrinate	NI	NI	RR							not compatible	s	Biobest
lambda-cyhalothrin				LD50-24h: 0.22 µg/bee; LD50-72h: 0.11 µg/bee	LD50-24h: 0.21 µg/bee; LD50-72h: 0.16 µg/bee							van der Steen et al. (2008)
	B. terrestris	cage test	dose-range						repellent at 400ml/ha			Gretenkord & Dresher (1996)
	NI	NI	RR							not compatible	s	Biobest

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality		Sublethal effects				Compatibility	Route	Ref.
permethrin	*B. terrestris*	NI	dose-range	LD50-24h:0.81 µg/bee								Thompson (2001)
				LD50-72h: 0.82 µg/bee								Thompson (2001)
	NI	NI	RR							not compatible	s	Biobest
resmethrin	NI	NI	RR							remove colonies before product application, retention time of 12h	s	Biobest
tau-fluvalinate					LD50-24h: 0.97 µg/bee; LD50-72h: 0.68 µg/bee							de Wael et al. (1995)
	NI	NI	RR	10-30%								van der Steen et al. (2008)
	NI	NI	RR							remove colonies before product application, retention time of 24h	s	Biobest
Organophosphates												
acephate	*B. terrestris*				LD50-24h: 3.52-135.5µg /bee							Thompson (2001); van der Steen et al. (2008)
					LD50-72h: 3.44-7.37 µg /bee							de Wael et al. (1995); van der Steen et al. (2008)
					LD50-48h: 3.69 µg/bee							van der steen et al. (2008)
	NI	NI	RR							not compatible	s	Biobest
azinfos-methyl	NI	NI	RR							not compatible	s	Biobest
bromophos	NI	NI	RR							not compatible	s	Biobest

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality				Sublethal effects				Compatibility	Route	Ref.
chlorfenvinphos	NI	NI	RR									remove colonies before product application, retention time of 36h	s	Biobest
chlorpyriphos	*B. terrestris*			LD50-24h: 2.39 µg ai/bee										Thompson (2001)
				LD50-72h: 1.58 µg ai/bee										Thompson (2001)
	NI	NI	RR									not compatible	s	Biobest
	B. impatiens	field					reduced brood biomass				non irigated: reduction of collony vitality; reduced worker biomass			Gels et al. (2002)
chlorpyrifos-methyl				LD50-24h: 0.09 µg/bee; LC50-72h: 0.09 µg/bee	LD50-24h: 0.02-0.38 µg/bee; LC50-48h: 0.05 µg/bee; LD50-72h: 0.051-0.36 µg/bee									de Wael et al. (1995) ; van der Steen et al. (2008)
	NI	NI	RR									not compatible	s	Biobest
chloropyrifos-ethyl	NI	NI	RR									not compatible	s	Biobest
demeton methyl	*B. lucorum*			LD50-24h: 1-2 µg ai/bee										50
				LD50-24h:6-24 µg ai/queen		weakly toxic $	no effect	total loss	50% reduction					Thompson (2001)
	B. pascuorum			LD50-24h: 1-3 µg ai/bee										Thompson (2001)
				LD50-24h: 10-24 µg ai/queen										Thompson (2001)
demeton-S-methyl	*B. terrestris*			LD50-24h: 3.27 µg ai/bee		moderately toxic $	total loss	25-50% reduction	50-75% reduction					Thompson (2001)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects				Compatibility	Route	Ref.
				LD50-72h: 2.68 µg ai/bee									Thompson (2001)
	NI	NI	RR		non-toxic $	no effect	25-50% reduction	50-75% reduction			not compatible	s	Biobest
diazinon	NI	NI	RR								not compatible	s	Biobest
dichlorvos	NI	NI	RR		highly toxic $	no effect	total loss	total loss			remove colonies before product application, retention time of 36h	s; st	Biobest
dimethoate	*B. terrestris*			LD50-24h: 4.1-13 µg ai/bee	non-toxic $	50% reduction	25-50% reduction	50-75% reduction					Thompson (2001)
				LD50-24-72h: 4.7 µg ai/bee									Thompson (2001)
	B. lucorum			LD50-24h: 2-5 µg ai/bee									Thompson (2001)
				LD50-24h: 5-20 µg ai/queen									Thompson (2001)
	B. pascuorum			LD50-24h:0.5-2 µg ai/bee									Thompson (2001)
				LD50-24h: 1-5 µg/quee n	non-toxic $	no effect	25-50% reduction	50-75% reduction					Thompson (2001)
	NI	NI	RR		weakly toxic $	50% reduction	total loss	25-50% reduction			not compatible	s	Biobest
disulfoton	*B. lucorum*			LD50-24h: 2-10 µg/bee									Thompson (2001)
				LD50-24h: > 40 µg/quee n	moderately toxic $	no effect	25-50% reduction	50% reduction					Thompson (2001)
	B. pascuorum			LD50-24h: 1-4 µg/bee									Thompson (2001)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects			Compatibility	Route	Ref.
				LD50-24h: 5-10 µg/queen		weakly toxic $	no effect	25-50% reduction	25-50% reduction			Thompson (2001)
etrimfos	NI	NI	RR							not compatible	s	Biobest
fenitrothion	NI	NI	RR			weakly toxic $	no effect	> 75% reduction	50-75% reduction	not compatible	s	Biobest
malathion	NI	NI	RR							not compatible	s	Biobest
methamidophos	NI	NI	RR							not compatible	s	Biobest
methidathion	NI	NI	RR			weakly toxic $	50% reduction	no effect	> 75% reduction	not compatible	s	Biobest
mevinphos	NI	NI	RR			moderately toxic $	no effect	25-50% reduction	50-75% reduction	remove colonies before product application, retention time of 24h	s	Biobest
naled	NI	NI	RR							remove colonies before product application, retention time of 48h	s	Biobest
	NI	NI	RR			non-toxic $	no effect	no effect		not compatible	f	Biobest
omethoate	NI	NI	RR			moderately toxic $	50-75% reduction	25-50% reduction	no effect	not compatible	s	Biobest
oxy-demeton-methyl	*B. terrestris*	cage test		high mortality at 1200 ml/ha								Thompson & Hunt (1999)
	B. terrestris				LD50-24h: 0.75 µg/bee							
	NI	NI	RR							remove colonies before product application	s	Koppert
parathion	NI	NI	RR							not compatible	s	Biobest
parathion-methyl	NI	NI	RR							not compatible	s	Biobest
phorate	*B. lucorum*			LD50-24h: 1-2 µg/bee								Thompson (2001)
				LD50-24h:6-23 µg/queen								Thompson (2001)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality		Sublethal effects				Compatibility	Route	Ref.
	B. pascuorum			LD50-24h: 1-2 µg/bee								Thompson (2001)
				LD50-24h: 1-5 µg/queen								Thompson (2001)
phosalone	*B. terrestris*			LD50-24h: 5.98 µg/bee; LD50-72h: 4.39 µg/bee	LD50-24h: 3.98-60 µg/bee; LD50-72h: 3.98 µg/bee							de Wael et al. (1995)
		cage test										Gretenkord & Dresher (1993)
	NI	NI	RR							cover the colonies before product application	s	Koppert
phosphamidon	NI	NI	RR							not compatible	s	Biobest
pirimiphos-methyl	NI	NI	RR							not compatible	s	Biobest
profenofos	NI	NI	RR							not compatible	s	Biobest
sulfotep	NI	NI	RR							not compatible	f	Biobest
tetrachlorvinphos	NI	NI	RR							not compatible	s	Biobest
triazophos	NI	NI	RR							not compatible	s	Biobest
trichlorfon	NI	NI	RR							not compatible	s	Biobest
vamidothion	NI	NI	RR							not compatible	s	Biobest
Neonicotinoids												
acetamiprid	*B. ignitus*	individual contact test (air dried)	1: 5000 v/v	16days: highly toxic*								Wu et al. (2010)
		individual oral test			LD50-48h: 2.3 mg/bee							Wu et al. (2010)
	B. hypocrita	individual contact test (air dried)	1: 5000 v/v	16days: highly toxic*								Wu et al. (2010)
		individual oral test			LD50-48h: 2.8 mg/bee							Wu et al. (2010)
	B. patagiatus	individual contact test (air dried)	1: 5000 v/v	16days: highly toxic*								Wu et al. (2010)
		individual oral test			LC50-48h: 2.1 mg/bee							Wu et al. (2010)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects			Compatibility	Route	Ref.
	NI	NI	RR							remove colonies before product application, retention time of 36h	s	Koppert
	NI	NI	RR							compatible	i	Biobest
clothianidin	*B. impatiens*	individual bees treated with potter spray tower	dose range	LC50-48h: 39 mg/l								Scott-Dupree et al. (2009)
imidacloprid	*B.terrestris*	micro-colony	200 (MFRC)		highly toxic $			total loss				Mommaerts et al. (2010b)
			dose-range			LC50-11w: 0.059 mg/l		EC50-11w: 37 µg/l				Mommaerts et al. (2010b)
		micro-colony including foraging	200 (MFRC)		highly toxic $			total loss				Mommaerts et al. (2010b)
			dose-range			20 µg AI/L (LC 50)		EC50-11w: 3.7 µg/l				Mommaerts et al. (2010b)
			dose-range	LD50-24h: 1.3 µg/bee; LD50-48h: 1.15 µg/bee; LD50-72h: 0.05 µg/bee		LD50-24h: 0.02 µg/bee; LD50-48h: 0.02 µg/bee; LD50-72h: 0.23 µg/bee		total loss				van der Steen et al. (2008)
		greenhouse and field	dose-range				no effect on colony development			foraging and homing: no effect		
		field	dose-range						reduced colony life-span 20 µg/l: all workers dead around food area; 10 µg/l: all workers dead in the nest; 2 µg/l safe			Mommaerts et al. (2010b)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects				Compatibility	Route	Ref.
		chronic feeding test of small colonies	0.002-0.005 (µg/bee)				reduced brood production and a lower number of larvae ejected						Tasei et al. (2000), Thompson (2003)
	B. impatiens	individual bees treated with potter spray tower	dose-range	LC50-48h: 322 mg/l									Scott-Dupree et al. (2009)
				72h: 100% mortality									Gradish et al. (2010)
		micro-colony	0.0192 mg/g						no oviposition	reduced life-span and pollen consumption			Gradish et al. (2010)
		field					when not irrigated reduced brood			when not irrigated reduced colony vitality and worker biomass			Gels et al. (2002)
	NI	NI	RR								not compatible	s; i	Biobest
nicotine	NI	NI	RR								remove colonies before product apllication, retention time of 24h (s) and 12h (f)	s; f	Biobest
thiacloprid	*B.terrestris*	micro-colony	120 (MFRC)	highly toxic $				total loss					Mommaerts et al. (2010b)
			dose-range	LC50-11w:18 mg/l				EC50-11w: 12 mg/l					Mommaerts et al. (2010b)
		micro-colony including foraging	12 mg/l	non-toxic $				> 75% reductio n					Mommaerts et al. (2010b)
	NI	NI	RR								remove colonies before product application, retention time of 24h	s; i	Biobest
thiamethoxam	*B.terrestris*	micro-colony	100 (MFRC)	highly toxic $				total loss					Mommaerts et al. (2010b)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality		Sublethal effects				Compatibility	Route	Ref.
					LC50-11w: 0.12 mg/l		EC50-11w: 35 µg/l					Mommaerts et al. (2010b)
		micro-colony including foraging	0.1 mg/l	non-toxic $	highly toxic $		> 75% reduction					Mommaerts et al. (2010b)
	NI	NI	RR							not compatible	s	Biobest
IGRs												
CSI												
buprofezin	*B.terrestris*	micro-colony	75 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	25-50% reduction	50% reduction			Mommaerts et al. (2006a)
			dose-range				harmless	LC50-11w: 69 mg/l	LC50-11w: 79 mg/l			Mommaerts et al. (2006a)
	NI	NI	RR							compatible	s	Biobest
chlorfluazuron	NI	NI	RR	non-toxic $	non-toxic $	non-toxic $	no effect	total loss	> 75% reduction	remove colonies before product application, retention time of 36h	s	Biobest
cyromazine	*B.terrestris*	micro-colony	100 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	LC50-11w: 1.3 mg/l	LC50-11w: 17 mg/l			Mommaerts et al. (2006a)
			dose-range				LC50-11w: 636 mg/l					Mommaerts et al. (2006a)
diflubenzuron	NI	NI	RR	non-toxic $	non-toxic $	non-toxic $	total loss	total loss	total loss	remove colonies before product application, retention time of 36h	s	Biobest
	B.terrestris	micro-colony	288 (MFRC)	non-toxic $	non-toxic $	non-toxic $	total loss	LC50-11w: 0.32 mg/l	LC50-11w: 0.95 mg/l			Mommaerts et al. (2006a)
			dose-range				LC50-11w: 25 mg/1			transovarial transport		Mommaerts et al. (2006a)
		individual contact test	24 961 dpm							24h: 44% cuticular penetration		Mommaerts et al. (2006a)
flucycloxuron	NI	NI	RR							not compatible	s	Biobest
	B.terrestris	micro-colony	125 (MFRC)	non-toxic $	non-toxic $	non-toxic $	> 75% reduction	no effect	total loss			Mommaerts et al. (2006a)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects			Compatibility	Route	Ref.
							LC50-11w: 132mg/l	harmless	LC50-11w: 0.78 mg/l			Mommaerts et al. (2006a)
flufenoxuron	*B.terrestris*	micro-colony	50 (MFRC)	non-toxic $	non-toxic $	non-toxic $	50% reduction	total loss	total loss			Mommaerts et al. (2006a)
			dose-range				LC50-11w: 167 mg/l	LC50-11w: 8.6 mg/l	LC50-11w: 9.3 mg/l			Mommaerts et al. (2006a)
		individual contact test	33 876 dpm						24h: 85% cuticular penetration			Mommaerts et al. (2006a)
	NI	NI	RR							not compatible	s	Biobest
hexaflumuron	NI	NI	RR							not compatible	s	Biobest
lufenuron	*B.terrestris*	micro-colony	50 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	total loss			Mommaerts et al. (2006a)
			dose-range			harmless	harmless	LC50-11w: 218 mg/l				Mommaerts et al. (2006a)
novaluron	NI	NI	RR							remove colonies before product application, retention time of 36h	s	Biobest
	B.terrestris	micro-colony	40 (MFRC)	non-toxic $	non-toxic $	non-toxic $	> 75% reduction	total loss	total loss			Mommaerts et al. (2006a)
			dose-range				LC50-11w: 11 mg/l	LC50-11w: 0.99 mg/l	LC50-11w: 6.2 mg/l			Mommaerts et al. (2006a)
	B.impatiens	individual workers treated with a potter spray tower	dose-range	LC50-48h: > 10 000 mg/l								Scott-Dupree et al. (2009)
	NI	NI	RR							remove colonies before product application, retention time of 48h	s	Biobest
teflubenzuron	*B. terrestris*	micro-colony	150 (MFRC)	non-toxic $	non-toxic $	non-toxic $	total loss	total loss	total loss			Mommaerts et al. (2006a)
			dose-range				LC50-11w: 47 mg/l	LC50-11w: 0.27 mg/l	LC50-11w: 1.7 mg/l			Mommaerts et al. (2006a)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects				Compatibility	Route	Ref.
		small colonies	30 µg/bee						arrested egg develop ment, larval mortality	decreased sucrose intake			de Wael et al. (1995); Thompson (2003)
	NI	NI	RR								not compatible	s	Biobest
JHAs													
fenoxycarb	*B. terrestris*	micro-colony	100 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				Mommaerts et al. (2006b)
	NI	NI	RR								compatible	s	Biobest
kinoprene	*B. terrestris*	micro-colony	650 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect on male production but higher number of larvae ejected	no effect on male production but higher number of larvae ejected	no effect				Mommaerts et al. (2006b)
			dose-range				LC50-11W: 524 x 106 mg/l	LC50-11w: 28 300 mg/l		2 times longer ovaries with more eggs after contact with 0,065 mg/l			Mommaerts et al. (2006b)
	NI	NI	RR										Biobest
methoprene	NI	NI	RR								compatible	s	Biobest
pyriproxyfen	*B. terrestris*	micro-colony	25 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect on male production but higher number of larvae ejected	no effect				Mommaerts et al. (2006b)
		individual contact test	28 907 dpm							24h: 34% cuticular penetration			Mommaerts et al. (2006b)
	NI	NI	RR								compatible	s	Biobest
MACs													
diofenolan	NI	NI	RR								compatible	s	Biobest
halofenozide	*B. terrestris*	individual contact test	5466 dpm							24h: 83% cuticular penetration			Mommaerts et al. (2006b)
methoxyfenozide	*B. terrestris*	micro-colony	96 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				Mommaerts et al. (2006b)
	NI	NI	RR								remove colonies before product application, retention time of 24h	s	Koppert

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects			Compatibility	Route	Ref.
				non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect			
tebufenozide	*B. terrestris*	micro-colony	240 (MFRC)									Mommaerts et al. (2006b)
	NI	NI	RR							compatible	s	Biobest
Biological insecticides												
Adoxophes orana Granulose Virus										compatible	s	Biobest
Bacillus thuringiensis var. aizawai	*B.terrestris*	micro-colony	15000 (MFRC)	non-toxic $	highly toxic $	non-toxic $	no effect	total loss	31% reduction			Mommaerts et al. (2010a)
			1500		non-toxic $			no effect		compatible	s	Biobest
Bacillus thuringiensis var. israelensis		micro-colony including foraging			non-toxic $			no effect		compatible	s	Biobest
Bacillus thuringiensis var. kurstaki	*B.terrestris*	micro-colony	160000 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect			Mommaerts et al. (2010a)
		micro-colony including foraging		non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect	compatible	s; d	Biobest; Mommaerts et al. (2010a)
Bacillus thuringiensis var. tenebrions										compatible	s	Biobest
Beauveria bassiana	*B.terrestris*	micro-colony	2500000000 (MFRC)	highly toxic $	weakly toxic $	non-toxic $	> 75% reduction	no effect	25% reduction			Mommaerts et al. (2009)
		micro-colony including foraging		non-toxic $				53% reduction of offspring; no effect on number of larvae ejected				Mommaerts et al. (2009)
	B.terrestris	individual workers treated with a potter spray tower	dose-range	54%								Hokkanen et al. (2004)
		flowers treated until drip-off	100000000 CFU/ml	30% after 2h 10% after 48h								Hokkanen et al. (2004)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality		Sublethal effects				Compatibility	Route	Ref.
				Transfer from infected to non infected bees occurs in the hive	non-toxic $	no effect	no effect	no effect				Hokkanen et al. (2004)
		field trial with maximum challenge test		15.4%								Hokkanen et al. (2004)
		field trial with treated flowers	100000000 CFU/ml	7%	non-toxic $	no effect	no effect					Hokkanen et al. (2004)
Cydia pomonella granulovirus	*B.terrestris*	micro-colony	6600000000000 (MFRC)	non-toxic $	non-toxic $							Mommaerts et al. (2009)
Metarhizium anisopliae	*B.terrestris*	maximum challenge test		73%								Hokkanen et al. (2004)
		individual bees treated with a potter spray tower	100000000 CFU/ml	63%								Hokkanen et al. (2004)
Paecilomyces fumosoroseus	*NI*	NI	RR							compatible	s	Biobest
spinetoram	*B.terrestris*	contact test	25 (MFRC)	wet: weakly toxic $; dry:moderately toxic $			total loss					Besard et al. (2011)
			dose-range	LC50-72h dry: 20.8 µg/l; LC50-72h wet: 50 µg/l								Besard et al. (2011)
		micro-colony	dose-range		LC50-72h: 20.8 µg/l; LC50-11w: 2.5 µg/l		no effect at 0.25 µg/l					Besard et al. (2011)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality		Sublethal effects			Compatibility	Route	Ref.
		micro-colony including foraging	dose-range	LC50-72h: 13.8 µg/l; LC50-7w: 1.9 µg/l		no effect at 0.25 µg/l					Besard et al. (2011)
Spinosad	*B. terrestris*	contact test	dose-range	LC50-72h dry: 40 µg/l; LC50-72h wet:14.3 µg/l							Besard et al. (2011)
		micro-colony	dose-range	LC50-72h: 80 µg/l; LC50-11w: 1.6 µg/l		no effect at 0.4 µg/l					Besard et al. (2011)
		micro-colony including foraging	dose-range	LC50-72h: 44.4 µg/l; LC50-7w: 3.8 µg/l		no effect at 0.4 µg/l					Besard et al. (2011)
	B. impatiens	individual bee treated with a potter spray tower	dose-range	LC50-48h: 895 mg/l					remove colonies before product application, retention time of 24h	s	Scott-Dupree et al. (2009)
		colony	dose-range				at realistic concentrations (0.2-0.8 µg/g) no effect on colony health	no effect on pollen consumption			Morandin et al. (2005)
		colony + flight cage	dose-range					slower foraging at 0.8 µg/g			Morandin et al. (2005)
Spodoptera exigua NPV	NI	NI	RR						compatible	s	Biobest
Verticillium lecanii	NI	NI	RR						compatible	s	Biobest
Others											
azadirachtin	NI	NI	RR						compatible	s	Biobest
chlorantraniliprole	*B. impatiens*	individual bees treated with a potter spray tower	dose-range	72h: harmless							Gradish et al. (2010)
		micro-colony	0.000615 mg/g				no effect on oviposition and number of ejected larvae	no effect on worker life-span and pollen consumption			Gradish et al. (2010)

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality		Sublethal effects			Compatibility	Route	Ref.
endosulfan					LD50-24h: 3,67 µg/bee; LC50-48h-72h: 1.72 µg/bee						van der Steen et al. (2008)
	NI	NI	RR						not compatible	s	Biobest
formetanate	NI	NI	RR						not compatible	s	Biobest
indoxacarb	NI	NI	RR						remove colonies before product application, retention time of 3d	s	Biobest
lindane	NI	NI	RR						not compatible	s	Biobest
matrine aqueous solution	*B. ignitus*	individual contact test (air dried)	1: 5000 v/v	16days: highly toxic *							Wu et al. (2010)
		individual oral test			LD50-48h: 0.5 mg/bee						Wu et al. (2010)
	B. hypocrita	individual contact test (air dried)	1: 5000 v/v	16days: highly toxic *							Wu et al. (2010)
		individual oral test			LD50-48h: 1.9 mg/bee						Wu et al. (2010)
	B. patagiatus	individual contact test (air dried)	1: 5000 v/v	16days: highly toxic *							Wu et al. (2010)
mineral oil		individual oral test			LC50-48h: 0.5 mg/bee				remove colonies before product application, retention time of 24h	s	Biobest
metaflumizone	*B. impatiens*	individual bees treated with a potter spray tower	dose-range	72h: moderately harmful							Gradish et al. (2010)
		micro-colony	0.003.32 mg/g				no effect on oviposition and number of ejected larvae	no effect on worker life-span and pollen consumption			Gradish et al. (2010)
Na-salts fatty acids	NI	NI	RR						compatibel	s	Biobest
neemoil	NI	NI	RR						compatible	s	Biobest
propargite	NI	NI	RR						compatible	s	Biobest
pymetrozine	NI	NI	RR						compatible	s; i	Biobest

Table 1. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects				Compatibility	Route	Ref.
pyrethrine	NI	NI	RR								remove colonies before product application, retention time of 24h	s	Biobest
rape seed oil	NI	NI	RR								compatible	s	Biobest
rotenone					LD50-24h: 0.38 µg/bee; LD50-72h: 0.36 µg/bee								de Wael et al. (1995)
	NI	NI	RR								remove colonies before product application, retention time of 12h	s	Biobest
thiocyclam	NI	NI	RR								compatible	s	Biobest
triazamate	NI	NI	RR								compatible	s; i	Biobest

Table 1. Overview of the toxicity of insecticides towards *Bombus* species, (NI: no information; RR: recommended rate; $: toxicity according to the IOBC classification for extended laboratory tests; * toxicity according to the IOBC classification for laboratory studies; £: compatibility according to the side-effect list; Route (s=spraying, st=space treatment, i= irrigation, d=dusting, f=fumigation)

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects				Compatibility	Route	Ref.
								Reproduction		Adult			
				contact	oral sugar water	oral pollen	contact	oral sugar water	oral pollen				
Acaricides													
abamectin	*B. terrestris*	micro-colony	18 (MFRC)	highly toxic $	highly toxic $	moderately toxic $	> 75% reduction		total loss				Besard et al. (2011)
			dose range		LC50-11w: 1.17								Besard et al. (2011)
	B. impatiens	individual bees treated with potter spray tower	10-100-1000	moderately toxic at 100 and 1000 mg/l*									Gradish et al. (2010)
		micro-colony	0.0000038 mg/g			no effect			initiation of oviposition was later (p<0.05), no effect on number of ejected larvae	consumed less pollen (p<0.05)			Gradish et al. (2010)
				LD50-72h: 0.14	LD50-72h: 0.07 µg/bee								de wael et al. (1995); Marletto et al. (2003)
	NI	NI	RR								remove colonies before product application, retention time of 24h	s	Biobest
acequinocyl	*B. terrestris*	micro-colony	150 (MFRC)	non-toxic $	non-toxic $	weakly toxic $	50% reduction	25-50% reduction	50-75% reduction				Besard et al. (2011)
	NI	NI	RR								remove adults before product application, retention time of 24h	s	Koppert
amitraz	*B. terrestris*	micro-colony	400 (MFRC)	non-toxic $	highly toxic $	moderately toxic $	no effect	total loss	50-75% reduction				Besard et al. (2011)
	NI	NI	RR								compatible	s	Biobest
azocyclotin	*B. terrestris*	micro-colony	750 (MFRC)	non-toxic $	moderately toxic $	moderately toxic $	no effect	25-50% reduction	50-75% reduction				Besard et al. (2011)
				non-toxic	non-toxic								van der Steen et al. (2008)
	NI	NI	RR								remove colonies before product application, retention time of 36h	s	Biobest
benzoximate	NI	NI	RR								compatible	s	Biobest

Table 2. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality			Sublethal effects			Compatibility	Route	Ref.
				non-toxic $	highly toxic $	weakly toxic $	no effect	total loss	50% reduction			
bifenazate	*B. terrestris*	micro-colony	96 (MFRC)	non-toxic $	highly toxic $	weakly toxic $	no effect	total loss	50% reduction			Besard et al. (2011)
			dose range		LC50-11W: 9.6							Besard et al. (2011)
	NI	NI	RR							compatible	s	Biobest
bifenthrin	*B. terrestris*	micro-colony	30 (MFRC)	highly toxic $	moderately toxic $	moderately toxic $	total loss	25-50% reduction	50-75% reduction			Besard et al. (2011)
			dose range		LC50-11w: 0.36					not compatible	s	Biobest
bromopropylate	*B. terrestris*	micro-colony	500 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	25-50% reduction	50-75% reduction			Besard et al. (2011)
	NI	NI	RR							compatible	s	Biobest
chlorfenapyr	*B. terrestris*	micro-colony	240 (MFRC)	non-toxic $	highly toxic $	highly toxic $	no effect	total loss	total loss			Besard et al. (2011)
clofentazine	*B. terrestris*	micro-colony	150 (MFRC)	non-toxic $	moderately toxic $	non-toxic $	50% reduction	25-50% reduction	50-75% reduction			Besard et al. (2011)
	NI	NI	RR							compatible	s	Biobest
cyhexatin	NI	NI	RR							remove colonies before product application, rentention time of 12h	s	Biobest
diafenthiuron	NI	NI	RR							remove colonies before product application, rentention time of 12h	s	Biobest
dicofol	NI	NI	RR							remove colonies before product application	s	Biobest
dienochlor	*B. terrestris*	micro-colony	500 (MFRC)	non-toxic $	highly toxic $	non-toxic $	no effect	25-50% reduction	50-75% reduction			Besard et al. (2011)
etoxazole	*B. terrestris*	micro-colony	55 (MFRC)	non-toxic $	highly toxic $	weakly toxic $	50% reduction	total loss	25-50% reduction			Besard et al. (2011)
	NI	NI	RR		LC50-11w: 4.4							Besard et al. (2011)
fenazaquin	*B. terrestris*	micro-colony	200 (MFRC)	non-toxic $	weakly toxic $	moderately toxic $	no effect	25-50% reduction	50% reduction			Besard et al. (2011)
	NI	NI	RR							remove colonies before product application, retention time of 12h	s	Biobest
fenbutanin oxide	*B. terrestris*	micro-colony	275 (MFRC)	non-toxic $	weakly toxic $	weakly toxic $	no effect	25-50% reduction	25-50% reduction			Besard et al. (2011)
	NI	NI	RR							compatible	s	Biobest
fenpyroximate	*B. terrestris*	micro-colony	50 (MFRC)	non-toxic $	highly toxic $	weakly toxic $	no effect	> 75% reduction	50-75% reduction			Besard et al. (2011)
	NI	NI	RR							remove colonies before product application, retention time of 36h	s	Koppert

Table 2. Continued

Active ingredient	Bumblebee species	Test method	Test concentration (mg/l)	Worker mortality		Sublethal effects			Compatibility	Route	Ref.
				non-toxic $	weakly toxic $	weakly toxic $	50% reduction	no effect	> 75% reduction				
fipronil	NI	NI	RR							not compatible	s; i	Biobest
flucycloxuron	*B. terrestris*	micro-colony	125 (MFRC)	non-toxic $	weakly toxic $	moderately toxic $	no effect	25-50% reduction	50-75% reduction			Besard et al. (2011)
hexythizox	*B. terrestris*	micro-colony	3 (MFRC)	non-toxic $	non-toxic $	moderately toxic $	no effect	25-50% reduction	50-75% reduction			Besard et al. (2011)
	NI	NI	RR							compatible	s	Biobest
milbemectin	B. terrestris	micro-colony	10 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect				Besard et al. (2011)
pyridaben	B. terrestris	micro-colony	75 (MFRC)	weakly toxic $	highly toxic $	moderately toxic $	50-75% reduction	25-50% reduction	no effect			Besard et al. (2011)
	NI	NI	RR							remove colonies before product application, retention time of 48h	s	Biobest
spirodiclofen	*B. terrestris*	micro-colony	96 (MFRC)	non-toxic $	non-toxic $	weakly toxic $	no effect	25-50% reduction	no effect			Besard et al. (2011)
	NI	NI	RR							not compatible	s	Koppert
spiromesifen	*B. terrestris*	micro-colony	0.8 (MFRC)	non-toxic $	non-toxic $	weakly toxic $	no effect	25-50% reduction	no effect			Besard et al. (2011)
	NI	NI	RR							compatible	s	Koppert
tebufenpyrad	*B. terrestris*	micro-colony	100 (MFRC)	non-toxic $	moderately toxic $	moderately toxic $	no effect	25-50% reduction	50-75% reduction			Besard et al. (2011)
			200 (MFRC)	non-toxic $	moderately toxic $	moderately toxic $	no effect	25-50% reduction	50-75% reduction			Besard et al. (2011)
			10 (MFRC)	non-toxic $	moderately toxic $	moderately toxic $	no effect	25-50% reduction	50-75% reduction			Besard et al. (2011)
			RR	10-30%								van der Steen et al. (2008)
	NI	NI	RR							remove colonies before product application, retention time of 12h	s	Biobest
tetradifon	NI	NI	RR							compatible	s	Biobest

Table 2. Overview of the toxicity of acaricides towards *Bombus* species, (NI: no information; RR: recommended rate; $: toxicity according to the IOBC classification for extended laboratory tests; * toxicity according to the IOBC classification for laboratory studies; £: compatibility according to the side-effect list; Route (s=spraying, i=irrigation)

Active ingredient	Bumblebee species	Test method	Tested concentration	Worker mortality			Sublethal effect				Compatibility	Route	Ref.
							reproduction			adult			
			(mg/l)	contact	oral sugar water	oral pollen	contact	oral sugar water	oral pollen	behaviour			
Chemical fungicides													
azoxystrobin	NI	NI	RR								compatible	s	Biobest
benomyl	NI	NI	RR								compatible	s	Biobest
bitertanol	NI	NI	RR								compatible	s	Biobest
boscalid + pyraclostrobin	*B. terrestris*	micro-colony	520 + 134 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				own unpublished data
bromuconazole	NI	NI	RR								compatible	s	Biobest
bupirimate	NI	NI	RR								compatible	s	Biobest
captan	NI	NI	RR								compatible	s	Biobest
carbendazim	NI	NI	RR								remove colonies before product application, retention time of 24h	s; d	Biobest
carbendazim + diethofencarb	*B. terrestris*	micro-colony	510 + 510 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				own unpublished data
chlorothalonil	NI	NI	RR								compatible	s	Biobest
copper abietate	*B. ignitus*	individual contact test (air-dried)	RR (1:5000 v/v)	30 days:non-toxic									Wu et al. (2010)
	B. patagiatus	individual contact test (air-dried)		30 days: non-toxic									Wu et al. (2010)
	B. hypocrita	individual contact test (air-dried)		30 days: non-toxic									Wu et al. (2010)
copper oxychloride	NI	NI	RR								compatible	s	Biobest
cymoxanil	NI	NI	RR								compatible	s	Biobest
cyproconazole	NI	NI	RR								compatible	s	Biobest
cyprodinil (+ fludioxonil)	*B. impatiens*	individual bees treated with potter spray tower	10-100-1000	non-toxic*									Gradish et al. (2010)

Table 3. Continued

Active ingredient	Bumblebee species	Test method	Tested concentration	Worker mortality			Sublethal effect				Compatibility	Route	Ref.
						no effect			no effect on oviposition and ejected larvea	no effect consumption (pollen)			
		micro-colony											Gradish et al. (2010)
	B. terrestris	micro-colony	375 + 250 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				own unpublished data
	NI	NI	RR								remove colonies before product application, retention time of 12h	s	Biobest
dichlofluanid	NI	NI	RR								compatible	s	Biobest
difenoconazole	*B. ignitus*	individual contact test (air-dried)	RR (1:1 000 v/v)	30 days:non-toxic									Wu et al. (2010)
	B. patagiatus			30 days: non-toxic									Wu et al. (2010)
	B. hypocrita			30 days: non-toxic									Wu et al. (2010)
	NI	NI	RR								compatible	s	Biobest
dimethomorph	NI	NI	RR								remove colonies before product application, retention time of 24h	s	Biobest
dinocap	NI	NI	RR								compatible	s	Biobest
dithianon	NI	NI	RR								compatible	s	Biobest
dodemorph	NI	NI	RR								compatible	s	Biobest
dodine	NI	NI	RR								remove colonies before product application, retention time of 48h	s	Koppert
ethirimol	NI	NI	RR								compatible	s	Biobest
etridiazole	NI	NI	RR								remove colonies before product application, retention time of 96h	s	Koppert
fenarimol	NI	NI	RR								compatible	s	Biobest
fenbuconazole	NI	NI	RR								compatible	s	Biobest

Table 3. Continued

Active ingredient	Bumblebee species	Test method	Tested concentration	Worker mortality			Sublethal effect				Compatibility	Route	Ref.
				non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				
fenhexamid	*B. terrestris*	micro-colony	750 (MFRC)								cover colonies before product application	s	own unpublished data
	NI	NI	RR									s	Biobest
fenpropimorph	NI	NI	RR								compatible	s	Biobest
flusilazole	NI	NI	RR								compatible	s	Biobest
flutriafol	NI	NI	RR								compatible	s	Biobest
folpet	NI	NI	RR								compatible	s	Biobest
fosetyl-aluminium	NI	NI	RR								remove colonies before product application, retention time of 48h	s	Biobest
hexaconazole	NI	NI	RR								compatible	s	Biobest
imazalil	NI	NI	RR								compatible	s	Biobest
iprodione	*B. terrestris*	micro-colony	1500 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				own unpublished data
	NI	NI	RR								compatible	s	Biobest
kresoxim-methyl	NI	NI	RR								cover colonies before product application	s	Koppert
mancozeb	NI	NI	RR								compatible	s	Biobest
maneb	NI	NI	RR								compatible	s; i	Biobest
mepanipyrim	*B. terrestris*	micro-colony	300 (MFRC)	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				own unpublished data
	NI	NI	RR								compatible	s	Biobest
metalaxyl	NI	NI	RR								compatible	s	Biobest
metiram	NI	NI	RR								compatible	s	Biobest
myclobutanil	*B. impatiens*	individual bees treated with potter spray tower	10-100-1000	non-toxic*									Gradish et al. (2010)
		micro-colony	0.011 mg/g			no effect			no effect on oviposition and ejected larvea	no effect on pollen consumption			Gradish et al. (2010)
	NI	NI	RR								compatible	s	Biobest
nuarimol	NI	NI	RR								compatible	s	Biobest
oxycarboxin	NI	NI	RR								compatible	s	Biobest

Table 3. Continued

Active ingredient	Bumblebee species	Test method	Tested concentration	Worker mortality			Sublethal effect				Compatibility	Route	Ref.
penconazole	NI	NI	RR								remove colonies before product application, retention time of 12h	s	Biobest
potasium bicarbonate	*B. impatiens*	micro-colony	0.081 mg/g			no effect	no effect on oviposition and ejected larvea	no effect on pollen consumption					Gradish et al. (2010)
procymidone	NI	NI	RR								compatible	s	Biobest
propamocarb	NI	NI	RR								compatible	s	Biobest
propiconazole	NI	NI	RR								compatible	s	Biobest
propineb	NI	NI	RR								compatible	s	Biobest
pyrazofos	NI	NI	RR								remove colonies before product application, retention time of 24h	s	Biobest
pyrifenox	NI	NI	RR								compatible	s	Biobest
pyrimethanil	NI	NI	RR								cover colonies before product application	s	Koppert
sulphur	NI	NI	RR								compatible	s; d; f	Biobest
tebuconazole	NI	NI	RR								remove colonies before product application, retention time of 24h	s	Biobest
tetraconazole	NI	NI	RR								compatible	s	Biobest
thiophanate-methyl	NI	NI	RR								compatible	s	Biobest
thiram	NI	NI	RR								compatible	s	Biobest
tolyfluanid	NI	NI	RR								compatible	s	Biobest
triadimefon	NI	NI	RR								compatible	s	Biobest
triadimenol	NI	NI	RR								compatible	s	Biobest
trifloxystrobine	NI	NI	RR								compatible	s	Koppert
triflumizole	NI	NI	RR								compatible	s	Biobest
triforine	NI	NI	RR								compatible	s	Biobest
vinclozolin	NI	NI	RR								compatible	s	Biobest
zineb	NI	NI	RR								compatible	s	Biobest
											not compatible	s; d; di	Biobest

Table 3. Continued

Active ingredient	Bumblebee species	Test method	Tested concentration	Worker mortality			Sublethal effect				Compatibility	Route	Ref.
Biological fungicides													
			CFU/l										
Ampelomyces quisqualis M-10	*B. terrestris*	micro-colony	3,5E+08	weakly toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				Momma erts et al. (2009)
		micro-colony including foraging			non-toxic $			no effect		no effect			Momma erts et al. (2009)
Hypocrea parapilulifera + Trichoderma atroviride; 1/1	*B. terrestris*	micro-colony	1,3E+05	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				Momma erts et al. (2009)
	B. terrestris	micro-colony	1,3E+05	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				Momma erts et al. (2009)
	B. terrestris	micro-colony	1,3E+06	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				Momma erts et al. (2009)
		micro-colony including foraging			non-toxic $			no effect		no effect			Momma erts et al. (2009)
Gliocladium catenulatum J1446	*B. terrestris*	micro-colony	7,5E+08	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				Momma erts et al. (2009)
		micro-colony including foraging			non-toxic $			no effect		no effect			Momma erts et al. (2009)
Bacillus subtilis QST713	*B. terrestris*	micro-colony	7,5E+10	highly-toxic $	highly-toxic $	non-toxic $	total loss	total loss	no effect				Momma erts et al. (2009)
Trichoderma harzianum T22	*B. terrestris*	micro-colony	6,0E+08	non-toxic $	non-toxic $	non-toxic $	no effect	no effect	no effect				Momma erts et al. (2009)
		micro-colony including foraging			non-toxic $			no effect		no effect			Momma erts et al. (2009)

Table 3. Overview of the toxicity of fungicides towards *Bombus* species, (NI: no information; RR: recommended rate; $: toxicity according to the IOBC classification for extended laboratory tests; * toxicity according to the IOBC classification for laboratory studies; £: compatibility according to the side-effect list; Route (s=spraying, i= irrigation, d=dusting, di= dipping; f=fumigation)

standardized (selection of a particular instar for exposure) and to test more concentrations in parallel. Similarly, also the field of behavioral changes lacks proper laboratory methods to assess behavioral changes in a lower tier. Here the development of a PER bioassay would allow to assess the impact on the memory and learning capacity of individual insects already in "tier 1". Furthermore, it is likely that also other endpoints will be identified for risk assessments due to the increasing knowledge of the insect body and its processes and because it is to be expected that new active substance will be found with other modes of action.

The obtained data showed that older insecticides (carbamates, pyrethroids and organophosphates) are more toxic than novel insecticides (IGRs, neonicotinoids and biological insecticides). Also low hazards can be expected based on the data for fungicides, whereas for the acaricides the side-effects are strongly dependent on the route of exposure. In addition, it was clear that over the different groups of PPPs bumblebees are in general less sensitive to pesticide toxicity than honeybees. However, the power of the linear regression between the LD_{50}-24h values of 17 insecticides in *B. terrestris* versus honeybees was poor. In conclusion, the identification and especially the knowledge of the consequences of sublethal effects for populations will lead to the development of IPM programs with low risks for pollinators. Reaching all these goals may be of little help if they are not accompanied by a proper communication with cultivators and farmers in the field.

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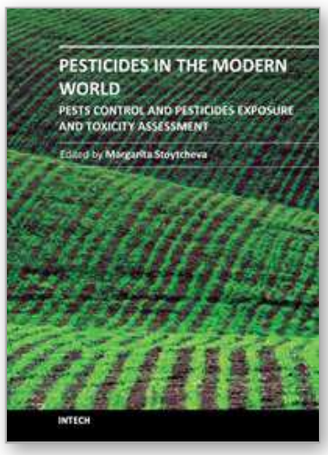

Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment

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The present book is a collection of selected original research articles and reviews providing adequate and up-to-date information related to pesticides control, assessment, and toxicity. The first section covers a large spectrum of issues associated with the ecological, molecular, and biotechnological approaches to the understanding of the biological control, the mechanism of the biocontrol agents action, and the related effects. Second section provides recent information on biomarkers currently used to evaluate pesticide exposure, effects, and genetic susceptibility of a number of organisms. Some antioxidant enzymes and vitamins as biochemical markers for pesticide toxicity are examined. The inhibition of the cholinesterases as a specific biomarker for organophosphate and carbamate pesticides is commented, too. The third book section addresses to a variety of pesticides toxic effects and related issues including: the molecular mechanisms involved in pesticides-induced toxicity, fish histopathological, physiological, and DNA changes provoked by pesticides exposure, anticoagulant rodenticides mode of action, the potential of the cholinesterase inhibiting organophosphorus and carbamate pesticides, the effects of pesticides on bumblebee, spiders and scorpions, the metabolic fate of the pesticide-derived aromatic amines, etc.

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