A review of the factors that influence pesticide residues in pollen and nectar: future research requirements for optimising the estimation of pollinator exposure

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Fiona Gierer[a]*, Sarah Vaughan[b], Mark Slater[b], Helen M. Thompson[b], J. Stephen Elmore[c] and Robbie D. Girling[a]

[a] School of Agriculture, Policy and Development, University of Reading, Reading, UK

[b] Syngenta Ltd, Jealott's Hill International Research Centre, Bracknell, Berkshire, UK

[c] Department of Food and Nutritional Sciences, University of Reading, Reading, UK

* Corresponding author. E-mail address: f.gierer@pgr.reading.ac.uk

Abstract

In recent years, the impact of Plant Protection Products (PPPs) on insect pollinator decline has stimulated significant amounts of research, as well as political and public interest. PPP residues have been found in various bee-related matrices, resulting in governmental bodies worldwide releasing guidance documents on methods for the assessment of the overall risk of PPPs to different bee species. An essential part of these risk assessments are PPP residues found in pollen and nectar, as they represent a key route of exposure. However, PPP residue values in these matrices exhibit large variations and are not available for many PPPs and crop species combinations, which results in inaccurate estimations and uncertainties in risk evaluation. Additionally, residue studies on pollen and nectar are expensive and practically challenging. An extrapolation between different cropping scenarios and PPPs is not yet justified, as the behaviour of PPPs in pollen and nectar is poorly understood. Therefore, this review aims to contribute to a better knowledge and understanding of the fate of PPP residues in pollen and nectar and to outline knowledge gaps and future research needs. The literature suggests that four primary factors, the crop type, the application method, the physicochemical properties of a compound and the environmental conditions have the greatest influence on PPP residues in pollen and nectar. However, these factors consist of many sub-factors and initial effects may be disguised by different sampling methodologies, impeding their exact characterisation. Moreover, knowledge about these factors is ambiguous and restricted to a few compounds and plant species. We propose that future research should concentrate on identifying relationships and common features amongst various PPP applications and crops, as well as an overall quantification of the described parameters; in order to enable a reliable estimation of PPP residues in pollen, nectar and other bee matrices.

Keywords

Pesticides; Risk Assessments; Pollinator; Pollen and Nectar; Residues

Capsule

Pesticide residue values within pollen and nectar have potentially significant consequences for the reliability of risk assessments for wild and managed bee populations, however, the reasons and mechanisms underlying variations in residues are poorly understood and require greater investigation.

Introduction

Usage, benefits and drawbacks of Plant Protection Products

The global population has increased rapidly, tripling since 1950 to a current total of 7.6 billion (Population Reference Bureau 2017), and is predicted to expand to 9.6 billion by 2050 (UN 2017). This growth has been facilitated by the intensification of crop production as a result of new developments and innovations (Carvalho 2006; Johnson 2000). As a consequence, the daily food supply per capita increased from 2196 kcal day^{-1} in 1960 to 2884 kcal day^{-1} in 2013, with cereal yields almost tripling in the same time period (FaoStat 2017). Concurrent increases in production, use and trade of Plant Protection Products (PPPs) indicate their contribution to these increases in food production (Atwood and Paisley-Jones 2017; Gilland 2002; Tilman 1999; Tilman et al. 2001; Zhang et al. 2011). Today, approximately 1000 active ingredients (a.i.s) (i.e. the components in PPPs which are active against pests/plant diseases) are globally available (Lewis et al. 2016).

The predominant use of PPPs is in the agricultural sector to protect crops from weeds, fungal pathogens and pests (Wilson and Tisdell 2001). Estimates suggest the losses in plant production without PPPs would be up to 80% for some crops with potentially severe economic consequences (Oerke and Dehne 2004; Oliveira et al. 2014; Pimentel 1997). Outside of the agricultural sector, PPPs are a cost and labour efficient method for the protection and maintenance of public spaces, for example weed control on railways and streets (Cooper and Dobson 2007). In the future, the targeted use of PPPs could further grow in importance; consequences of globalisation and climate change are predicted to change the distribution and life cycles of many pest species, which could render previous control strategies ineffective (Hulme 2017; Rosenzweig et al. 2001). Therefore, there is a strong argument to suggest that PPPs currently make a significant contribution to stable and reliable crop yields, high food quality and the prevention of economic losses, which is a key factor in enabling the global food system to continue to operate in its current format.

Nevertheless, PPPs are toxic chemicals and, in the absence of mitigation, some exposure to non-target organisms and the ecosystem is inevitable. Due to their wide range of applications, PPP residues and their metabolites can be found in many ecosystems, with the potential to cause various effects on humans, soil and water organisms, birds, mammals and invertebrates (Mostafalou and Abdollahi 2017; Pimentel 2005; Tilman 1999).

PPPs and insect pollinators

In recent years, high overwintering losses of honey bee colonies and declines in populations of other insect pollinator species in Europe and North America (Lee et al. 2015; Ollerton 2017; Potts et al. 2010b; Seitz et al. 2016) have raised concerns about the contribution of PPPs to these losses (IPBES 2016). Managed and wild pollinator species provide vital ecosystem services, particularly for agro-ecosystems (Albrecht et al. 2012; Klein et al. 2007; Vanbergen et al. 2014; Veddeler et al. 2008) and

Gallai et al. (2009) calculated the total economic value of pollination worldwide to be € 153 billion. As a result, the toxic effects of PPPs on pollinators, particularly neonicotinoids, has become the focus of significant amounts of research, and political and public interest. There is a broad consensus amongst researchers in the field that declines are the result of a combination of factors including habitat loss, pests/diseases and PPPs (Goulson et al. 2015; IPBES 2016; Potts et al. 2010a). Whilst the overall role of PPPs on pollinator declines is still debated, there is clear evidence for both the exposure of bees to a range of chemical products via contact and oral exposure (e.g. Botias et al. 2017; Chauzat et al. 2010; Johnson et al. 2010; Kiljanek et al. 2017; Tosi et al. 2018) and the toxicity of PPPs to bees in laboratory toxicity studies (e.g. Kasiotis et al. 2014; Pettis et al. 2012; Sanchez-Bayo et al. 2017; Woodcock et al. 2017; Wu et al. 2011).

Overall, there is a difficult trade-off between permitting the use of products upon which modern agriculture relies for the protection of crops and maintaining vital environmental goods and services, which themselves have an important role in sustainable food production. Therefore, in order to ensure the safety of PPPs, complex and highly regulated processes of environmental risk assessments have been developed.

With respect to pollinators, the European Food Safety Authority (EFSA 2013) published a Guidance Document on the risk assessment of PPPs on bees (including honey bees, *Apis mellifera* L., Bumblebees, *Bombus spp*. and solitary bee species), to outline a process by which PPPs can be evaluated for their potential risks in causing unacceptable harm to bees. Similar approaches have been published in the US, Canada and Brazil (Cham et al. 2017; USEPA 2014). An important component in these approaches are PPP residue levels in pollen and nectar. They represent a key route of exposure for pollinators as, in many species, all life stages feed to some extent upon these food sources (Rortais et al. 2017; Villa et al. 2000). However, knowledge to enable a more accurate prediction of PPP residues in pollen and nectar is limited and a number of barriers, which are discussed in more detail in the next section, inhibit a clear assessment of residue levels used in risk estimation.

Aim of the review

In this review our aim was to identify and compile existing literature data on the behaviour and fate of residues in pollen and nectar following PPP applications, and outline the manifold parameters which appear to influence these residues. In doing so we identify knowledge gaps concerning the variability of PPP residue values in pollen or nectar and highlight future research needs, in order to enable a precise prediction of residue levels for pollinator risk assessments in future and to encourage, initiate and facilitate further research in this field.

Pollinator risk assessments and evidence base

Current methodological approaches for pollinator risk assessments

Current approaches for pollinator risk assessments (e.g. Cham et al. 2017; EFSA 2013; USEPA 2014) pursue similar strategies and methodologies. In general, effect studies (e.g. laboratory adult acute oral toxicity studies, larvae toxicity studies) and exposure estimates (contact or oral) are combined in a tiered approach to assess the risk of PPPs to pollinators, ranging from very conservative estimates to more realistic scenarios. While the latter requires high data input and more extensive studies, in the lower, more conservative tiers, worst-case default values can be applied. Theoretically, such an approach allows for more rapid and cost-effective initial assessments that are robust enough to separate those PPPs that pose a potential risk to bees from those that can be considered of low risk. To assess the risk from oral exposure of bees to PPPs, the guidance documents (Cham et al. 2017; EFSA 2013; USEPA 2014) provide general default residue values in pollen and nectar for different application scenarios, which aim to be protective. If the assessment fails in lower tiers and risk mitigation is not possible, the guidance documents cited above suggest a refinement of the assessment in higher tiers, for example by using representative "real" PPP residue values in pollen or nectar or compound and crop specific data, which can be further refined by conducting field trials.

Barriers associated with PPP residues in bee products and their implementation in risk assessments

A recent proposal made in reference to EFSA's risk assessment from the European Crop Protection Authority (ECPA 2017), which represents the industrial sector, concluded that the current guidance is over-conservative and that even substances known to be non-toxic to bees fail at lower tiers. They assert that, in most cases, a higher tier refinement is required. In order to facilitate higher tier assessments, oral exposure estimates must be refined using representative residue data (Cham et al. 2017; EFSA 2013; USEPA 2014). However, data on residue levels in floral resources vary widely and are unknown for many PPPs and crop species (EFSA 2013; Lundin et al. 2015). Table 1 provides a brief overview of PPP residue data recorded in pollen from spray applications, illustrating the variation of PPP residues from different active ingredients and in different crops. These data are taken from a recent meta-study (Kyriakopoulou et al. 2017) and from the pollinator risk assessment published by EFSA (2013), both providing a comprehensive overview and summary of data on the available residue data in bee-relevant matrices and products, which were gathered from Draft Assessment Reports (DARs), literature and peer reviews of active ingredients.

Overall, there is wide variation in residues, with differences not only between various active ingredients and crop combinations, but also within each of these groups. For instance, aggregated residues from various PPPs vary considerably not only within *Brassica* pollen, but also from uses of individual PPPs, such as teflubenzuron on *Brassica*. Similar findings can be observed for residues in nectar (Table S1).

Both publications highlighted the fact that the available studies differed considerably in design, sampling timing, sampling methodology and application scenarios, or lacked data for certain types of active ingredients. Thus residue data is difficult to compare. Overall, knowledge about PPP residues in pollen or nectar is fragmentary and only a small proportion of treatments and crops have been taken into account, with the majority of residue values provided for neonicotinoids and oilseed rape (OSR) (*Brassica napus* L.). Pollinator risk evaluation is therefore based on extrapolated residue data and as a consequence, on an incomplete dataset. However, the knowledge regarding PPP residues present in pollen and nectar is too limited to allow extrapolations or conclusions to be drawn from those crops where data are available.

Yet, if risk assessments are based on residue values that are not representative for the treatment scenario and cropping system, the risk posed from PPPs to pollinators might be incorrectly estimated (Lundin et al. 2015), resulting in false negatives (i.e. misuses of concern), or in false positives, which may result in unnecessary higher tier testing. The currently available data sets can neither mitigate the variability and incompleteness, nor rationalise how this should be addressed in risk assessments or why these variations in PPP residues occur.

PPP residue studies in pollen and nectar

Extensive studies are needed to derive reliable PPP residue values in pollen and nectar. However, the exact determination of residues in bee-attractive plants is expensive and time consuming. Relatively large volumes of the target matrices are required for the chemical analyses needed to quantify the PPP residues, but pollen and nectar are typically produced only in small quantities. Furthermore, numerous active substances, crop species and application scenarios must be considered. The ECPA (2017) claimed that, in order to meet the requirements of the EFSA guidance document (EFSA 2013), for a single product used on five different bee-attractive crops, up to 75 residue studies would need to be conducted, with associated costs of approximately € 7.5m. Consequently, the development and registration of new products and innovations, in addition to the re-authorisation of already approved PPPs, are likely to incur large costs, which may limit the availability of PPPs. According to the ECPA, minor use crops are most likely to be affected, which are often economically important for their growers and for crop diversity, but not of significant importance to the industry to justify high costs for research and development.

Furthermore, with new insights and findings becoming apparent and a better comprehension of risks posed by PPPs in recent decades, it is likely that regulatory requirements will be further increased and adapted and that applicants for active ingredient and PPP registrations, PPP producers and responsible authorities will need to deliver more detailed data regarding the fate of PPPs in plant matrices important to pollinators.

Thus, to ensure the accurate protection of pollinators and to permit scope for developments in crop protection, methods need to be devised to enable an accurate estimation of PPP residues in pollen and other bee-important matrices that require reduced effort and expenditure. In order to achieve this, a better knowledge and understanding of the fate of PPP residues in pollen and nectar is necessary. The identification of patterns and relationships of PPP residues within the plant and between different species may provide an opportunity to identify better methods for accurate estimation of residue levels for diverse PPPs and cultivation methods. However, little is understood about the behaviour and relationship of residues in floral resources, which can be altered and influenced by numerous factors.

Factors influencing PPP residues in pollen, nectar and other related matrices

An assessment of the literature suggests that there are four primary factors which could influence the level of PPP residues in pollen and nectar and other related matrices: i) crop related parameters ii) discrepancies in PPP application method, timing and dose rate iii) physicochemical properties of the active ingredient and iv) environmental conditions. These primary factors consist in turn of several sub-factors which can all potentially contribute to variable PPP residues in pollen or related crop matrices. The first two factors listed are considered more often in the literature, since they are tangible and relatively easy to determine under constant conditions. By contrast, the effects of environmental conditions are more difficult to isolate, as they can, for example, influence the chemical properties of an active ingredient, as well as the development and physiology of a plant. Hence, there are a wide range of factors influencing PPP residue levels in pollinator relevant matrices, which are strongly interdependent and form a complex system. Another factor which can unintentionally influence the results of PPP residue levels in pollen and nectar is the sampling methodology. For instance, OSR flowers are often collected and then incubated for a certain time period and temperature to facilitate pollen release (e.g. Botias et al. 2015). The loss of water might result in higher PPP concentrations, but conversely the high temperatures can initiate a dissipation of PPP residues. In other studies pollen is collected by grinding the anthers to powder (e.g. Jiang et al. 2018), collecting pollen in boxes as it falls naturally from plants (Schmuck et al. 2001) or by using bees (e.g. Choudhary and Sharma 2008). These discrepancies in sampling are often not scrutinised in studies but might influence the comparability of results.

General findings

Although high variability is typically observed in PPP residues in pollen and nectar, there are some instances that permit comparisons among a wide range of PPPs/crop systems. Kyriakopoulou et al. (2017) detected statistically significant differences among sampling matrices, with the residue levels in both pollen and nectar being highest when extracted directly from flowers than from bees. Such differences could be caused by dilution effects, when bees mix pollen from untreated and treated crops (Bonmatin et al. 2015; Rolke et al. 2016). In many studies a dilution effect, cross contamination from other fields (e.g. Kunz et al. 2015) or chemical alterations cannot be excluded when pollen and nectar is collected by free flying bees and it is often difficult to directly link the residues found to the previous PPP treatment, unless studies are conducted using bee-sampled pollen collected from tunnelled crops (i.e. no alternative sources of pollen are available).

Furthermore, Kyriakopoulou et al. (2017) detected higher residues in pollen than in nectar, a phenomenon which has been reported in several other studies, which employ a range of treatment regimens (e.g. Choudhary and Sharma 2008; Cowles and Eitzer 2017; Dively and Kamel 2012; EFSA 2012; Goulson 2013; Jiang et al. 2018). Reasons for this difference have not been investigated thus far; however, several possible mechanisms can be proposed. If bee- collected matrices are analysed, the effect could be caused by the partial metabolism of residues in nectar within the bees (Gong and Diao 2017; Sanchez-Bayo and Goka 2014). However, similar results have also been reported from samples taken directly from the plant. Cowles and Eitzer (2017) suggested a relationship between residue levels in pollen and nectar and whether nectaries and anthers are supplied by phloem or xylem. Choudhary and Sharma (2008) presumed analytical impediments, for example the active ingredient could form conjugates with sugars in nectar, thus becoming difficult to extract, or that, due to morphological differences, there may be differences in either the initial levels of PPPs or in their rates of degradation. Overall, the meta-analysis by Kyriakopoulou et al. (2017) found a strong correlation between the residue levels in pollen and nectar, though none of the individual studies included in the meta- analysis has directly compared this parameter thus far.

Crop related parameters

Although few crop species are considered in studies on PPP residues in pollen and nectar, there is evidence that crop traits have an influence on the residue levels in bee-important matrices. Differences in residue levels in various plant parts can be explained by a dilution effect with plant growth (more biomass) (Holland et al. 1996), plant height (Kleier 1994) and even plant age (Bonmatin et al. 2015), for example when PPPs have the ability to be adsorbed to plant compounds like lignin (Fujisawa et al. 2002). Overall, these effects are strongly related to the physicochemical properties of a compound (see section below for full discussion on the effects of physicochemical properties). Soil treatments of the systemic compound imidacloprid demonstrated that there is a clear gradient with respect to residue levels from the leaves at the bottom of the plant up to the leaves at the top of the plant, and eventually to the flowers and pollen (Alsayeda et al. 2007; Bonmatin et al. 2005; Johnson 2012; Laurent and Rathahao 2003; Stoner and Eitzer 2012).

This raises questions as to whether conclusions drawn from the PPP residue levels found in foliage can be applied to those in pollen/nectar and whether crops with lower biomass exhibit higher residue levels in leaves and consequently in pollen or nectar. Balfour et al. (2016) found that neonicotinoid concentrations in the tissues of flowering maize (*Zea mays* L.) and OSR are negatively correlated with plant mass, however, they did not directly compare these results with pollen and nectar collected from the same plots.

Dively and Kamel (2012) found a strong correlation of imidacloprid residues in squash (*Cucurbita pepo* L.) between leaves and pollen, and leaves and nectar ($r = 0.94$ and $r = 0.88$, respectively; $p < 0.001$) following different soil application treatments. This, however, was analysed only during the course of one year and the trend was not replicated for metabolites of imidacloprid or other investigated neonicotinoids. Dively and Kamel (2012) suggested that the diverse chemical properties of the investigated compounds, mainly the solubility, were the reason for a varying uptake and translocation rate, and consequently higher residue levels of other neonicotinoids. However, the differences in residue levels could also be due to the fact that they randomly selected leaves for analysis during their study. Considering the dilution effect and gradient, different results might have been found by using leaves of similar size and position on the plant. Such an approach was employed by Jiang et al. (2018), who collected only newly expanded leaves of cotton (*Gossypium* sp.) over a one-month period. Although no correlations were observed in nectar, correlations between imidacloprid and thiamethoxam residues in leaves and pollen ($r = 0.88$ and $r = 0.90$, respectively; $P < 0.001$) were found. However, it is unclear whether these observations also apply for other crops, other PPPs (i.e. those with non-systemic properties) and different application scenarios.

Demonstrating similarities between species has proven to be problematic, with even varieties of the same species resulting in different residue levels. This was demonstrated by Bonmatin et al. (2003), who investigated several sunflower (*Helianthus annuus* L.) varieties after a seed treatment with imidacloprid. The final concentration in flowers was dependent on the variety, with ranges from 2.7 $\mu g\ g^{-1}$ up to 7 $\mu g\ g^{-1}$. The authors did not provide any information about habitus or other species-specific characteristics, but an acropetal decrease of residues in foliage, as described above, was detected for all varieties. In addition, during the formation of the capitula of the sunflower there was a sudden increase in imidacloprid residue levels in the upper parts of the plants. Similar findings were reported by Laurent and Rathahao (2003), analysing different parts of sunflowers. Moreover, PPP concentrations in pollen were similar to those found in the floret dish. It can therefore be concluded that the pollen was contaminated by the late shift of PPP residues in sunflowers. The authors presumed a remobilisation process, in which compounds accumulated in older leaves were transferred towards the upper part of plants during the reproductive stage. However, imidacloprid is a xylem-mobile PPP; hence, it should not re-translocate (Sur and Stork 2003).

Laurent and Rathahao (2003) provided another explanation for the phenomenon, suggesting that it was a consequence of the differential root system of sunflowers. This consists of fascicular roots, which grow horizontally in the superficial layer of the soil, and a deeper root system; thus, various soil levels can be penetrated. Sunflowers are particularly capable of recovering PPP residues from soils, which can be attributed to this extensive root system (Bonmatin et al. 2003; Mitton et al. 2016). Consequently, the more pronounced root system of an older plant can take up more PPPs from the soil, leading to an increase of residues during the flowering period. The root system is also an important parameter concerning the PPP uptake from soil in other species, for example from the *Cucurbita* family (Otani et al. 2007). For instance, cucumbers *(Cucumis sativus)* grafted with high uptake root stocks could recover up to 70% more dieldrin (an organochlorine insecticide) than those with a low uptake root stock (Otani and Seike 2007), giving a further explanation as to why different varieties exhibit different residue levels from soil treatments. Regarding the ability of different root systems to shift the PPP residues in plants, plant density and whether experiments are conducted in field or pots might also be important parameters to understand the variability of residues in flowers and should be considered in soil-applied PPP residue studies.

Obviously, these observations are less relevant for foliar-sprayed or non-systemic PPPs (see section below for full discussion of the effects of application method). The PPP’s chemical properties, the morphology and the structure of the leaves, flowers and cuticle determine the uptake rate of the product and hence the likelihood of translocation to pollen or nectar (DiTomaso 1999; Kirkwood 1999;

Price and Anderson 1985). For example, a hairy or waxy leaf structure affects the retention time of chemicals on the surface (Yu et al. 2009); this can alter the uptake rate of the PPPs and hence the chemicals' exposure to environmental conditions. Therefore, even under similar conditions, different plant species will show different residue levels and behaviour. Kyriakopoulou et al. (2017) found species-related differences in pollen and nectar residues. In particular, OSR showed the highest residue values in comparison to other plants. However, there were more data available for OSR and the majority of other species was summarised to one group. Therefore, there is limited confidence as to whether OSR genuinely is a crop which accumulates a high level of PPP residues in pollen or nectar. For a summary of this section and problems regarding pollinator risk assessments see Figure 1.

Application Method, Application Timing, Dose Rate

The application method, timing of the application and the dose rate of an applied PPP are strongly interdependent. For example, by using a seed treatment, the longest possible time period between application and flowering of the plant is attained. In contrast, many fungicides are sprayed directly onto the plant shortly before or during flowering, especially when they have been assessed as non-harmful for bees. Furthermore, seed dressings often contain less active ingredient per hectare and therefore may be considered to be more environmentally friendly. This is reflected in the residue levels of foliar applications and seed dressings reviewed by EFSA (2012, 2013), with residues from seed dressings being substantially lower than from spray applications. Evidence regarding the effect of dose rate on PPP residues in pollen was provided by Bonmatin et al. (2005), who used three different doses of the systemic active ingredient imidacloprid, applied as a seed dressing to sunflower seed. The concentration of imidacloprid in the capitula of several varieties became higher when the dose rate was increased. Furthermore, the ascent of imidacloprid during flowering (see section above) was more pronounced when the doses of the seed dressing were high. However, studies directly comparing the effect on residues in pollen and nectar at different dose rates of foliar applied or non-systemic PPPs are scarce, although it is possible to discern a certain trend from the detailed values provided by EFSA (2012), indicating that higher dose rates cause higher residues in pollen and nectar.

Yet, it cannot be concluded that a high application of PPPs naturally results in a high exposure for bees or in high residues in relation to the dose rate. Choudhary and Sharma (2008) applied a range of PPPs to mustard (*Brassica juncea* Czern.) using foliar application, each with a defined dose rate, and showed that PPPs applied at higher rates indeed tend to result in higher residues in pollen and nectar (Table 2). Interestingly, RUDs - the residue unit employed in risk assessments to account for different dose rates (RUD = concentration in nectar/ pollen (mg kg^{-1}) at an application rate of 1 kg ha^{-1} or 1 mg $seed^{-1}$) - exhibited an opposing trend in this experiment. Lambda-cyhalothrin afforded the highest PPP residues relative to the dose rate and endosulfan, though applied at the highest dose, afforded the lowest residues with respect to the dose. Thus, the ratio of residues from different PPPs relative to the dose rate is not equal for all compounds, it is rather influenced by other factors, for example the different chemical properties of the active ingredients, which are responsible for varying uptake and accumulation in floral parts.

Nevertheless, Byrne et al. (2014) observed higher residues in nectar with a doubled dose rate compared to the normal dose rate when treating citrus trees with a soil drench application of imidacloprid. This effect was reinforced at later sampling dates, i.e. with a longer time period between application and flowering. It is assumed that a longer time period between application and flowering results in lower residues because of the dilution, metabolism and dissipation in plants. For imidacloprid, however, to a certain extent the contrary was shown. Whether this effect is similar to that described by Bonmatin et al. (2003) and Laurent and Rathahao (2003) in the above section is not verifiable. It does, however, illustrate that the timing of the application can have a significant impact on residue levels in pollen and nectar. These findings can also be important when comparing varieties and cropping systems. For instance, Pohorecka et al. (2012) found substantially lower residues of thiamethoxam in bee foraging products from winter OSR than spring OSR. It is hypothesised that the longer time period between treatment and bloom of winter OSR led to a higher degradation of the active ingredient.

Cowles and Eitzer (2017) also detected late imidacloprid accumulation in sunflower pollen, but under different experimental conditions. Their extensive experimental setup considered three neonicotinoids, applied at different times with different application methods to sunflower and swamp milkweed (*Asclepias incarnata* L.). Again, a low rate imidacloprid soil drench application was the only scenario (application rate, method, and insecticide) found to result in increasing concentrations as the time post-application increased; which meant a soil drench application performed 10 weeks prior to bloom was the only timing for this application scenario that exceeded the designated “toxicity threshold” for bees in pollen concentrations at the lowest dose rate. In contrast, dinotefuran soil drench applications led to higher residues when they were applied closer to the blooming period. The authors concluded that dinotefuran has a better solubility and higher mobility than imidacloprid and therefore the uptake is faster, whereas the uptake of imidacloprid takes longer and so residues accumulate later in pollen. This finding might be especially important for the estimation of residues in crops with a pronounced short or long life cycle and shows that the physicochemical properties of a compound must always be taken into account (see section below for full discussion of physicochemical properties).

Cowles and Eitzer (2017) demonstrated that higher application rates resulted in higher residues in pollen and nectar, depending on the chemical applied. However, the method of application had the strongest influence on pollen and nectar residue levels. Soil drench applications resulted overall in higher residues than the foliar applications, even if both were applied only two weeks before bloom. This indicates that, even though the uptake via leaves is good, it cannot be compared with the uptake and transport via the roots and should be considered separately for the assessment of residues in floral matrices. In contrast to these findings, the tables provided by EFSA (2012, 2013) indicate that residue values from foliar applications are higher compared to soil treatments. However, those tables only consider seed dressings, which contain significantly less active ingredient than soil drenches or foliar sprays. Furthermore, many residue values for foliar applications are derived from applications performed during bloom or shortly before, whereas the latest foliar application in Cowles' and Eitzer's experiment was applied two weeks before bloom.

Dively and Kamel (2012) showed that foliar-applied neonicotinoids in squash resulted in higher residues in pollen compared to a soil drip and drench application, especially when squash was sprayed at full bloom. In contrast, the PPP residues from foliar applications in nectar were lower after a spray application or did not differ from soil drench and drip irrigation. This leads to the assumption that systemic PPPs are provided over a longer period from the inside of the plant and thus have a greater probability to accumulate and express in nectar. Dively and Kamel found the lowest residue levels from imidacloprid bedding tray soil applications. This was the most distant application method relative to bloom and no increase in residues could be observed. However, the dose rate was very low compared to the other treatment regimes. In total, contrary to Cowles and Eitzer's (2017) experiment, the timing of the application and dose rate seemed to play a more important role than the application method, confirming the assumption that applications closer to bloom result in higher residues. Both Kubik et al. (1999) and Wallner (2009) showed that there is a lag period between the application of fungicides and the maximum residue level in pollen, although the compounds were sprayed directly onto the plant before and during bloom in cherry trees and OSR, respectively. More studies with different PPPs are necessary to confirm these results, especially for foliar applications (Figure 2). Overall, it can be concluded that there is a strong interdependence between the time available for the accumulation of the compound and the time for dissipation, metabolism and translocation in the plant, influenced by the chemical properties of a PPP and the application method.

Physicochemical Properties

A detailed knowledge about the physical and chemical properties of a chemical compound is a necessary prerequisite to understand its general behaviour in metabolism, analytical methods, formulations, and the environment (Tsipi et al. 2015). Therefore, these parameters are usually studied under well-defined conditions and are required for the registration of a PPP. Physicochemical properties determine the uptake of a compound into the plant, its translocation, as well as its dissipation and metabolisation in the plant and the environment.

For PPPs applied before bloom, it can be assumed that every parameter influencing the uptake of a compound and its acropetal translocation will influence the residues in floral resources. Some key physicochemical properties include the solubility in water, the partition coefficient octanol/water (log K_{ow}), the dissociation coefficient (pK_a), the molecular size of a compound, the root concentration factor and the transpiration stream concentration factor. These properties can be altered by additives and vary depending on the formulation type (Bonmatin et al. 2015; Farha et al. 2016; Hsu and Kleier 1996; Trapp 2004).

Overall, PPPs can be classified according to their behaviour in and on plants. Non-systemic or contact compounds are not distributed in the plant and will probably cause only residues in floral matrices if the flower or pollen comes directly into contact with the PPP. On the contrary, translaminar PPPs are taken up and redistributed from one face of a leaf to the opposite face of a leaf, an important parameter for many fungicides (Klittich et al. 2008), whereas systemic PPPs are distributed within the whole plant, either acropetally via the transpiration stream to older leaves in xylem or in both, acropetal and basipetal directions to new growth in the phloem sap. The most common way for the translocation of (non-ionised) plant systemic insecticides is the unidirectional acropetal translocation in xylem (Wyss and Bolsinger 1997).

One key parameter describing PPP translocation for non-ionised compounds in the plant is the partition coefficient octanol/water (log K_{ow}). It describes the compound's lipophilicity and its ability to move through bio membranes; thus it determines the uptake of a PPP through the leaf cuticle and its distribution within the plant (Briggs and Bromilow 1994; Kirkwood 1999; Klittich et al. 2008; Wang and Liu 2007).

In general, compounds with a log $K_{ow} < 0$ are considered to be hydrophilic and compounds with a log $K_{ow} > 0$ lipophilic (Wang and Liu 2007). Lipophilic compounds tend to cross bio membranes but are partitioned into lipophilic tissue along the symplastic pathway (Sicbaldi et al. 1997). Therefore, the optimum uptake and translocation in xylem occurs for non-ionised PPPs with intermediate log K_{ow} values of 1–3. Translaminar distributed compounds can show log K_{ow} values up to 4.5. Highly polar and highly non-polar compounds are poorly translocated within a plant (Bromilow and Chamberlain 1989; Bromilow and Chamberlain 1995; Sicbaldi et al. 1997; Vryzas 2016).

Non-ionised compounds with a lower log K_{ow} can also be distributed in the phloem sap, though entering the symplast is impeded (Bromilow et al. 1987). In contrast, more lipophilic compounds can readily enter the phloem, but also easily move between xylem and phloem. However, as the xylem is moving faster than the phloem, compounds are eventually translocated in the xylem (Peterson and Edgington 1976; Wyss and Bolsinger 1997).

In general, most phloem-mobile compounds appear to be weak acids (Trapp 2004) and their translocation is highly dependent on a favourable combination of log K_{ow} and the dissociation coefficient (pK_a) (Wyss and Bolsinger 1997). The pK_a describes the acid strength and ability of a compound to dissociate; it can be regarded as the pH at which a particular acid or base group is 50% ionised (Bromilow and Chamberlain 1995). Plant compartments exhibit different pH values across membranes, ranging from pH 5 in the apoplast to pH 8 in the phloem sap (Chamberlain et al. 1998). Accordingly, a weak acid will appear at low pH in its un-dissociated state, having the ability to easily enter the symplast. Once in the symplast, due to the higher pH, it dissociates and is not able to cross back through the membranes (i.e. the ion trap theory) (Briggs et al. 1987; Bromilow and Chamberlain 1995; Chamberlain et al. 1998; Pessarakli 2014; Tyree et al. 1979).

It is understood that pollen and nectar, as part of reproductive organs, are a sink for photosynthetic products, even though nectaries can be supplied by phloem and xylem depending on the crop and variety (Heil 2011; Pacini et al. 2016; Wist and Davis 2006). However, many PPPs already found in these matrices, mainly insecticides and fungicides, are considered to be more xylem-mobile according to their physicochemical properties. Thus, an acropetal movement in the plant is conceivable but the exact mechanism as to how these chemicals are transferred into the pollen is not yet understood. Aajoud et al. (2008) demonstrated that the low transpiration stream of different parts of a sunflower head cannot be responsible for all of the fipronil residues found in this tissue. Although fipronil is more likely to move in xylem due to its high log K_{ow} (= 4.0), Aajoud et al. showed under laboratory conditions that fipronil is transported from sources (older leaves) to sinks (growing parts). In general, for non-ionised compounds like fipronil or neonicotinoids the ion trap theory does not apply and the active ingredient can move freely between phloem and xylem according to its bio membrane permeability (Sur and Stork 2003). Aajoud et al. (2008) suggested that both xylem and phloem pathways are involved in the transfer of fipronil to the flower head. Transfer via xylem from the roots to the leaves has been previously demonstrated and depends upon the rate of leaf transpiration, in addition to the compound concentration in the xylem, whereas the phloem pathway seems to be an influencing factor in the translocation to the flower parts and hence to pollen or nectar.

Another explanation for unexpected phloem transport is that biotransformation in plants can alter the compounds' properties. For example, due to its physicochemical parameters, imidacloprid is transported in xylem and accumulates in leaves, but some of its metabolites (e.g. 6-chloronicotinic acid) were shown to have properties which are potentially phloem-mobile (Buchholz and Nauen 2002; Chamberlain et al. 1995; Nauen et al. 1999). Furthermore, transformed compounds can form conjugates with glucose, isomaltose and amino acids, which could change the translocation pathway (Jiang et al. 2009; Oliver and Hewitt 2014; Sur and Stork 2003; Wu et al. 2012).

These findings could perhaps explain the increase in imidacloprid in upper plant parts, as described in the earlier section about crop-related parameters, and rationalise the presence of PPP residues in physiological sinks like pollen and nectar. Nevertheless, these conclusions might not apply for compounds with other physicochemical properties, especially for PPPs which are considered to be non-systemic.

In pollen residue studies, physicochemical properties are often mentioned to describe and explain the reason for differences in residue levels but, to our knowledge, no study has tried to link these PPP characteristics experimentally to the residues found in pollen or other matrices. Kyriakopoulou et al. (2017) found weak correlations between the residue levels in nectar and the solubility in water, although Bromilow and Chamberlain (1995) considered water solubility as a rather poor guide to systemic behaviour. Thorbek and Hyder (2006) used artificial neural networks to examine the relationship between physicochemical properties of different PPPs and residues in food products. In their opinion, the physicochemical properties and the crop type explained up to 50% of the variation. Thorbek and Hyder concluded that these properties control important aspects of the processes leading to residues in food commodities. These findings may be transferred to the residue occurrence in bee-important plant matrices.

In general, the uptake of PPPs and their half lives in plants are very well studied, primarily because risk assessments on human exposure or their environmental fate are required for the registration of PPPs, as well as the setting of Maximum Residue Levels (MRLs). However, the process determining the residues in bee-important matrices is not well understood, and more research is required to link the physicochemical properties of a compound to the translocation to pollen or nectar and to the fate and dissipation after the application of a PPP (Figure 3).

Environmental conditions

PPPs applied to a crop enter a complex system, which is greatly influenced by its surrounding environment and underlying manifold interactions. These variations are reflected in the fluctuating PPP residues reported in pollen or nectar, especially in field experiments. Laurent and Rathahao (2003) reported significantly higher variations in pollen residues in a lysimeter experiment compared to greenhouse experiments. Jiang et al. (2018) experienced varying residue fluctuations across the course of one month in field experiments and Rolke et al. (2016) observed high variations even within different sub-areas of one field. Even small-scale weather incidents can change the result of a chemical treatment, for example when the compound is washed off the leaves by rain shortly after application. Additionally, plant growth and physiology are dependent on the surrounding conditions and will influence the behaviour of the compound. This of course makes the comparability of PPP residues in pollen or nectar from different studies difficult, although the environmental fate of all kinds of PPPs are well studied.

Key parameters influencing both the chemical fate and the plant are water and temperature. Physicochemical properties are usually assessed under defined laboratory conditions and are therefore likely to change under varying conditions (Hornsby et al. 1995; Tsipi et al. 2015). For example, cuticle permeability was shown to increase rapidly with increasing temperatures (Baur et al. 1997; Baur and Schönherr 1995). Degradation processes in soil, vegetation and air are all accelerated at higher temperatures (Bloomfield et al. 2006), whereas colder temperatures limit biological and chemical reaction activities, resulting in longer half-lives and slower dissipation rates (Farha et al. 2016). Humidity can increase compound uptake into leaves (Hull 1970; Ramsey et al. 2002), while rain can lead to wash-off and leaching (Hunsche et al. 2007; Radolinski et al. 2018) and water stress was shown to affect the distribution of systemic insecticides in plant leaves (Stamm et al. 2016). Soil conditions are affected by temperature and water availability; organic matter content, microbial life and clay content play a key role in the fate and uptake of soil applied PPPs (Cessna et al. 2017; Di et al. 1998; Gevao et al. 2000; Zhang et al. 2018). PPPs with a long half-life in soil or exposed to conditions that prevent a breakdown in soil are more likely to be taken up during flowering. The transport of xylem-mobile compounds is, inter alia, dependent on the transpiration stream. Therefore, environmental conditions and plant species which enable a high transpiration will enhance acropetal movement and consequently the likelihood of translocation of PPP residues to pollen or nectar.

In general, flowers are also exposed to these conditions, however, they may show a different susceptibility to environmental conditions and a different uptake compared to the rest of the plant, due to the different structure and often hydrophilic properties of their surface (Baker and Hunt 1981;

Koch et al. 2008). Furthermore, flower opening, the dispersal of pollen, as well as the amount and composition of pollen and nectar produced is dependent on the surrounding environment, especially temperature and humidity (Heil 2011; Pacini et al. 2006; Vidal et al. 2006). PPPs applied during or shortly before bloom will contact the flowers directly and the presence of residues is therefore likely at least in pollen, even for compounds with a short half-life. All factors favouring a fast dissipation or degradation can thus decrease the residues in pollen or nectar. Choudhary and Sharma (2008) recorded a general faster dissipation of PPP residues in pollen than in nectar depending on the active ingredient. They attributed this faster degradation to the fact that pollen is more exposed to environmental conditions than the nectaries, which are typically deeply embedded within the flower. Consequently, the presentation of pollen, the arrangement of anthers and nectaries within the flower and their protection by flower petals could influence the impact of environmental conditions on residue behaviour in pollen and nectar. For example, compounds with a relatively low photo stability, such as pyrethroids, might dissipate faster in pollen grains presented openly to pollinators, compared to residues in nectar. None of the available studies considered or compared the influence of environmental conditions on PPP residues in pollen or nectar (Fig. 4). However, a field study conducted in consecutive years detected correlations in PPP residues from one year to another, despite varying environmental conditions (Dively and Kamel 2012). Nevertheless, the factors acting in different environments on PPPs availability in floral resources are complex and not well understood. Different climates and soils, for example across Europe, are currently accounted for in risk assessments for bees by conducting residue trials at multiple sites across broad geographic regions. However, environmental influences are not understood well enough to allow an extrapolation or comparison between different sites and may require further attention depending on the mode of application and properties of the active ingredient (e.g. soil uses with systemic compounds, UV stability). Controlled-environment studies looking at the effect of for example temperature on residues could provide further insights.

Conclusions

Overall, PPP residues in bee-important plant matrices are subject to manifold influences and many parameters can potentially impact their level and residence time in pollen or nectar. Several plant-related parameters, such as species and variety (including morphology), habitus and structure, were identified as contributing factors to the variation observed in PPP residue levels in pollen or nectar. Furthermore, the application mode, especially the dose rate and the timing of the application, were considered as a key source of residue variations. Nevertheless, the highest variations can probably be explained by the physicochemical properties of different compounds and, above all, by the influence of environmental conditions. However, we also demonstrate that studies which focus on these influencing factors are scarce and the complex processes which determine the residue level in bee-important matrices are not well understood (Fig. 1-4).

Thus far, research has typically concentrated on the influences of the broad application areas of neonicotinoids, thereby mainly on soil applications, which are not representative of most other insecticides. Investigations into the variability of non-systemic products in floral resources is notably neglected in research, whilst further research into residues of fungicides and herbicides in pollen and nectar is also required.

It is questionable whether the currently available data sets on residue levels can mitigate the described variability and whether they are representative enough to be used for conducting reliable risk assessments on pollinators. More wide-ranging and well replicated studies, which reflect different cropping scenarios, are necessary to obtain reliable residue levels in these specific matrices. In addition, PPPs are designed to have the best possible uptake rate and retention time on and in the plant to be effective against pests and to simultaneously avoid environmental pollution. This conflicts with the aim to achieve low residues in pollen or nectar. Therefore, application modes and circumstances in which PPP residues in flower parts are low or dissipate fast should be clarified.

It would be extremely difficult to assess the fate in pollen or nectar for all active ingredients, in all bee-important plants and under different climates. Therefore, methods are needed which enable an accurate estimation and extrapolation of PPP residues in these ecologically important matrices, which are also able to consider the numerous influences they are exposed to. In order to enable this, comparable results are required, which do not just reflect a snapshot of a single randomly selected field area and environmental conditions, but also reveal a broader knowledge which can be transferred to further situations.

This can only be achieved by improving the understanding of residue behaviour and their dynamics in these complex tissues. A fundamental challenge for future research will be to quantify the effects of different dynamics and interacting factors on PPP residue levels. Future research should aim to investigate relationships, interdependences and common features amongst various PPP applications, which may allow conclusions to be drawn on residues in pollen and nectar and, as a result, permit suitable systems to be identified which can act as model scenarios or be consulted for worst-case estimations, enabling all other scenarios to be adequately covered.

Achievement of this will permit risk assessments to be conducted with considerably less effort and expenditure, whilst simultaneously enabling rapid and accurate assessment of the risks for pollinators posed by PPPs.

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Conflict of interest

We wish to draw attention to the fact that the corresponding author’s PhD project is funded by Syngenta Ltd, a company manufacturing and selling plant protection products. Furthermore, some of the co-authors are employed by Syngenta.

Bibliography

Aajoud, A., Raveton, M., Azrou-Isghi, D., Tissut, M., and Ravanel, P. (2008). How can the fipronil insecticide access phloem? J Agric Food Chem 56, 3732-3737.

Albrecht, M., Schmid, B., Hautier, Y., and Müller, C. B. (2012). Diverse pollinator communities enhance plant reproductive success. Proceedings of the Royal Society B: Biological Sciences 279, 4845-4852.

Alsayeda, H., Pascal-Lorber, S., Nallanthigal, C., Debrauwer, L., and Laurent, F. (2007). Transfer of the insecticide [14C] imidacloprid from soil to tomato plants. Environ Chem Lett 6, 229-234.

Atwood, D., and Paisley-Jones, C. (2017). "Pesticides industry sales and usage: 2008- 2012 market estimates." U.S. Environmental Protection Agency, Washington, DC. https://www.epa.gov/pesticides/pesticides-industry-sales-and-usage-2008-2012-market-estimates retrieved 04.12.2017

Baker, E. A., and Hunt, G. M. (1981). Developmental changes in leaf epicuticular waxes in relation to foliar penetration. New Phytol 88, 731-747.

Balfour, N. J., Carreck, N. L., Blanchard, H. E., and Ratnieks, F. L. (2016). Size matters: Significant negative relationship between mature plant mass and residual neonicotinoid levels in seed-treated oilseed rape and maize crops. Agric, Ecosyst Environ 215, 85-88.

Baur, P., Buchholz, A., and Schönherr, J. (1997). Diffusion in plant cuticles as affected by temperature and size of organic solutes: similarity and diversity among species. Plant, Cell Environ 20, 982-994.

Baur, P., and Schönherr, J. (1995). Temperature dependence of the diffusion of organic compounds across plant cuticles. Chemosphere 30, 1331-1340.

Bloomfield, J. P., Williams, R. J., Gooddy, D. C., Cape, J. N., and Guha, P. (2006). Impacts of climate change on the fate and behaviour of pesticides in surface and groundwater--A UK perspective. Sci Total Environ 369, 163-177.

Bonmatin, J. M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E. A., Noome, D. A., Simon-Delso, N., and Tapparo, A. (2015). Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research (international) 22, 35-67.

Bonmatin, J. M., Moineau, I., Charvet, R., Colin, M. E., Fleche, C., and Bengsch, E. (2005). Behaviour of imidacloprid in fields. Toxicity for honey bees. Environ Chem 5, 483-494.

Bonmatin, J. M., Moineau, I., Charvet, R., Fleche, C., Colin, M. E., and Bengsch, E. R. (2003). A LC/APCI-MS/MS Method for Analysis of Imidacloprid in Soils, in Plants, and in Pollens. Anal Chem 75, 2027-2033.

Botias, C., David, A., Hill, E. M., and Goulson, D. (2017). Quantifying exposure of wild bumblebees to mixtures of agrochemicals in agricultural and urban landscapes. Environ Pollut 222, 73-82.

Botias, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E., and Goulson, D. (2015). Neonicotinoid Residues in Wildflowers, a Potential Route of Chronic Exposure for Bees. Environ Sci Technol 49, 12731-12740.

Briggs, G. G., and Bromilow, R. H. (1994). Influence of Physicochemical Properties on Uptake and Loss of Pesticides and Adjuvants from the Leaf Surface. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1-26.

Briggs, G. G., Rigitano, R. L. O., and Bromilow, R. H. (1987). Physico-chemical factors affecting uptake by roots and translocation to shoots of weak acids in barley. Pestic Sci 19, 101-112.

Bromilow, R. H., and Chamberlain, K. (1989). Designing molecules for systemicity. BR PLANT GR, 113-128.

Bromilow, R. H., and Chamberlain, K. (1995). Principles governing uptake and transport of chemicals. Plant contamination: Modeling and simulation of organic chemical processes, 37-68.

Bromilow, R. H., Rigitano, R. L., Briggs, G. G., and Chamberlain, K. (1987). Phloem translocation of non-ionised chemicals in Ricinus communis. Pestic Sci 19, 85-99.

Buchholz, A., and Nauen, R. (2002). Translocation and translaminar bioavailability of two neonicotinoid insecticides after foliar application to cabbage and cotton. Pest Manage Sci 58, 10-16.

Byrne, F. J., Visscher, P. K., Leimkuehler, B., Fischer, D., Grafton-Cardwell, E. E., and Morse, J. G. (2014). Determination of exposure levels of honey bees foraging on flowers of mature citrus trees previously treated with imidacloprid. Pest Manage Sci 70, 470-482.

Carvalho, F. P. (2006). Agriculture, pesticides, food security and food safety. Environ Sci Policy 9, 685-692.

Cessna, A. J., Knight, J. D., Ngombe, D., and Wolf, T. M. (2017). Effect of temperature on the dissipation of seven herbicides in a biobed matrix. Can J Soil Sci 97, 717-731.

Cham, K. d. O., Rebelo, R. M., Oliveira, R. d. P., Ferro, A. A., Vianasilva, F. E. d. C., Borges, L. d. O., Saretto, C. O. S. D., Tonelli, C. A. M., and Macedo, T. C. (2017). "Manual de avaliação de risco ambiental de agrotóxicos para abelhas." Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (Ibama), Brasília.

Chamberlain, K., Patel, S., and Bromilow, R. H. (1998). Uptake by roots and translocation to shoots of two morpholine fungicides in barley. Pestic Sci 54, 1-7.

Chamberlain, K., Tench, A. J., Williams, R. H., and Bromilow, R. H. (1995). Phloem translocation of pyridinecarboxylic acids and related imidazolinone herbicides in Ricinus communis. Pest Manage Sci 45, 69-75.

Chauzat, M.-P., Martel, A.-C., Blanchard, P., Clément, M.-C., Schurr, F., Lair, C., Ribière, M., Wallner, K., Rosenkranz, P., and Faucon, J.-P. (2010). A case report of a honey bee colony poisoning incident in France. J Apic Res 49, 113-115.

Choudhary, A., and Sharma, D. C. (2008). Dynamics of pesticide residues in nectar and pollen of mustard (Brassica juncea (L.) Czern.) grown in Himachal Pradesh (India). Environ Monit Assess 144, 143-150.

Cooper, J., and Dobson, H. (2007). The benefits of pesticides to mankind and the environment. Crop Protect 26, 1337-1348.

Cowles, R. S., and Eitzer, B. D. (2017). Residues of neonicotinoid insecticides in pollen and nectar from model plants. Journal of Environmental Horticulture 35, 24-34.

Di, H. J., Aylmore, L. A. G., and Kookana, R. S. (1998). Degradation rates of eight pesticides in surface and subsurface soils under laboratory and field conditions. Soil Science 163, 404-411.

DiTomaso, J. M. (1999). Barriers to foliar penetration and uptake of herbicides. In "Proceedings of the California Weed Science Society", Vol. 51, pp. 150-155.

Dively, G. P., and Kamel, A. (2012). Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. J Agric Food Chem 60, 4449-4456.

ECPA (2017). "Proposal for a protective and workable regulatory European bee risk assessment scheme based on the EFSA bee guidance and other new data and available approaches." European Crop Protection Authority. http://www.ecpa.eu retrieved 08.04.2018

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 Journal 10, 2668.

EFSA (2013). Guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees). EFSA Journal 11, 3295.

FaoStat (2017). "FAOSTAT Database- Statistical databases." FAO- Food and Agriculture Organization of the United Nations Rome, Italy. www.faostat.org retrieved 23.11.2017

Farha, W., Abd El-Aty, A. M., Rahman, M. M., Shin, H. C., and Shim, J. H. (2016). An overview on common aspects influencing the dissipation pattern of pesticides: a review. Environ Monit Assess 188, 693.

Fujisawa, T., Ichise, K., Fukushima, M., Katagi, T., and Takimoto, Y. (2002). Improved Uptake Models of Nonionized Pesticides to Foliage and Seed of Crops. J Agric Food Chem 50, 532-537.

Gallai, N., Salles, J.-M., Settele, J., and Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol Econ 68, 810-821.

Gevao, B., Semple, K. T., and Jones, K. C. (2000). Bound pesticide residues in soils: a review. Environ Pollut 108, 3-14.

Gilland, B. (2002). World population and food supply: can food production keep pace with population growth in the next half-century? Food Policy 27, 47-63.

Gong, Y., and Diao, Q. (2017). Current knowledge of detoxification mechanisms of xenobiotic in honey bees. Ecotoxicology 26, 1-12.

Goulson, D. (2013). REVIEW: An overview of the environmental risks posed by neonicotinoid insecticides. J Appl Ecol 50, 977-987.

Goulson, D., Nicholls, E., Botias, C., and Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347, 1255957.

Heil, M. (2011). Nectar: generation, regulation and ecological functions. Trends Plant Sci 16, 191-200.

Hornsby, A. G., Wauchope, R. D., and Herner, A. (1995). "Pesticide Properties in the Environment," Springer Science & Business Media.

Hsu, F. C., and Kleier, D. A. (1996). Phloem mobility of xenobiotics VIII. A short review. J Exp Bot 47, 1265-1271.

Hull, H. M. (1970). Leaf structure as related to absorption of pesticides and other compounds. In "Residue Reviews/Rückstands-Berichte", 1-150. Springer Verlag, Berlin, Heidelberg.

Hulme, P. E. (2017). Climate change and biological invasions: evidence, expectations, and response options. Biol Rev Camb Philos Soc 92, 1297-1313.

Hunsche, M., Damerow, L., Schmitz-Eiberger, M., and Noga, G. (2007). Mancozeb wash-off from apple seedlings by simulated rainfall as affected by drying time of fungicide deposit and rain characteristics. Crop Protect 26, 768-774.

IPBES (2016). Summary for policymakers of the assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. (S.G. Potts, V. L. Imperatriz-Fonseca, H. T. Ngo, J. C. Biesmeijer, T. D. Breeze, L. V. Dicks, L. A. Garibaldi, R. Hill, J. Settele, A. J. Vanbergen, M. A. Aizen, S. A. Cunningham, C. Eardley, B. M. Freitas, N. Gallai, P. G. Kevan, A. Kovács-Hostyánszki, P. K. Kwapong, X. L. J. Li, D. J. Martins, G. Nates-Parra, J. S. Pettis, R. Rader and B. F. Viana, eds.). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany, pp. 36.

Jiang, D.-X., Lu, X.-L., Hu, S., Zhang, X.-B., and Xu, H.-H. (2009). A new derivative of fipronil: Effect of adding a glycinyl group to the 5-amine of pyrazole on phloem mobility and insecticidal activity. Pestic Biochem Physiol 95, 126-130.

Jiang, J., Ma, D., Zou, N., Yu, X., Zhang, Z., Liu, F., and Mu, W. (2018). Concentrations of imidacloprid and thiamethoxam in pollen, nectar and leaves from seed-dressed cotton crops and their potential risk to honeybees (Apis mellifera L.). Chemosphere 201, 159-167.

Johnson, D. G. (2000). Population, food, and knowledge. The American Economic Review 90, 1-14.

Johnson, J. (2012). "The role of pesticides on honey bee health and hive maintenance with an emphasis on the neonicotinoid, imidacloprid," University of Maryland, Baltimore.

Johnson, R. M., Ellis, M. D., Mullin, C. A., and Frazier, M. (2010). Pesticides and honey bee toxicity – USA. Apidologie 41, 312-331.

Kasiotis, K. M., Anagnostopoulos, C., Anastasiadou, P., and Machera, K. (2014). Pesticide residues in honeybees, honey and bee pollen by LC–MS/MS screening: Reported death incidents in honeybees. Sci Total Environ 485, 633-642.

Kiljanek, T., Niewiadowska, A., Gawel, M., Semeniuk, S., Borzecka, M., Posyniak, A., and Pohorecka, K. (2017). Multiple pesticide residues in live and poisoned honeybees - Preliminary exposure assessment. Chemosphere 175, 36-44.

Kirkwood, R. C. (1999). Recent developments in our understanding of the plant cuticle as a barrier to the foliar uptake of pesticides. Pest Manage Sci 55, 69-77.

Klein, A. M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., and Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B 274, 303-13.

Klittich, C., Jr., Green, F. R., 3rd, Ruiz, J. M., Weglarz, T., and Blakeslee, B. A. (2008). Assessment of fungicide systemicity in wheat using LC-MS/MS. Pest Manage Sci 64, 1267-77.

Koch, K., Bhushan, B., and Barthlott, W. (2008). Diversity of structure, morphology and wetting of plant surfaces. Soft Matter 4, 1943-1963.

Kubik, M., Nowacki, J., Pidek, A., Warakomska, Z., Michalczuk, L., and Goszczyñski, W. (1999). Pesticide residues in bee products collected from cherry trees protected during blooming period with contact and systemic fungicides. Apidologie 30, 521-532.

Kunz, N., Frommberger, M., Dietzsch, A. C., Wirtz, I. P., Stähler, M., Frey, E., Illies, I., Dyrba, W., Alkassab, A., and Pistorius, J. (2015). Neonicotinoids and bees: A large scale field study investigating residues and effects on honeybees, bumblebees and solitary bees in oilseed rape grown from clothianidin-treated seed. Julius-Kühn-Archiv 450, 155.

Kyriakopoulou, K., Kandris, I., Pachiti, I., Kasiotis, K. M., Spyropoulou, A., Santourian, A., Kitromilidou, S., Pappa, G., and Glossioti, M. (2017). Collection and analysis of pesticide residue data for pollen and nectar – Final Report. EFSA Supporting Publications 14.

Laurent, F. M., and Rathahao, E. (2003). Distribution of [14C] imidacloprid in sunflowers (Helianthus annuus L.) following seed treatment. J Agric Food Chem 51, 8005-8010.

Lee, K. V., Steinhauer, N., Rennich, K., Wilson, M. E., Tarpy, D. R., Caron, D. M., Rose, R., Delaplane, K. S., Baylis, K., Lengerich, E. J., Pettis, J., Skinner, J. A., Wilkes, J. T., Sagili, R., and vanEngelsdorp, D. (2015). A national survey of managed honey bee 2013–2014 annual colony losses in the USA. Apidologie 46, 292-305.

Lewis, K. A., Tzilivakis, J., Warner, D. J., and Green, A. (2016). An international database for pesticide risk assessments and management. Human and Ecological Risk Assessment: An International Journal 22, 1050-1064.

Lundin, O., Rundlof, M., Smith, H. G., Fries, I., and Bommarco, R. (2015). Neonicotinoid Insecticides and Their Impacts on Bees: A Systematic Review of Research Approaches and Identification of Knowledge Gaps. PLoS One 10, e0136928.

Mitton, F. M., Gonzalez, M., Monserrat, J. M., and Miglioranza, K. S. (2016). Potential use of edible crops in the phytoremediation of endosulfan residues in soil. Chemosphere 148, 300-306.

Mostafalou, S., and Abdollahi, M. (2017). Pesticides: an update of human exposure and toxicity. Arch Toxicol 91, 549-599.

Nauen, R., Reckmann, U., Armborst, S., Stupp, H. P., and Elbert, A. (1999). Whitefly-active metabolites of imidacloprid: Biological efficacy and translocation in cotton plants. Pest Manage Sci 55, 265-271.

Oerke, E. C., and Dehne, H. W. (2004). Safeguarding production—losses in major crops and the role of crop protection. Crop Protect 23, 275-285.

Oliveira, C. M., Auad, A. M., Mendes, S. M., and Frizzas, M. R. (2014). Crop losses and the economic impact of insect pests on Brazilian agriculture. Crop Protect 56, 50-54.

Oliver, R., and Hewitt, H. G. (2014). "Fungicides in Crop Protection," 2/Ed. CABI Publishing.

Ollerton, J. (2017). Pollinator diversity: distribution, ecological function, and conservation. Annual Review of Ecology, Evolution, and Systematics 48.

Otani, T., and Seike, N. (2007). Rootstock control of fruit dieldrin concentration in grafted cucumber (Cucumis sativus). J Pestic Sci 32, 235-242.

Otani, T., Seike, N., and Sakata, Y. (2007). Differential uptake of dieldrin and endrin from soil by several plant families andCucurbitagenera. Soil Sci Plant Nutr 53, 86-94.

Pacini, E., Guarnieri, M., and Nepi, M. (2006). Pollen carbohydrates and water content during development, presentation, and dispersal: a short review. Protoplasma 228, 73-77.

Pacini, E., Nepi, M., and Vesprini, J. L. (2016). Nectar biodiversity: a short review. Plant Syst Evol 238, 7-21.

Pessarakli, M. (2014). "Handbook of plant and crop physiology," CRC Press.

Peterson, C. A., and Edgington, L. V. (1976). Entry of pesticides into the plant symplast as measured by their loss from an ambient solution. Pestic Sci 7, 483-491.

Pettis, J. S., vanEngelsdorp, D., Johnson, J., and Dively, G. (2012). Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema. Naturwissenschaften 99, 153-158.

Pimentel, D. (1997). Pest management in agriculture In "Techniques for Reducing Pesticide Use: Environmental and Economic Benefits. " (D. Pimentel, ed.), 456. John Wiley & Sons, Chichester, UK.

Pimentel, D. (2005). Environmental and economic costs of the application of pesticides primarily in the United States. Environ Dev Sustainability 7, 229-252.

Pohorecka, K., Skubida, P., Miszczak, A., Semkiw, P., Sikorski, P., Zagibajło, K., Teper, D., Kołtowski, Z., Skubida, M., Zdańska, D., and Bober, A. (2012). Residues of Neonicotinoid Insecticides in Bee Collected Plant Materials from Oilseed Rape Crops and their Effect on Bee Colonies. J Apic Sci 56, 115- 134.

Population Reference Bureau (2017). "2017 World Population Data Sheet," Washington, DC. https://www.prb.org/international/indicator/population/snapshot/ retrieved 08.12.2017

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W. E. (2010a). Global pollinator declines: trends, impacts and drivers. Trends Ecol Evol 25, 345-53.

Potts, S. G., Roberts, S. P. M., Dean, R., Marris, G., Brown, M. A., Jones, R., Neumann, P., and Settele, J. (2010b). Declines of managed honey bees and beekeepers in Europe. J Apic Res 49, 15-22.

Price, C. E., and Anderson, N. H. (1985). Uptake of chemicals from foliar deposits: effects of plant species and molecular structure. Pest Manage Sci 16, 369-377.

Radolinski, J., Wu, J., Xia, K., and Stewart, R. (2018). Transport of a neonicotinoid pesticide, thiamethoxam, from artificial seed coatings. Sci Total Environ 618, 561-568.

Ramsey, R. J. L., Stephenson, G. R., and Hall, J. C. (2002). Effect of relative humidity on the uptake, translocation, and efficacy of glufosinate ammonium in wild oat (Avena fatua). Pestic Biochem Physiol 73, 1-8.

Rolke, D., Persigehl, M., Peters, B., Sterk, G., and Blenau, W. (2016). Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in northern Germany: residues of clothianidin in pollen, nectar and honey. Ecotoxicology 25, 1691-1701.

Rortais, A., Arnold, G., Dorne, J. L., More, S. J., Sperandio, G., Streissl, F., Szentes, C., and Verdonck, F. (2017). Risk assessment of pesticides and other stressors in bees: Principles, data gaps and perspectives from the European Food Safety Authority. Sci Total Environ 587-588, 524-537.

Rosenzweig, C., Iglesias, A., Yang, X., Epstein, P. R., and Chivian, E. (2001). Climate change and extreme weather events; implications for food production, plant diseases, and pests. Global Change Hum Health 2, 90-104.

Sanchez-Bayo, F., Belzunces, L., and Bonmatin, J. M. (2017). Lethal and sublethal effects, and incomplete clearance of ingested imidacloprid in honey bees (Apis mellifera). Ecotoxicology 26, 1199-1206.

Sanchez-Bayo, F., and Goka, K. (2014). Pesticide residues and bees--a risk assessment. PLoS One 9, e94482.

Schmuck, R., Schöning, R., Stork, A., and Schramel, O. (2001). Risk posed to honeybees (Apis mellifera L, Hymenoptera) by an imidacloprid seed dressing of sunflowers. Pest Manage Sci 57, 225-238.

Seitz, N., Traynor, K. S., Steinhauer, N., Rennich, K., Wilson, M. E., Ellis, J. D., Rose, R., Tarpy, D. R., Sagili, R. R., Caron, D. M., Delaplane, K. S., Rangel, J., Lee, K., Baylis, K., Wilkes, J. T., Skinner, J. A., Pettis, J. S., and vanEngelsdorp, D. (2016). A national survey of managed honey bee 2014–2015 annual colony losses in the USA. J Apic Res 54, 292-304.

Sicbaldi, F., Sacchi, G. A., Trevisan, M., and Del Re, A. A. (1997). Root uptake and xylem translocation of pesticides from different chemical classes. Pest Manage Sci 50, 111-119.

Stamm, M. D., Heng-Moss, T. M., Baxendale, F. P., Siegfried, B. D., Blankenship, E. E., and Nauen, R. (2016). Uptake and translocation of imidacloprid, clothianidin and flupyradifurone in seed-treated soybeans. Pest Manage Sci 72, 1099-1109.

Stoner, K. A., and Eitzer, B. D. (2012). Movement of soil-applied imidacloprid and thiamethoxam into nectar and pollen of squash (Cucurbita pepo). PLoS One 7, e39114.

Sur, R., and Stork, A. (2003). Uptake, translocation and metabolism of imidacloprid in plants. Bull Insectol 56, 35-40.

Thorbek, P., and Hyder, K. (2006). Relationship between physicochemical properties and maximum residue levels and tolerances of crop-protection products for crops set by the USA, European Union and Codex. Food Addit Contam 23, 764-776.

Tilman, D. (1999). Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proceedings of the National Academy of Sciences 96, 5995-6000.

Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H., Simberloff, D., and Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. Science 292, 281-284.

Tosi, S., Costa, C., Vesco, U., Quaglia, G., and Guido, G. (2018). A 3-year survey of Italian honey bee-collected pollen reveals widespread contamination by agricultural pesticides. Sci Total Environ 615, 208-218.

Trapp, S. (2004). Plant uptake and transport models for neutral and ionic chemicals. Environ Sci Pollut R 11, 33-39.

Tsipi, D., Botitsi, H., and Economou, A. (2015). Pesticide Chemistry and Risk Assessment. In "Mass Spectrometry for the Analysis of Pesticide Residues and Their Metabolites", 1-34. John Wiley & Sons, Inc.

Tyree, M. T., Peterson, C. A., and Edgington, L. V. (1979). A Simple Theory Regarding Ambimobility of Xenobiotics with Special Reference to the Nematicide, Oxamyl. Plant Physiol 63, 367-374.

UN (2017). "World Population Prospects: The 2017 Revision, Key Findings and Advance Tables.." United Nations, Department of Economic and Social Affairs, Population Division, New York.

USEPA (2014). "Guidance for Assessing Pesticide Risks to Bees ". United States Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Washington, D.C.

Vanbergen, A. J., Heard, M. S., Breeze, T., Potts, S. G., and Hanley, N. (2014). Status and value of pollinators and pollination services. Department for Environment, Food and Rural Affairs 53pp.

Veddeler, D., Olschewski, R., Tscharntke, T., and Klein, A.-M. (2008). The contribution of non-managed social bees to coffee production: new economic insights based on farm-scale yield data. Agroforest Syst 73, 109-114.

Vidal, M. d. G., Jong, D. d., Wien, H. C., and Morse, R. A. (2006). Nectar and pollen production in pumpkin (Cucurbita pepo L.). Brazilian Journal of Botany 29, 267-273.

Villa, S., Vighi, M., Finizio, A., and Serini, G. B. (2000). Risk assessment for honeybees from pesticide-exposed pollen. Ecotoxicology 9, 287-297.

Vryzas, Z. (2016). The Plant as Metaorganism and Research on Next-Generation Systemic Pesticides – Prospects and Challenges. Frontiers in Microbiology 7, 1968.

Wallner, K. (2009). Sprayed and seed dressed pesticides in pollen, nectar and honey of oilseed rape. Julius-Kühn-Archiv 423, 152-153.

Wang, C. J., and Liu, Z. Q. (2007). Foliar uptake of pesticides—Present status and future challenge. Pestic Biochem Physiol 87, 1-8.

Wilson, C., and Tisdell, C. (2001). Why farmers continue to use pesticides despite environmental, health and sustainability costs. Ecol Econ 39, 449-462.

Wist, T. J., and Davis, A. R. (2006). Floral nectar production and nectary anatomy and ultrastructure of Echinacea purpurea (Asteraceae). Ann Bot 97, 177-93.

Woodcock, B., Bullock, J., Shore, R., Heard, M., Pereira, M., Redhead, J., Ridding, L., Dean, H., Sleep, D., and Henrys, P. (2017). Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science 356, 1393-1395.

Wu, H. X., Yang, W., Zhang, Z. X., Huang, T., Yao, G. K., and Xu, H. H. (2012). Uptake and phloem transport of glucose-fipronil conjugate in Ricinus communis involve a carrier-mediated mechanism. J Agric Food Chem 60, 6088-6094.

Wu, J. Y., Anelli, C. M., and Sheppard, W. S. (2011). Sub-lethal effects of pesticide residues in brood comb on worker honey bee (Apis mellifera) development and longevity. PLoS One 6, e14720.

Wyss, P., and Bolsinger, M. (1997). Translocation of pymetrozine in plants. Pestic Sci 50, 195-202.

Yu, Y., Zhu, H., Frantz, J. M., Reding, M. E., Chan, K. C., and Ozkan, H. E. (2009). Evaporation and coverage area of pesticide droplets on hairy and waxy leaves. Biosys Eng 104, 324-334.

Zhang, P., Ren, C., Sun, H., and Min, L. (2018). Sorption, desorption and degradation of neonicotinoids in four agricultural soils and their effects on soil microorganisms. Sci Total Environ 615, 59-69.

Zhang, W., Jiang, F., and Ou, J. (2011). Global pesticide consumption and pollution: with China as a focus. Proceedings of the International Academy of Ecology and Environmental Sciences 1, 125.

Tables

Table 1 Summary of selected PPP residues in pollen expressed as Residue Unit Dose (RUD) (mg a.i. kg^{-1} pollen at an application rate of 1 kg a.i. ha^{-1}) derived from two different sources. The minimum, maximum and mean values demonstrate the high variability of residues found in pollen from spray applications. Some calculations were not derived from a single crop or active ingredient, but many different crops/active ingredients were summarised ("various").

Crop	**Active ingredient**	**Min** (RUD mg kg^{-1})	**Max** (RUD mg kg^{-1})	**Mean** (RUD mg kg^{-1})	**Source***
various	various	0.0002	149.8	6.1 ± 30.704 (SD)	a
various	various	0.004	366	65.06 ± 89.421 (SD)	b
various	alpha- Cypermethrin	11.370	366.3	167.3 ± 121.438 (SD)	b
Brassica sp.	various	2.083	366.3	87.06 ± 102.8 (SD)	b
	Teflubenzuron	21.7	149.8	**	a
	Acetamiprid	3.4	14.8	**	a

Examples for PPP residues of the same active ingredient in different crops

Active ingredient	**Crop**			**Source***
	Melon (*Cucurbitaceae*)	*Phacelia tanacetifolia* L.	*Brassica sp.*	
Spirotetramat	2.2	63.5	83.1	a

*Sources: a) EFSA 2013, see Annex F; b) Kyriakopoulou et al. 2017

** Only two residue values were provided for this active ingredient/ crop combination

Table 2: Relationship between dose rate, residues in pollen (ppm) and Residue Unit Dose (RUD) for three active ingredients. Data from Choudhary and Sharma (2007). Application of 750 L ha^{-1} water in mustard (*Brassica juncea* Czern.) in 2003/2004.

Active ingredient	**Dose rate** (g a.i. ha^{-1})	**Residues pollen** ppm	**RUD**
Endosulfan	525	2.126	4.05
Spiromesifen	225	2.052	9.12
Lambda- Cyhalothrin	75	1.607	21.43