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A meta-analysis comparing the sensitivity of bees to pesticides

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Abstract The honey bee *Apis mellifera*, the test species used in the current environmental risk assessment procedure, is generally considered as extremely sensitive to pesticides when compared to other bee species, although a quantitative approach for comparing the difference in sensitivity among bees has not yet been reported. A systematic review of the relevant literature on the topic followed by a meta-analysis has been performed. Both the contact and oral acute LD_{50} and the chronic LC_{50} reported in laboratory studies for as many substances as possible have been extracted from the papers in order to compare the sensitivity to pesticides of honey bees and other bee species (Apiformes). The sensitivity ratio R between the endpoint for the species *a* (*A. mellifera*) and the species *s* (bees other than *A. mellifera*) was calculated for a total of 150 case studies including 19 bee species. A ratio higher than 1 indicated that the species *s* was more sensitive to pesticides than honey bees. The meta-analysis showed a high variability of sensitivity among bee species (*R* from 0.001 to 2085.7), however, in approximately 95 % of the cases the sensitivity ratio was below 10. The effect of pesticides in domestic and wild bees is dependent on the intrinsic sensitivity of single bee species as well as their specific life cycle, nesting activity and foraging behaviour. Current data indicates a need for more comparative information between honey bees and non-*Apis* bees as well as separate pesticide risk assessment procedures for non-*Apis* bees.

Keywords Toxicity · Environmental risk assessment · *Apis mellifera* · Apiformes · Pollinators · Comparative ecotoxicology

Introduction

Bees, including managed and wild bees, play an important role in the creation and conservation of biodiversity providing important pollination services to most wild plant species (Kwak et al. 1998; Ashman et al. 2004). They are also economically important as several crops depend partially, or completely, on their pollination for fruit and seed production. In Europe, 84 % of productive crop species depend, at least to some extent, upon animal pollination (Williams 1994). Crop pollination has become a model system for the study of ecosystem services because crop plants accounting for 35 % of the global food supply (in terms of Metric tonnes of food production) require animal-mediated pollination (Klein et al. 2007). The direct and indirect benefits provided by bees to agriculture are estimated at $10–33 billion annually in the United States.

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Overall, the ecosystem service of pollinators was estimated to an annual value of 153 billion Euros, which is equivalent to 9.5 % of the total value of world agricultural production for human consumption (Gallai et al. 2009).

Although many species are known to provide pollination services, in many parts of the world crop pollination relies solely on a single domesticated pollinator species, the European honey bee *Apis mellifera* (Winfree et al. 2007). Honey bees remain the most economically valuable pollinator of crop monocultures worldwide and their absence can cause a 90 % decrease in the yield of some fruit, seed and nut crops. Although honey bees are versatile, cheap, convenient and easy to manage compared to several wild bees, for some crops they are not the most effective pollinators on a per flower basis (Klein et al. 2007). Wild bees are reported to be the main pollinators for many crops and often their pollination service cannot be replaced by honey bees (Klein et al. 2007). Visitation by wild and domesticated bees promotes fruit set independently, thus, honey bees foraging on flower supplements, rather than substitutes, pollination by wild bees (Garibaldi et al. 2013). For this reason, relying only on single pollinator species can constitute a risk as it exposes crop pollination to various decline factors such as parasites and diseases which are species-specific (Winfree et al. 2007). Apart from the domesticated bee *A. mellifera*, there are at least 16,000 other described bee species globally but only few of them are managed commercially as crop pollinators (Bosch et al. 2008).

Unfortunately, in the last decades, a number of sources have reported that the abundance of wild bees has declined simultaneously to the losses of honey bees in numerous countries worldwide (Committee on the Status of Pollinators in North America 2007; Neumann and Carreck 2010; Potts et al. 2010). At the same time, the decline of the pollination service and the degradation of the interaction network between plants and pollinator insects have been reported both in Europe and in North America (Biesmeijer et al. 2006; Burkle et al. 2013). Many factors, including pathogens, parasites, availability of resources due to habitat fragmentation and loss, climate change and pollutants, acting singularly or simultaneously, are suggested to contribute to the explanation of this phenomenon (Neumann and Carreck 2010; Potts et al. 2010). Although the role and interaction of these factors is still unclear and under evaluation, the extensive use of chemical pesticides against pest insects for crop protection may have contributed to the loss of pollinators (Maini et al. 2010; Blacquiere et al. 2012).

The honey bee is generally considered as extremely sensitive to pesticides compared to other insect species, making this species a good environmental indicator of pesticide pollution (Porrini et al. 2003). The high sensitivity of honey bees seems to be confirmed by the lower number of genes encoding xenobiotic detoxifying enzymes in the *A. mellifera* genome compared with other insects species (Claudianos et al. 2006). However, within the group of bees (Apiformes), the deficit of detoxification genes may not be exclusive of *A. mellifera* but rather be a specific adaptation in the eusocial insects. In the last years, some reviews aiming at comparing the sensitivity of *A. mellifera* to pesticides, as well as that of other bees or even other insects, have already been published (Tasei 2002; Devillers et al. 2003; Hardstone and Scott 2010). Although these papers showed a wide range of sensitivity both among bee species and between honey bees and other insects, a quantitative approach for comparing the difference in sensitivity has yet to be performed.

Following the decline of pollinators, concerns have been raised by different levels of the society, including regulators and governments from EU member states, members of the European Parliament and beekeepers, on the appropriateness of the current risk assessment scheme (EPPO/OEPP 2010; European Commission 2002) for the approval and authorisation of pesticide active substances. The current risk assessment scheme focuses on *A. mellifera* and suggests then to extrapolate data from honey bees to other bee species but without any specification of how to account for the inter-specific differences. As highlighted in the EFSA Opinion on the science behind the development of a risk assessment of plant protection products on bees (EFSA 2012), the extrapolation of a risk assessment with honey bees to other bees may not be appropriate. In fact, both the differences in the level of exposure and the response to pesticides may vary significantly between different bee taxa.

The aim of this work was to compare the sensitivity of *A. mellifera* and other bee species (Apiformes) to pesticides in order to fix a reliable assessment factor, which can assure the maximum level of protection of other species of bees as *A. mellifera* is used as surrogate in environmental risk assessment of pesticides.[1]

To reach the goal we performed a meta-analysis, a well-established statistical methodology used to find generalities in collections of studies that have varied or conflicting outcomes (Cresswell 2011), of both the contact and oral acute LD_{50} and the chronic LC_{50} reported in toxicological laboratory studies for pesticides.

[1] In 2011, EFSA received a mandate by the EU Commission to develop a Guidance Document on the risk assessment of plant protection products on bees, including honey bees, bumblebees and solitary bees. The EFSA proposal was published in July 2013 (http://www.efsa.europa.eu/it/efsajournal/pub/3295.htm) but to be adopted and implemented, the new Guidance Document have to be approved by the EU Member States and the EU Commission. At the time of the publication of this paper the process was on going.

Only the endpoints regarding adult bees were considered for the analysis due to the lack of data on larval stages.

Materials and methods

Literature search

A systematic review (SR) was conducted to find the relevant literature on the topic (EFSA 2010). The main scope of the SR is to ensure transparency and reproducibility by well-documenting each single step.

The first step is the definition of a clear and explicit scientific question to be addressed in the systematic review. The review question which was considered as the most appropriate for the scope of the review was: 'Are honey bees more sensitive than other species of bees when exposed to pesticides?' Being a closed-framed question on the effects of an intervention, the four key elements are (PICO): honey bees as population of interest (P), pesticides as intervention of interest (I), other species of bees as comparator (a control or reference intervention—C) and the effects as the outcomes of interest (O).

As second step, a search strategy has been designed. The literature search was carried out in ISI Web of Knowledge using the option "All Databases" (comprising Web of Science 1975–2013, Current Contents Connect 1998–2013, CABI: CAB Abstracts 1910–2013, FSTA®—the food science resource 1969–2013, MEDLINE 1950–2013, Journal Citation Reports) and the following terms and formula: '=((bee* OR honeybee* OR Apis OR bumblebee* OR Bombus OR "solitary bee*" OR Osmia OR Megachile OR Nomia OR Stingless) AND (pesticide* OR PPP OR plant protection product* OR agrochemical* OR insecticide*) AND (toxicity OR LD_{50} OR lethal* OR LC_{50}))'. Grey literature was also searched using Google scholar while Draft Assessment Reports for the approval of pesticide active substances (DARs) were obtained through the EFSA website. No limitations of dates and languages were applied to the search strategy.

For the purpose of a systematic review, the inclusion/exclusion criteria were set a priori. From a total of 1,405 references, a first selection of relevant papers was made using title and abstracts; this process involved two reviewers. The endpoints from a paper were identified for inclusion in the analysis if a pair of LD_{50} contact or/and LD_{50} oral or/and LC_{50} values was available in the same study for *A. mellifera* and at least another bee species. The LD_{50} was expressed as µg/bee, while the LC_{50} as µg/g of active substance. Due to the limited number of studies dealing with more than one species, those papers where only one species was tested were also included and the comparison of the same endpoint was considered consistent for the meta-analysis purpose only in case the same experimental design was applied in the selected studies.

It was decided to focus on laboratory test studies as more standardised experimental designs are available for comparison than in semi-field and field studies. Review studies have been used to cross-check the search strategy results aiming at including as many relevant publications as possible (Thompson 2001; Tasei 2002; Devillers et al. 2003; Hardstone and Scott 2010).

Finally, we selected endpoints from 53 pesticides of different chemical classes (carbamates, neonicotinoids, organochlorines, organophosphates, pyrethroids and miscellaneous), included in 44 publications, i.e. 32 papers, 9 DARs, 2 reports of the European Commission and 1 report of the World Health Organization (WHO).

Data extraction

Data from the selected publications were extracted and entered into a database including several variables: active substance, bee species, treatment specifications (exposure, endpoint, time-scale), value of the endpoint and references (see Supplementary Material). A dataset including the contact LD_{50} of 47 substances, the oral LD_{50} of 19 substances and the LC_{50} of 3 substances to 19 different species (*Apis* spp., stingless bees, bumblebees and solitary bees), was constructed.

Data analysis

The sensitivity ratio R between the endpoint for the species *a* (*A. mellifera*) and the species *s* (other than *A. mellifera*) ($R = LD_{50}a/LD_{50}s$ or $LC_{50}a/LC_{50}s$) was calculated for a total of 150 case studies. A case study is defined as a unique pair combination of pesticide, endpoint and exposure for *A. mellifera* and any other bee species. A pesticide was considered suitable for the meta-analysis only if the same endpoint values (LD_{50} contact or/and LD_{50} oral or/and LC_{50}) were available in the same study for *A. mellifera* and at least another bee species. The data were also considered suitable when reported in different studies, assuring that the same time-scale and the same experimental design were applied. In the latter case, when more than one value was available in different studies the lowest LD_{50} or LC_{50} value was selected for the comparative analysis. A ratio of 1 indicated that the species *s* had the same sensitivity to pesticide as *A. mellifera*. A ratio higher than 1 indicated that the species *s* was more sensitive to pesticides than *A. mellifera*.

Statistical analyses

For each species and systematic group of bees, as well for each substance and chemical class, the median, the range

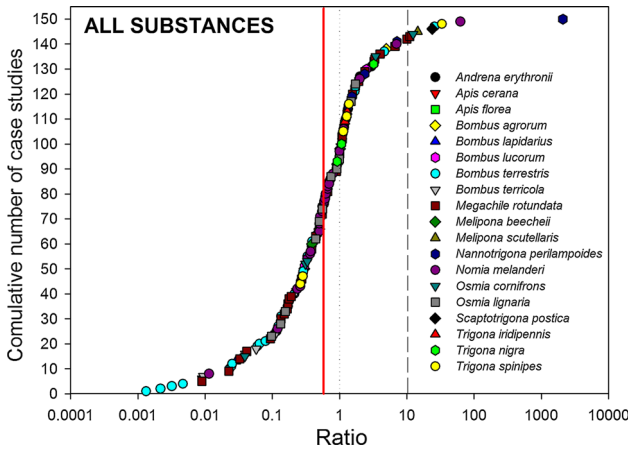

Fig. 1 Overall distribution of the sensitivity ratios *R* for all bee species. A ratio of 1 indicates that the species *s* has the same sensitivity to pesticide as *Apis mellifera*, lower values indicate higher sensitivity of honey bees. The *solid line* and the *dashed line* indicate the median and the 95th percentile, respectively

(min, max) and the 95th percentile *R* values have been calculated. Statistical correlation between the LD_{50} or LC_{50} values of the species *a* (*A. mellifera*) and the species *s* (other bees) was investigated using Spearman's correlation.

Results

The overall sensitivity ratio *R* (considering all bee species and pesticides) showed a high variability among cases, ranging from 0.001 to 2085.7 (Fig. 1). However, the median *R* was 0.57, meaning that the species *a* (*A. mellifera*) was often more sensitive to pesticides than species *s* (other bee species). The species *s* showed a higher sensitivity to pesticides than *A. mellifera* in 53 out of 150 cases (35.3 %) and only in 8 cases (5.3 %) the *R* was higher than 10. Considering the single bee species, the median *R* was higher than 1 (the species *s* more sensitive than species *a*) in 9 out of 19 species (Table 1), and in particular for: the Asian honey bees, *Apis cerana* and *Apis florea*; the stingless bees (Meliponini), *Melipona scutellaris*, *Nannotrigona perilampoides*, *Scaptotrigona postica*, *Trigona iridipennis*, *Trigona nigra* and *Trigona spinipes*, and the solitary bee *Andrena erythronii*. Among the groups of bees the median *R* increased in the following order: Andreninae > Meliponini > Apini > Nomiinae > Megachilini > Osmiini > Bombini. When looking at the 95th percentile, the sensitivity among the bee groups increased in the order Meliponini > Megachilini > Osmiini > Nomiinae > Bombini > Andreninae > Apini. Considering the substances singularly, the median *R* was higher than 1 in 20 out of 53 substances (Table 2). Across the six chemical classes of pesticides, the species *s* were more sensitive than honey bees (median *R* values higher than 1) only for neonicotinoids. Based on the median *R* values, the chemical groups ranked: neonicotinoids > miscellaneous > organochlorines > carbamates > organophosphates > pyrethroids. Within each chemical class, the *R* values were higher than 10 in two organochlorines (22.2 % of the cases), two miscellaneous (13.3 %), two neonicotinoids (11.1 %), one pyrethroid (5.3 %) and one organophosphate (1.6 %). In the case of carbamates, the *R* values were always lower than 10 (Fig. 2).

For the acute contact LD_{50}, a total number of 111 case studies were identified, including 47 substances and 18 bee species (Supplementary Material). A significant positive correlation was found between the LD_{50} values of species *a* and species *s* (Spearman's rank correlation $r_s = 0.75$, $p < 0.05$). The median *R* was 0.63, indicating that the other bee species were overall less sensitive than *A. mellifera*. The sensitivity ratios ranged from 0.002 to 2085.7 but only in 6.3 % of the cases the *R* values were higher than 10 (Fig. 3). Only in 7 cases the comparison of the acute contact LD_{50} values showed a tenfold higher sensitivity for a bee species other than *A. mellifera* and specifically: acetamiprid to *Osmia cornifrons* (12-fold), cyhalothrin to *Megachile rotundata* (11-fold), endosulfan to *T. spinipes* (33-fold), fipronil to *M. scutellaris* (14-fold) and *S. postica* (24-fold), thiacloprid to *N. perilampoides* (2,086-fold) and toxaphene to *Nomia melanderi* (63-fold).

Comparisons of the acute oral LD_{50} were made for 33 cases, including 19 substances and 5 bee species (Supplementary Material). A significant positive correlation was found between the LD_{50} values of *A. mellifera* and the other bee species (Spearman's rank correlation $r_s = 0.60$, $p < 0.05$). The sensitivity ratios ranged from 0.001 to 25.88 and only in one comparison (3.0 % of the cases) the R value was higher than 10. The median sensitivity ratio was 0.39 (Fig. 4). Only for the substance phosalone, *A. mellifera* showed to be less sensitive than *Bombus terrestris* with a factor higher than 10 (26-fold).

The chronic oral LC_{50}s were available only for 3 substances and two species (Supplementary Material). No significant correlation was found between the LC_{50}s of the species *s* and species *a* (Spearman's rank correlation $r_s = 0.67$, $p > 0.05$). The median sensitivity ratio was 0.85 (range 0.44–1.15), implying that chronic sensitivity between *A. mellifera* and the other bee species (*M. rotundata* and *N. melanderi*) was similar (Fig. 5).

Discussion and conclusions

The group of Apiformes or bees (superfamily Apoidea), comprises 7 families and more than 16,000 species

Table 1 Median, min, max and 95th percentile of the sensitivity ratio for the all bees, for each systematic group and for each single species

Family (subfamily/tribe)[a]	Species	Number of cases	Sensitivity ratio ($LD_{50}a/LD_{50}s$)		
			Median	Range (min–max.)	95th Percentile
7 Systematic groups	19 Species	150	0.57	0.001–2085.7	10.55
Andrenidae (Andreninae)	1 Species	6	1.47	0.709–3.00	2.75
	Andrena erythronii Robertson	6	1.47	0.709–3.00	2.75
Apidae (Apinae/Apini)	2 Species	5	1.09	1.040–1.51	1.45
	Apis cerana Fabricius	3	1.09	1.040–1.51	1.47
	Apis florea Fabricius	2	1.14	1.089–1.18	1.18
Apidae (Apinae/Bombini)	5 Species	45	0.21	0.001–25.88	4.20
	Bombus agrorum (Fabricius)	3	0.5	0.30–5.00	4.55
	Bombus lapidarius (L.)	1	0.03		
	Bombus lucorum (L.)	3	0.5	0.300–2.50	2.30
	Bombus terrestris (L.)	32	0.20	0.001–25.88	3.02
	Bombus terricola Kirby	6	0.05	0.009–0.23	0.20
Apidae (Apinae/Meliponini)	7 Species	22	1.29	0.263–2085.7	32.92
	Melipona beecheii Bennett	3	0.92	0.390–1.02	1.01
	Melipona scutellaris Latreille	1	14.46		
	Nannotrigona perilampoides (Cresson)	6	1.95	1.157–2085.7	1566.1
	Scaptotrigona postica (Latreille)	1	24.07		
	Trigona iridipennis Smith	2	1.25	1.196–1.30	1.29
	Trigona nigra Provancher	3	1.07	0.917–3.23	3.01
	Trigona spinipes (Fabricius)	6	1.21	0.263–33.38	25.38
Halictidae (Nomiinae)	1 Species	27	0.59	0.012–62.61	5.52
	Nomia melanderi Cockerell	27	0.59	0.012–62.61	5.52
Megachilidae (Megachilinae/Megachilini)	1 species	29	0.55	0.009–11.00	8.67
	Megachile rotundata (Fabricius)	29	0.55	0.009–11.00	8.67
Megachilidae (Megachilinae/Osmiini)	2 Species	16	0.53	0.039–12.42	5.69
	Osmia cornifrons (Radoszkowski)	5	0.33	0.039–12.42	10.63
	Osmia lignaria Say	11	0.56	0.097–1.72	1.60

[a] Michener (2007)

(Michener 2007) each with different life cycles, behavioral, morphological and physiological features. Even though there is a wide variability among bees, only a few species have been used in ecotoxicological studies. In this meta-analysis study we compared the sensitivity of 19 species of bees to pesticides in relation with the sensitivity of *A. mellifera*, which is the standard test species used for the risk assessment of pesticides (EPPO/OEPP 2010; European Commission 2002; Regulation (EC) 544/2011). By the assessment of 150 case studies we demonstrated a high variability of sensitivity when comparing *A. mellifera* with all the other bee species but in approximately 95 % of the cases the sensitivity ratio *R* was below 10. The sensitivity ratios ranged from 0.001 to 2085.7 with a median value of 0.57, indicating that in most cases the sensitivity of *A. mellifera* was higher than other bee species. However, in 35.3 % of the cases the sensitivity of other bee species was higher than *A. mellifera* and, in 5.3 % of the cases, it was tenfold higher. Considering the acute contact toxicity, the median *R* was 0.63 and only in 7 cases it was higher than 10, indicating that an assessment factor of 10 would cover the 94 % of the cases when LD_{50} contact from *A. mellifera* is used in the pesticide risk assessment. Concerning the contact exposure, this assessment factor could be considered sufficiently robust due to the large number of case studies (111), the high number of different species (18) and substances (47) included in our dataset.

The median *R* calculated with the acute oral LD_{50} values showed some uncertainties as it was based on fewer cases (33), bee species (5) and substances (19). Moreover, the different modality of feeding between social and solitary bees (group *vs* individual feeding) makes the comparison among bee species more difficult for the oral toxicity tests than for the acute contact toxicity tests where all bees are

Table 2 Median, min, max and 95th percentile of the sensitivity ratio for the all substances, for each chemical class and for each single substance

Chemical class	Substances	Number of cases	Sensitivity ratio ($LD_{50}a/LD_{50}s$)		
			Median	Range (min–max.)	95th Percentile
6 Chemical groups	53 Substances	150	0.57	0.001–2085.7	10.55
Carbamates	8 Substances	27	0.59	0.009–2.37	1.81
	Aldicarb	4	0.41	0.235–0.80	0.75
	Aldicarb sulfone	4	0.74	0.332–2.37	2.15
	Aldicarb sulfoxide	4	1.12	0.699–1.29	1.27
	Aminocarb	2	1.03	0.039–2.02	1.92
	Carbaryl	3	0.06	0.009–0.71	0.64
	Methomyl	7	0.39	0.023–1.33	1.28
	Mexacarbate	2	0.58	0.058–1.11	1.06
	Pirimicarb	1	0.47		
Neonicotinoids	4 Substances	18	1.06	0.005–2085.7	323.42
	Acetamiprid	1	12.42		
	Imidacloprid	12	0.96	0.005–2.36	1.78
	Thiacloprid	1	2085.71		
	Thiamethoxam	4	1.14	1.089–1.53	1.48
Organochlorines	5 Substances	9	0.67	0.094–62.61	50.92
	DDT	2	3.56	0.094–7.03	6.68
	Dieldrin	3	0.26	0.167–1.28	1.18
	Endosulfan	1	33.38		
	Lindane	1	0.26		
	Toxaphene	2	31.64	0.667–62.61	59.51
Organophosphates	23 Substances	62	0.50	0.001–25.88	4.95
	Acephate	1	0.001		
	Carbophenothion	1	10		
	Chlorpyrifos-ethyl	1	0.02		
	Chlorpyrifos-methyl	2	0.99	0.289–1.69	1.62
	Demeton	2	0.34	0.171–0.50	0.48
	Demeton-methyl	2	0.50	0.50–0.50	0.50
	Demeton-*S*-methyl	1	0.08		
	Diazinon	7	0.92	0.151–1.92	1.69
	Dicrotophos	2	2.17	1.00–3.33	3.22
	Dimethoate	13	0.39	0.065–3.44	2.31
	Disulfoton	2	3.75	2.500–5.00	4.88
	Fenitrothion	2	0.97	0.226–1.71	1.64
	Malathion	2	2.28	0.556–4.00	3.83
	Mevinphos	2	0.30	0.009–0.59	0.56
	Naled	2	0.27	0.033–0.50	0.48
	Oxydemeton-methyl	3	0.37	0.023–1.33	1.24
	Parathion	2	1.10	0.191–2.00	1.91
	Phorate	2	0.30	0.30–0.30	0.30
	Phosalone	3	1.72	0.736–25.88	23.46
	Phosmet	1	0.31		
	Phosphamidon	2	3.52	0.370–6.67	6.35
	TEPP	2	0.33	0.042–0.63	0.60
	Trichlorfon	5	0.27	0.009–1.24	1.09

Table 2 continued

Chemical class	Substances	Number of cases	Sensitivity ratio ($LD_{50}a/LD_{50}s$)		
			Median	Range (min–max.)	95th Percentile
Pyrethroids	7 Substances	19	0.33	0.002–11.00	7.53
	Alpha-cypermethrin	2	0.15	0.115–0.18	0.17
	Cyfluthrin	2	0.20	0.002–0.39	0.37
	Cyhalothrin	2	5.81	0.611–11.00	10.48
	Cypermethrin	1	0.28		
	Deltamethrin	2	0.07	0.003–0.13	0.13
	Lambda-cyhalothrin	3	0.33	0.214–4.62	4.19
	Permethrin	7	1.38	0.112–7.14	5.97
Miscellaneous	6 Substances	15	0.91	0.012–24.07	17.35
	Captan	2	0.87	0.742–1.00	0.98
	Diafenthiuron	1	1.51		1.51
	Fipronil	4	8.86	0.012–24.07	22.63
	Propiconazole	3	1.48	0.910–1.72	1.70
	RH7988	2	0.61	0.550–0.67	0.66
	Spinosad	3	0.13	0.120–0.29	0.27

treated topically (Ladurner et al. 2003). However, with the data available up to date for oral exposure, in most cases the sensitivity of *A. mellifera* was higher than other bees (median $R = 0.39$) and in 97 % of the cases the R was lower than 10.

Similarly, the median *R* calculated for the chronic toxicity showed more uncertainties as it is based only on 6 cases, 2 species and 3 substances, however, in all these cases, the sensitivity of other bee species was lower or similar to honey bees (median $R = 0.85$).

In Scott-Dupree et al. (2009), the direct contact toxicity of two neonicotinoids, clothianidin and imidacloprid, was determined in three species of non-*Apis* bees (*Bombus impatiens*, *M. rotundata* and *Osmia lignaria*) using a Potter spray tower. These data were not included in our dataset because this modality of exposure is not comparable with the topical application in the acute toxicity test. However, comparing the LC_{50}s (expressed as percentage of solution, wt:vol) obtained for each bee and compound in Scott-Dupree et al. (2009) with the data on honey bees from Bailey et al. (2005) using the same protocol, we found a R higher than 10 only for imidacloprid in *M. rotundata* (13-fold) and *O. lignaria* (31-fold). *B. impatiens* was more tolerant to the direct contact applications than *M. rotundata* and *O. lignaria*, and showed *R* values lower than 1 for both neonicotinoids.

Overall, based on the results and considering, for both the acute contact and oral LD_{50}, the similar trend of values in honey bees and in the other bee species, it can be concluded that when *A. mellifera* is used as surrogate test species in environmental risk assessment, an assessment factor of 10 applied to honey bees LD_{50} endpoints would also be protective for other bee species LD_{50} in 95 % of the cases.

However, we acknowledge some limitations in our analysis, such as a limited dataset for the chronic toxicity test endpoint (LC_{50}) and the lack of data related to the larval stage. For this reason, it was not possible to also establish an assessment factor for the chronic LC_{50} in adults and the larvae endpoints.

When looking at the median and 95th percentile *R* values, stingless bees (*M. scutellaris*, *N. perilampoides*, *S. postica*, *T. iridipennis*, *T. nigra* and *T. spinipes*) compared to honey bees appeared to be more sensitive to pesticides than other bee species. However, it is difficult to draw a conclusion and identify the most sensitive species due to the low number of cases available for several taxonomic groups of bees. Although we collected data related to 19 different bee species, included in 7 systematic groups, most of the case studies are only allocated to four species: *B. terrestris*, *M. rotundata*, *N. melanderi*, *O. lignaria*. Overall (considering the median value of *R*), bumblebees are less sensitive than honey bees, followed by the solitary bees, even though, in some cases, the sensitivity of these species can be tenfold higher than honey bees. The observed differences in sensitivity of bee species to pesticides are difficult to explain. Within a single species, a correlation between bumblebee body weight and sensitivity to pesticides was found in van der Steen (1994). In Thompson (2001), small differences in sensitivity were found between

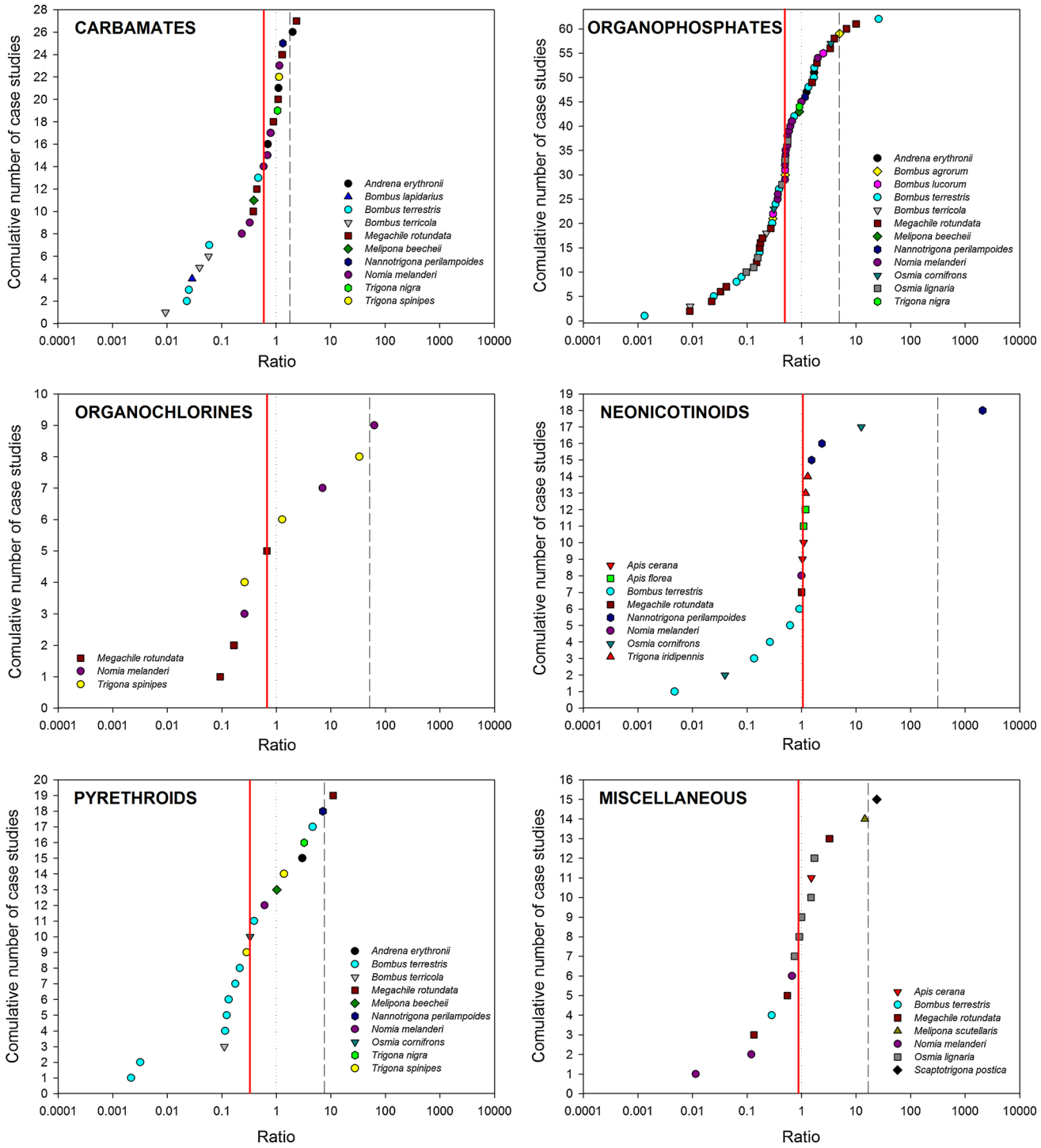

Fig. 2 Distribution of the sensitivity ratios *R* of bee species for each chemical class. A ratio of 1 indicates that the species *s* has the same sensitivity to pesticide as *Apis mellifera*, lower values indicate higher sensitivity of honey bees. The *solid line* and the *dashed line* indicate the median and the 95th percentile, respectively

A. mellifera and *Bombus* spp. when the LD_{50} values were normalized with respect to body weight. Comparing the adverse effects of 158 pesticides to *A. mellifera*, *Bombus* spp., *M. rotundata* and *N. melanderi*, Devillers et al. (2003) found that *M. rotundata* is the most susceptible to pesticides followed by *N. melanderi*, while no difference in sensitivity was found between *A. mellifera* and *Bombus* spp. Devillers et al. (2003) affirmed that the sensitivity of different bee species is generally inversely proportional to their mean body weight. In average, bumblebees

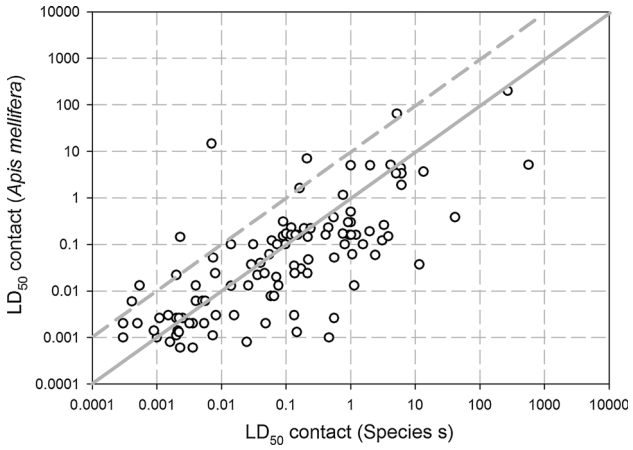

Fig. 3 Relationship between the LD_{50} acute contact (μg/bee) of *Apis mellifera* and the other bee species. The *solid line* delineates the 1:1 relationship. The *dashed line* delineates a factor of 10

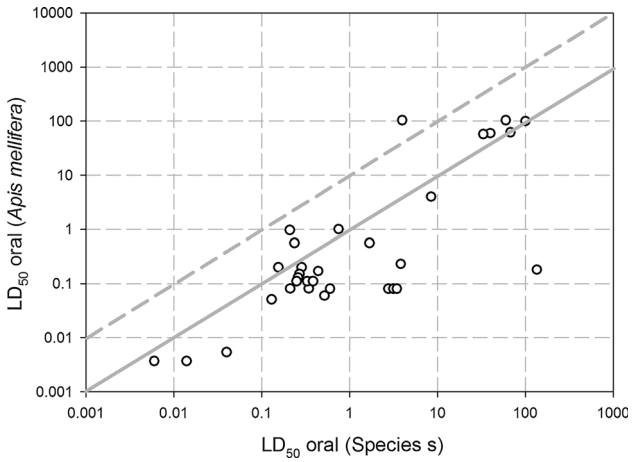

Fig. 4 Relationship between the LD_{50} acute oral (μg/bee) of *Apis mellifera* and the other bee species. The *solid line* delineates the 1:1 relationship. The *dashed line* delineates a factor of 10

(*B. terrestris*, 200–250 mg) are heavier than honey bees (*A. mellifera*, 100–120 mg), the weight of which is about 4 times higher than that of alfalfa leafcutting bees (*M. rotundata*, 25–30 mg). However, similar relationship between body weight and sensitivity was not confirmed in other studies (Helson et al. 1994). In some cases the higher sensitivity to pesticides of *M. rotundata* compared with *N. melanderi* and *A. mellifera* was explained by the higher pH of its haemolymph (Ahmad and Johansen 1973), while in other studies it was related to its greater surface-to-volume ratio (Johansen 1972). Feeding pre-adaptation and sociality level could also explain the different susceptibility between species. For instance, Cresswell et al. (2012) hypothesized the different sensitivity in feeding rate (one of the possible endpoints) between bumblebees and honey bees by the better pre-adaptation of honey bees to feed on nectar containing alkaloids, such as imidacloprid. In our meta-

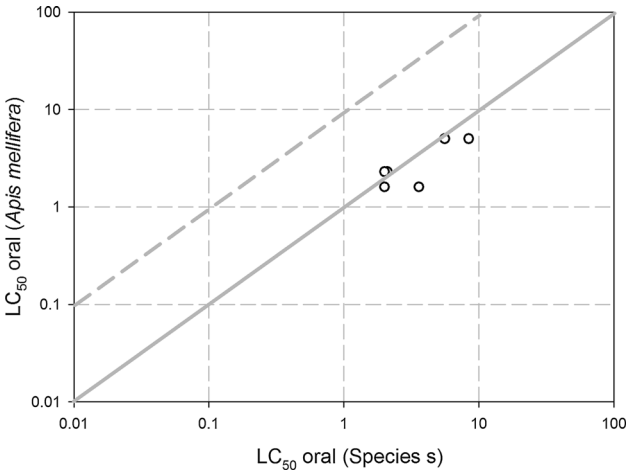

Fig. 5 Relationship between the LC_{50} chronic oral (μg/g) of *Apis mellifera* and the other bee species. The *solid line* delineates the 1:1 relationship. The *dashed line* delineates a factor of 10

analysis the sensitivity of stingless bees was in general higher than bumblebees and solitary bees. This higher sensitivity could be explained by their relatively smaller body size together with their higher level of sociality. In fact, it could be argued that the complex and evolved social immune systems in highly social bees (honey bees and stingless bees) can prevent exposure of the colony to diseases and xenobiotics with behavioural, physiological and spatial mechanisms, such as the removal of contaminated individuals. Conversely these mechanisms could make the single bees more sensitive to pesticides (Cremer et al. 2007). The limited number of data collected for other species, i.e. *A. erythronii*, *A. florea*, *A. cerana*, *Bombus lapidarius*, *M. scutellaris* and *S. postica*, prevented us from drawing conclusions about their specific sensitivities.

Comparing the sensitivity ratio of the different classes of pesticides, the neonicotinoids showed the highest median *R* as well the highest 95th percentile. For this chemical class, the other bee species were more sensitive than honey bees in 55.6 % of the cases and *R* values were higher than 10 in 11.2 % of the cases. The highest values of R were observed in the cyano-substituted neonicotinoids (acetamiprid and thiacloprid) which exhibited a much lower toxicity to honey bees than nitro-substituted neonicotinoids (imidacloprid and thiamethoxam). Lower values of *R* were observed for the other chemical classes, in particular in the group of carbamates where *R* was always below 10 and only in 33.3 % of the cases the sensitivity of other bees was higher than for honey bees.

Although several endpoints or biomarkers could be considered (feeding activity, learning ability, longevity) to compare the sensitivity of bees to pesticides, here endpoints such as the LD_{50} or the LC_{50} were focused considering literature data availability, which allowed building

a larger dataset for the meta-analysis. For each of these endpoints, different bee species can have different sensitivities. Cresswell and Laycock (2011) analyzed the sensitivity of bumblebees and honey bees to a toxicological stressor, comparing dose–response relationships of two different endpoints (feeding rate in bumblebees vs. sublethal performances in honey bees). Despite the substantial disparity in the LD_{50} values for imidacloprid, similar dose-dependent responses between the feeding rate of bumblebees and the sublethal performances in honey bees was found. Moreover, they showed that different endpoints can substantially differ in sensitivity even within species. For bumblebees, fecundity (Mommaerts et al. 2010; Laycock et al. 2012; Whitehorn et al. 2012) and feeding rate (Cresswell et al. 2012) appeared to be the most sensitive endpoints among those investigated, while, for honey bees, the proboscis extension reflex (PER) test, which revels effects on learning ability and memory, and the homing study seem the most sensitive assays (Decourtye et al. 2005; Henry et al. 2012; Schneider et al. 2012; Matsumoto 2013).

In the risk assessment of pesticides to bees the exposure must be considered together with their sensitivity to the test compound. Pollen and nectar consumption varies largely between bee species as well as different routes of exposure. Unlike honey bees, non-*Apis* bees can be also highly exposed to pesticide residues in the soil as many bees nest underground or use mud as nesting material. Moreover, non-*Apis* larvae are much more exposed to pesticide residues in pollen because they consume large provisions of unprocessed pollen. In addition, many life-history traits influencing the vulnerability to pesticides suggest that bumblebees and solitary bees could be more susceptible compared to honey bees, in particular, when the application of a pesticide coincides with the nesting period in solitary bees or with colony establishment in bumblebees. In addition, unlike honey bees, wild bees cannot be temporarily moved during pesticide spraying and, in the case of solitary bees, the death of a nesting female results in the end of the reproductive activity, while in social bees deficits following spraying may be compensated by workers and also by new bees emerging from the brood. Floral specialisation, shorter nesting period and limited foraging range are other factors that could make non-*Apis* bees more susceptible to pesticide compared to honey bees (Thompson and Hunt 1999; Brittain and Potts 2011; EFSA 2012). These differences among bee species (both in exposure routes and in sensitivity) highlight the need to include more bee species in the standard ecotoxicity data set required for the authorization of pesticides in order to achieve the same level of protection for honey bees and wild bees alike. Moreover, further comparison of the oral toxicity studies with *A. mellifera* and other bee species are necessary to confirm the appropriateness of the assessment factor of 10 when honey bee endpoint is used as surrogate.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Ahmad Z, Johansen C (1973) Selective toxicity of carbophenothion and trichlorfon to the honey bee and the alfalfa leafcutting bee. Environ Entomol 2(1):27–30

Ashman TL, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, Morgan MT, Wilson WG (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology 85(9):2408–2421

Bailey J, Scott-Dupree C, Harris R, Tolman J, Harris B (2005) Contact and oral toxicity to honey bees (*Apis mellifera*) of agents registered for use for sweet corn insect control in Ontario, Canada. Apidologie 36(4):623–633

Biesmeijer JC, Roberts SP, Reemer M, Ohlemuller R, Edwards M, Peeters T, Schaffers AP, Potts SG, Kleukers R, Thomas CD, Settele J, Kunin WE (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313(5785):351–354

Blacquiere T, Smagghe G, van Gestel CAM, Mommaerts V (2012) Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotoxicology 21(4):973–992

Bosch J, Sgolastra F, Kemp WP (2008) Life cycle ecophysiology of Osmia mason bees used as crop pollinators. In: James RR, Pitts-Singer TL (eds) Bee pollination in agricultural ecosystems. Oxford University Press, Oxford

Brittain C, Potts SG (2011) The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. Basic Appl Ecol 12(4):321–331

Burkle LA, Marlin JC, Knight TM (2013) Plant-pollinator interactions over 120 years: loss of species, co-occurrence, and function. Science 339(6127):1611–1615

Claudianos C, Ranson H, Johnson RM, Biswas S, Schuler MA, Berenbaum MR, Feyereisen R, Oakeshott JG (2006) A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. Insect Mol Biol 15(5):615–636

Committee on the Status of Pollinators in North America NRC, (2007) Status of Pollinators in North America. National Academies Press, Washington, DC

Cremer S, Armitage SAO, Schmid-Hempel P (2007) Social immunity. Curr Biol 17:693–702

Cresswell JE (2011) A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. Ecotoxicology 20(1):149–157

Cresswell JE, Laycock I (2011) Towards the comparative ecotoxicology of bees: the response–response relationship. In: 11th International symposium of the ICP-BR Bee Protection Group, Wageningen, 2–4 November 2011

Cresswell JE, Page CJ, Uygun MB, Holmbergh M, Li YR, Wheeler JG, Laycock I, Pook CJ, de Ibarra NH, Smirnoff N, Tyler CR (2012) Differential sensitivity of honey bees and bumble bees to a dietary insecticide (imidacloprid). Zoology 115(6):365–371

Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, Cluzeau S, Pham-Delegue MH (2005) Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. Arch Environ Contam Toxicol 48(2):242–250

Devillers J, Decourtye A, Budzinski H, Pham-Delegue MH, Cluzeau S, Maurin G (2003) Comparative toxicity and hazards of pesticides to APIS and non-APIS bees. A chemometrical study. SAR QSAR Environ Res 14(5–6):389–403

EFSA (2010) Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA J 8(6):1637

EFSA (2012) Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA J 10(5): 2668

EPPO/OEPP (2010) PP 3/10 (3): chapter 10: honeybees. EPPO Bull 40(3):323–331

European Commission (2002) SANCO/10329/2002 Rev 2 guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC

Gallai N, Salles JM, Settele J, Vaissiere BE (2009) Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 68(3):810–821

Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalheiro LG, Harder LD, Afik O, Bartomeus I, Benjamin F, Boreux V, Cariveau D, Chacoff NP, Dudenhoffer JH, Freitas BM, Ghazoul J, Greenleaf S, Hipolito J, Holzschuh A, Howlett B, Isaacs R, Javorek SK, Kennedy CM, Krewenka KM, Krishnan S, Mandelik Y, Mayfield MM, Motzke I, Munyuli T, Nault BA, Otieno M, Petersen J, Pisanty G, Potts SG, Rader R, Ricketts TH, Rundlof M, Seymour CL, Schuepp C, Szentgyorgyi H, Taki H, Tscharntke T, Vergara CH, Viana BF, Wanger TC, Westphal C, Williams N, Klein AM (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339(6127):1608–1611

Hardstone MC, Scott JG (2010) Is *Apis mellifera* more sensitive to insecticides than other insects? Pest Manag Sci 66(11): 1171–1180

Helson BV, Barber KN, Kingsbury PD (1994) Laboratory toxicology of 6 forestry insecticides to 4 species of bee (Hymenoptera, Apoidea). Arch Environ Contam Toxicol 27(1):107–114

Henry M, Beguin M, Requier F, Rollin O, Odoux JF, Aupinel P, Aptel J, Tchamitchian S, Decourtye A (2012) A common pesticide decreases foraging success and survival in honey bees. Science 336(6079):348–350

Johansen CA (1972) Toxicity of field-weathered insecticide residues to four kinds of bees. Environ Entomol 1(3):393–394

Klein AM, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. Proc Biol Sci 274(1608): 303–313

Kwak MM, Velterop O, van Andel J (1998) Pollen and gene flow in fragmented habitats. Appl Veg Sci 1(1):37–54

Ladurner E, Bosch J, Maini S, Kemp WP (2003) A method to feed individual bees (Hymenoptera: Apiformes) known amount of pesticides. Apidologie 34:594–602

Laycock I, Lenthall KM, Barratt AT, Cresswell JE (2012) Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker Bumble bees (Bombus terrestris). Ecotoxicology 21(7):1937–1945

Maini S, Medrzycki P, Porrini C (2010) The puzzle of honey bee losses: a brief review. Bull Insectology 63(1):153–160

Matsumoto T (2013) Reduction in homing flights in the honey bee *Apis mellifera* after a sublethal dose of neonicotinoid insecticides. Bull Insectology 66(1):1–9

Michener CD (2007) The bees of the world, 2nd edn. The John Hopkins University Press, Baltimore

Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G, Smagghe G (2010) Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. Ecotoxicology 19(1):207–215

Neumann P, Carreck NL (2010) Honey bee colony losses. J Apic Res 49(1):1–6

Porrini C, Sabatini AG, Girotti S, Fini F, Monaco L, Celli G, Bortolotti L, Ghini S (2003) The death of honey bees and environmental pollution by pesticides: the honey bees as biological indicators. Bull Insectology 56(1):147–152

Potts SG, Roberts SPM, Dean R, Marris G, Brown MA, Jones R, Neumann P, Settele J (2010) Declines of managed honey bees and beekeepers in Europe. J Apic Res 49(1):15–22

Regulation (EC) 544/2011. Commission Regulation (EU) No 544/2011 of June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards to the data requirements for active substances

Schneider CW, Tautz J, Grünewald B, Fuchs S (2012) RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. PLoS One 7(1):e30023

Scott-Dupree CD, Conroy L, Harris CR (2009) Impact of currently used or potentially useful insecticides for canola agroecosystems on Bombus impatiens (Hymenoptera: Apidae), *Megachile rotundata* (Hymentoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). J Econ Entomol 102(1):177–182

Tasei JN (2002) Impact of agrochemicals on non-*Apis* bees. Honey bees: estimating the environmental impact of chemicals. Taylor & Francis, London

Thompson HM (2001) Assessing the exposure and toxicity of pesticides to bumblebees (Bombus sp.). Apidologie 32(4):305–321

Thompson HM, Hunt LV (1999) Extrapolating from honeybees to bumblebees in pesticide risk assessment. Ecotoxicology 8(3): 147–166

van der Steen JJM (1994) Method development for the determination of the contact LD 50 of pesticides for bumble bees (Bombus terrestris L.). Apidologie 25(5):463–465

Whitehorn PR, O'Connor S, Wackers FL, Goulson D (2012) Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336(6079):351–352

Williams IH (1994) The dependence of crop production within the European Union on pollination by honey bees. Agric Sci Rev 6:229–257

Winfree R, Williams NM, Dushoff J, Kremen C (2007) Native bees provide insurance against ongoing honey bee losses. Ecol Lett 10(11):1105–1113