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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1   **A review of the factors that influence pesticide residues in pollen and nectar:**

2   **future research requirements for optimising the estimation of pollinator**

3   **exposure**

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10   **Abstract**

11   In recent years, the impact of Plant Protection Products (PPPs) on insect pollinator decline has

12   stimulated significant amounts of research, as well as political and public interest. PPP residues have

13   been found in various bee-related matrices, resulting in governmental bodies worldwide releasing

14   guidance documents on methods for the assessment of the overall risk of PPPs to different bee

15   species. An essential part of these risk assessments are PPP residues found in pollen and nectar, as

16   they represent a key route of exposure. However, PPP residue values in these matrices exhibit large

17   variations and are not available for many PPPs and crop species combinations, which results in

18   inaccurate estimations and uncertainties in risk evaluation. Additionally, residue studies on pollen and

19   nectar are expensive and practically challenging. An extrapolation between different cropping

20   scenarios and PPPs is not yet justified, as the behaviour of PPPs in pollen and nectar is poorly

21   understood. Therefore, this review aims to contribute to a better knowledge and understanding of

22   the fate of PPP residues in pollen and nectar and to outline knowledge gaps and future research needs.

23   The literature suggests that four primary factors, the crop type, the application method, the

24   physicochemical properties of a compound and the environmental conditions have the greatest

1

25 influence on PPP residues in pollen and nectar. However, these factors consist of many sub-factors

26 and initial effects may be disguised by different sampling methodologies, impeding their exact

27 characterisation. Moreover, knowledge about these factors is ambiguous and restricted to a few

28 compounds and plant species. We propose that future research should concentrate on identifying

29 relationships and common features amongst various PPP applications and crops, as well as an overall

30 quantification of the described parameters; in order to enable a reliable estimation of PPP residues in

31 pollen, nectar and other bee matrices.

32 **Keywords**

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

34 **Capsule**

35 Pesticide residue values within pollen and nectar have potentially significant consequences for the

36 reliability of risk assessments for wild and managed bee populations, however, the reasons and

37 mechanisms underlying variations in residues are poorly understood and require greater

38 investigation.

39 **Introduction**

40 **Usage, benefits and drawbacks of Plant Protection Products**

41 The global population has increased rapidly, tripling since 1950 to a current total of 7.6 billion

42 (Population Reference Bureau 2017), and is predicted to expand to 9.6 billion by 2050 (UN 2017). This

43 growth has been facilitated by the intensification of crop production as a result of new developments

44 and innovations (Carvalho 2006; Johnson 2000). As a consequence, the daily food supply per capita

45 increased from 2196 kcal day^{-1} in 1960 to 2884 kcal day^{-1} in 2013, with cereal yields almost tripling in

46 the same time period (FaoStat 2017). Concurrent increases in production, use and trade of Plant

47 Protection Products (PPPs) indicate their contribution to these increases in food production (Atwood

48 and Paisley-Jones 2017; Gilland 2002; Tilman 1999; Tilman et al. 2001; Zhang et al. 2011). Today,

49 approximately 1000 active ingredients (a.i.s) (i.e. the components in PPPs which are active against

50 pests/plant diseases) are globally available (Lewis et al. 2016).

51 The predominant use of PPPs is in the agricultural sector to protect crops from weeds, fungal

52 pathogens and pests (Wilson and Tisdell 2001). Estimates suggest the losses in plant production

53 without PPPs would be up to 80% for some crops with potentially severe economic consequences

54 (Oerke and Dehne 2004; Oliveira et al. 2014; Pimentel 1997). Outside of the agricultural sector, PPPs

55 are a cost and labour efficient method for the protection and maintenance of public spaces, for

56 example weed control on railways and streets (Cooper and Dobson 2007). In the future, the targeted

57 use of PPPs could further grow in importance; consequences of globalisation and climate change are

58 predicted to change the distribution and life cycles of many pest species, which could render previous

59 control strategies ineffective (Hulme 2017; Rosenzweig et al. 2001). Therefore, there is a strong

60 argument to suggest that PPPs currently make a significant contribution to stable and reliable crop

61 yields, high food quality and the prevention of economic losses, which is a key factor in enabling the

62 global food system to continue to operate in its current format.

63 Nevertheless, PPPs are toxic chemicals and, in the absence of mitigation, some exposure to non-target

64 organisms and the ecosystem is inevitable. Due to their wide range of applications, PPP residues and

65 their metabolites can be found in many ecosystems, with the potential to cause various effects on

66 humans, soil and water organisms, birds, mammals and invertebrates (Mostafalou and Abdollahi

67 2017; Pimentel 2005; Tilman 1999).

68 **PPPs and insect pollinators**

69 In recent years, high overwintering losses of honey bee colonies and declines in populations of other

70 insect pollinator species in Europe and North America (Lee et al. 2015; Ollerton 2017; Potts et al.

71 2010b; Seitz et al. 2016) have raised concerns about the contribution of PPPs to these losses (IPBES

72 2016). Managed and wild pollinator species provide vital ecosystem services, particularly for agro-

73 ecosystems (Albrecht et al. 2012; Klein et al. 2007; Vanbergen et al. 2014; Veddeler et al. 2008) and

74 Gallai et al. (2009) calculated the total economic value of pollination worldwide to be € 153 billion. As

75 a result, the toxic effects of PPPs on pollinators, particularly neonicotinoids, has become the focus of

76 significant amounts of research, and political and public interest. There is a broad consensus amongst

77 researchers in the field that declines are the result of a combination of factors including habitat loss,

78 pests/diseases and PPPs (Goulson et al. 2015; IPBES 2016; Potts et al. 2010a). Whilst the overall role

79 of PPPs on pollinator declines is still debated, there is clear evidence for both the exposure of bees to

80 a range of chemical products via contact and oral exposure (e.g. Botias et al. 2017; Chauzat et al. 2010;

81 Johnson et al. 2010; Kiljanek et al. 2017; Tosi et al. 2018) and the toxicity of PPPs to bees in laboratory

82 toxicity studies (e.g. Kasiotis et al. 2014; Pettis et al. 2012; Sanchez-Bayo et al. 2017; Woodcock et al.

83 2017; Wu et al. 2011).

84 Overall, there is a difficult trade-off between permitting the use of products upon which modern

85 agriculture relies for the protection of crops and maintaining vital environmental goods and services,

86 which themselves have an important role in sustainable food production. Therefore, in order to ensure

87 the safety of PPPs, complex and highly regulated processes of environmental risk assessments have

88 been developed.

89 With respect to pollinators, the European Food Safety Authority (EFSA 2013) published a Guidance

90 Document on the risk assessment of PPPs on bees (including honey bees, *Apis mellifera* L.,

91 Bumblebees, *Bombus spp*. and solitary bee species), to outline a process by which PPPs can be

92 evaluated for their potential risks in causing unacceptable harm to bees. Similar approaches have been

93 published in the US, Canada and Brazil (Cham et al. 2017; USEPA 2014). An important component in

94 these approaches are PPP residue levels in pollen and nectar. They represent a key route of exposure

95 for pollinators as, in many species, all life stages feed to some extent upon these food sources (Rortais

96 et al. 2017; Villa et al. 2000). However, knowledge to enable a more accurate prediction of PPP

97 residues in pollen and nectar is limited and a number of barriers, which are discussed in more detail

98 in the next section, inhibit a clear assessment of residue levels used in risk estimation.

99 **Aim of the review**

100 In this review our aim was to identify and compile existing literature data on the behaviour and fate

101 of residues in pollen and nectar following PPP applications, and outline the manifold parameters which

102 appear to influence these residues. In doing so we identify knowledge gaps concerning the variability

103 of PPP residue values in pollen or nectar and highlight future research needs, in order to enable a

104 precise prediction of residue levels for pollinator risk assessments in future and to encourage, initiate

105 and facilitate further research in this field.

106 **Pollinator risk assessments and evidence base**

107 **Current methodological approaches for pollinator risk assessments**

108 Current approaches for pollinator risk assessments (e.g. Cham et al. 2017; EFSA 2013; USEPA 2014)

109 pursue similar strategies and methodologies. In general, effect studies (e.g. laboratory adult acute oral

110 toxicity studies, larvae toxicity studies) and exposure estimates (contact or oral) are combined in a

111 tiered approach to assess the risk of PPPs to pollinators, ranging from very conservative estimates to

112 more realistic scenarios. While the latter requires high data input and more extensive studies, in the

113 lower, more conservative tiers, worst-case default values can be applied. Theoretically, such an

114 approach allows for more rapid and cost-effective initial assessments that are robust enough to

115 separate those PPPs that pose a potential risk to bees from those that can be considered of low risk.

116 To assess the risk from oral exposure of bees to PPPs, the guidance documents (Cham et al. 2017;

117 EFSA 2013; USEPA 2014) provide general default residue values in pollen and nectar for different

118 application scenarios, which aim to be protective. If the assessment fails in lower tiers and risk

119 mitigation is not possible, the guidance documents cited above suggest a refinement of the

120 assessment in higher tiers, for example by using representative "real" PPP residue values in pollen or

121 nectar or compound and crop specific data, which can be further refined by conducting field trials.

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

123   A recent proposal made in reference to EFSA's risk assessment from the European Crop Protection

124   Authority (ECPA 2017), which represents the industrial sector, concluded that the current guidance is

125   over-conservative and that even substances known to be non-toxic to bees fail at lower tiers. They

126   assert that, in most cases, a higher tier refinement is required. In order to facilitate higher tier

127   assessments, oral exposure estimates must be refined using representative residue data (Cham et al.

128   2017; EFSA 2013; USEPA 2014). However, data on residue levels in floral resources vary widely and

129   are unknown for many PPPs and crop species (EFSA 2013; Lundin et al. 2015). Table 1 provides a brief

130   overview of PPP residue data recorded in pollen from spray applications, illustrating the variation of

131   PPP residues from different active ingredients and in different crops. These data are taken from a

132   recent meta-study (Kyriakopoulou et al. 2017) and from the pollinator risk assessment published by

133   EFSA (2013), both providing a comprehensive overview and summary of data on the available residue

134   data in bee-relevant matrices and products, which were gathered from Draft Assessment Reports

135   (DARs), literature and peer reviews of active ingredients.

136   Overall, there is wide variation in residues, with differences not only between various active

137   ingredients and crop combinations, but also within each of these groups. For instance, aggregated

138   residues from various PPPs vary considerably not only within *Brassica* pollen, but also from uses of

139   individual PPPs, such as teflubenzuron on *Brassica*. Similar findings can be observed for residues in

140   nectar (Table S1).

141   Both publications highlighted the fact that the available studies differed considerably in design,

142   sampling timing, sampling methodology and application scenarios, or lacked data for certain types of

143   active ingredients. Thus residue data is difficult to compare. Overall, knowledge about PPP residues in

144   pollen or nectar is fragmentary and only a small proportion of treatments and crops have been taken

145   into account, with the majority of residue values provided for neonicotinoids and oilseed rape (OSR)

146   (*Brassica napus* L.). Pollinator risk evaluation is therefore based on extrapolated residue data and as a

147   consequence, on an incomplete dataset. However, the knowledge regarding PPP residues present in

148   pollen and nectar is too limited to allow extrapolations or conclusions to be drawn from those crops

149   where data are available.

150   Yet, if risk assessments are based on residue values that are not representative for the treatment

151   scenario and cropping system, the risk posed from PPPs to pollinators might be incorrectly estimated

152   (Lundin et al. 2015), resulting in false negatives (i.e. misuses of concern), or in false positives, which

153   may result in unnecessary higher tier testing. The currently available data sets can neither mitigate

154   the variability and incompleteness, nor rationalise how this should be addressed in risk assessments

155   or why these variations in PPP residues occur.

156   **PPP residue studies in pollen and nectar**

157   Extensive studies are needed to derive reliable PPP residue values in pollen and nectar. However, the

158   exact determination of residues in bee-attractive plants is expensive and time consuming. Relatively

159   large volumes of the target matrices are required for the chemical analyses needed to quantify the

160   PPP residues, but pollen and nectar are typically produced only in small quantities. Furthermore,

161   numerous active substances, crop species and application scenarios must be considered. The ECPA

162   (2017) claimed that, in order to meet the requirements of the EFSA guidance document (EFSA 2013),

163   for a single product used on five different bee-attractive crops, up to 75 residue studies would need

164   to be conducted, with associated costs of approximately € 7.5m. Consequently, the development and

165   registration of new products and innovations, in addition to the re-authorisation of already approved

166   PPPs, are likely to incur large costs, which may limit the availability of PPPs. According to the ECPA,

167   minor use crops are most likely to be affected, which are often economically important for their

168   growers and for crop diversity, but not of significant importance to the industry to justify high costs

169   for research and development.

170   Furthermore, with new insights and findings becoming apparent and a better comprehension of risks

171   posed by PPPs in recent decades, it is likely that regulatory requirements will be further increased and

172   adapted and that applicants for active ingredient and PPP registrations, PPP producers and responsible

173　authorities will need to deliver more detailed data regarding the fate of PPPs in plant matrices

174　important to pollinators.

175　Thus, to ensure the accurate protection of pollinators and to permit scope for developments in crop

176　protection, methods need to be devised to enable an accurate estimation of PPP residues in pollen

177　and other bee-important matrices that require reduced effort and expenditure. In order to achieve

178　this, a better knowledge and understanding of the fate of PPP residues in pollen and nectar is

179　necessary. The identification of patterns and relationships of PPP residues within the plant and

180　between different species may provide an opportunity to identify better methods for accurate

181　estimation of residue levels for diverse PPPs and cultivation methods. However, little is understood

182　about the behaviour and relationship of residues in floral resources, which can be altered and

183　influenced by numerous factors.

184

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

186　An assessment of the literature suggests that there are four primary factors which could influence the

187　level of PPP residues in pollen and nectar and other related matrices: i) crop related parameters ii)

188　discrepancies in PPP application method, timing and dose rate iii) physicochemical properties of the

189　active ingredient and iv) environmental conditions. These primary factors consist in turn of several

190　sub-factors which can all potentially contribute to variable PPP residues in pollen or related crop

191　matrices. The first two factors listed are considered more often in the literature, since they are

192　tangible and relatively easy to determine under constant conditions. By contrast, the effects of

193　environmental conditions are more difficult to isolate, as they can, for example, influence the chemical

194　properties of an active ingredient, as well as the development and physiology of a plant. Hence, there

195　are a wide range of factors influencing PPP residue levels in pollinator relevant matrices, which are

196　strongly interdependent and form a complex system. Another factor which can unintentionally

197　influence the results of PPP residue levels in pollen and nectar is the sampling methodology. For

198   instance, OSR flowers are often collected and then incubated for a certain time period and

199   temperature to facilitate pollen release (e.g. Botias et al. 2015). The loss of water might result in higher

200   PPP concentrations, but conversely the high temperatures can initiate a dissipation of PPP residues.

201   In other studies pollen is collected by grinding the anthers to powder (e.g. Jiang et al. 2018), collecting

202   pollen in boxes as it falls naturally from plants (Schmuck et al. 2001) or by using bees (e.g. Choudhary

203   and Sharma 2008). These discrepancies in sampling are often not scrutinised in studies but might

204   influence the comparability of results.

205   **General findings**

206   Although high variability is typically observed in PPP residues in pollen and nectar, there are some

207   instances that permit comparisons among a wide range of PPPs/crop systems. Kyriakopoulou et al.

208   (2017) detected statistically significant differences among sampling matrices, with the residue levels

209   in both pollen and nectar being highest when extracted directly from flowers than from bees. Such

210   differences could be caused by dilution effects, when bees mix pollen from untreated and treated

211   crops (Bonmatin et al. 2015; Rolke et al. 2016). In many studies a dilution effect, cross contamination

212   from other fields (e.g. Kunz et al. 2015) or chemical alterations cannot be excluded when pollen and

213   nectar is collected by free flying bees and it is often difficult to directly link the residues found to the

214   previous PPP treatment, unless studies are conducted using bee-sampled pollen collected from

215   tunnelled crops (i.e. no alternative sources of pollen are available).

216   Furthermore, Kyriakopoulou et al. (2017) detected higher residues in pollen than in nectar, a

217   phenomenon which has been reported in several other studies, which employ a range of treatment

218   regimens (e.g. Choudhary and Sharma 2008; Cowles and Eitzer 2017; Dively and Kamel 2012; EFSA

219   2012; Goulson 2013; Jiang et al. 2018). Reasons for this difference have not been investigated thus

220   far; however, several possible mechanisms can be proposed. If bee- collected matrices are analysed,

221   the effect could be caused by the partial metabolism of residues in nectar within the bees (Gong and

222   Diao 2017; Sanchez-Bayo and Goka 2014). However, similar results have also been reported from

223 samples taken directly from the plant. Cowles and Eitzer (2017) suggested a relationship between

224 residue levels in pollen and nectar and whether nectaries and anthers are supplied by phloem or

225 xylem. Choudhary and Sharma (2008) presumed analytical impediments, for example the active

226 ingredient could form conjugates with sugars in nectar, thus becoming difficult to extract, or that, due

227 to morphological differences, there may be differences in either the initial levels of PPPs or in their

228 rates of degradation. Overall, the meta-analysis by Kyriakopoulou et al. (2017) found a strong

229 correlation between the residue levels in pollen and nectar, though none of the individual studies

230 included in the meta- analysis has directly compared this parameter thus far.

231 **Crop related parameters**

232 Although few crop species are considered in studies on PPP residues in pollen and nectar, there is

233 evidence that crop traits have an influence on the residue levels in bee-important matrices.

234 Differences in residue levels in various plant parts can be explained by a dilution effect with plant

235 growth (more biomass) (Holland et al. 1996), plant height (Kleier 1994) and even plant age (Bonmatin

236 et al. 2015), for example when PPPs have the ability to be adsorbed to plant compounds like lignin

237 (Fujisawa et al. 2002). Overall, these effects are strongly related to the physicochemical properties of

238 a compound (see section below for full discussion on the effects of physicochemical properties). Soil

239 treatments of the systemic compound imidacloprid demonstrated that there is a clear gradient with

240 respect to residue levels from the leaves at the bottom of the plant up to the leaves at the top of the

241 plant, and eventually to the flowers and pollen (Alsayeda et al. 2007; Bonmatin et al. 2005; Johnson

242 2012; Laurent and Rathahao 2003; Stoner and Eitzer 2012).

243 This raises questions as to whether conclusions drawn from the PPP residue levels found in foliage can

244 be applied to those in pollen/nectar and whether crops with lower biomass exhibit higher residue

245 levels in leaves and consequently in pollen or nectar. Balfour et al. (2016) found that neonicotinoid

246 concentrations in the tissues of flowering maize (*Zea mays* L.) and OSR are negatively correlated with

247   plant mass, however, they did not directly compare these results with pollen and nectar collected

248   from the same plots.

249   Dively and Kamel (2012) found a strong correlation of imidacloprid residues in squash (*Cucurbita pepo*

250   L.) between leaves and pollen, and leaves and nectar (r = 0.94 and r = 0.88, respectively; p < 0.001)

251   following different soil application treatments. This, however, was analysed only during the course of

252   one year and the trend was not replicated for metabolites of imidacloprid or other investigated

253   neonicotinoids. Dively and Kamel (2012) suggested that the diverse chemical properties of the

254   investigated compounds, mainly the solubility, were the reason for a varying uptake and translocation

255   rate, and consequently higher residue levels of other neonicotinoids. However, the differences in

256   residue levels could also be due to the fact that they randomly selected leaves for analysis during their

257   study. Considering the dilution effect and gradient, different results might have been found by using

258   leaves of similar size and position on the plant. Such an approach was employed by Jiang et al. (2018),

259   who collected only newly expanded leaves of cotton (*Gossypium* sp.) over a one-month period.

260   Although no correlations were observed in nectar, correlations between imidacloprid and

261   thiamethoxam residues in leaves and pollen (r= 0.88 and r = 0.90, respectively; P < 0.001) were found.

262   However, it is unclear whether these observations also apply for other crops, other PPPs (i.e. those

263   with non-systemic properties) and different application scenarios.

264   Demonstrating similarities between species has proven to be problematic, with even varieties of the

265   same species resulting in different residue levels. This was demonstrated by Bonmatin et al. (2003),

266   who investigated several sunflower (*Helianthus annuus* L.) varieties after a seed treatment with

267   imidacloprid. The final concentration in flowers was dependent on the variety, with ranges from 2.7

268   µg g^{-1} up to 7 µg g^{-1}. The authors did not provide any information about habitus or other species-

269   specific characteristics, but an acropetal decrease of residues in foliage, as described above, was

270   detected for all varieties. In addition, during the formation of the capitula of the sunflower there was

271   a sudden increase in imidacloprid residue levels in the upper parts of the plants. Similar findings were

272 reported by Laurent and Rathahao (2003), analysing different parts of sunflowers. Moreover, PPP

273 concentrations in pollen were similar to those found in the floret dish. It can therefore be concluded

274 that the pollen was contaminated by the late shift of PPP residues in sunflowers. The authors

275 presumed a remobilisation process, in which compounds accumulated in older leaves were

276 transferred towards the upper part of plants during the reproductive stage. However, imidacloprid is

277 a xylem-mobile PPP; hence, it should not re-translocate (Sur and Stork 2003).

278 Laurent and Rathahao (2003) provided another explanation for the phenomenon, suggesting that it

279 was a consequence of the differential root system of sunflowers. This consists of fascicular roots,

280 which grow horizontally in the superficial layer of the soil, and a deeper root system; thus, various soil

281 levels can be penetrated. Sunflowers are particularly capable of recovering PPP residues from soils,

282 which can be attributed to this extensive root system (Bonmatin et al. 2003; Mitton et al. 2016).

283 Consequently, the more pronounced root system of an older plant can take up more PPPs from the

284 soil, leading to an increase of residues during the flowering period. The root system is also an

285 important parameter concerning the PPP uptake from soil in other species, for example from the

286 *Cucurbita* family (Otani et al. 2007). For instance, cucumbers *(Cucumis sativus)* grafted with high

287 uptake root stocks could recover up to 70% more dieldrin (an organochlorine insecticide) than those

288 with a low uptake root stock (Otani and Seike 2007), giving a further explanation as to why different

289 varieties exhibit different residue levels from soil treatments. Regarding the ability of different root

290 systems to shift the PPP residues in plants, plant density and whether experiments are conducted in

291 field or pots might also be important parameters to understand the variability of residues in flowers

292 and should be considered in soil-applied PPP residue studies.

293 Obviously, these observations are less relevant for foliar-sprayed or non-systemic PPPs (see section

294 below for full discussion of the effects of application method). The PPP's chemical properties, the

295 morphology and the structure of the leaves, flowers and cuticle determine the uptake rate of the

296 product and hence the likelihood of translocation to pollen or nectar (DiTomaso 1999; Kirkwood 1999;

297   Price and Anderson 1985). For example, a hairy or waxy leaf structure affects the retention time of

298   chemicals on the surface (Yu et al. 2009); this can alter the uptake rate of the PPPs and hence the

299   chemicals' exposure to environmental conditions. Therefore, even under similar conditions, different

300   plant species will show different residue levels and behaviour. Kyriakopoulou et al. (2017) found

301   species-related differences in pollen and nectar residues. In particular, OSR showed the highest

302   residue values in comparison to other plants. However, there were more data available for OSR and

303   the majority of other species was summarised to one group. Therefore, there is limited confidence as

304   to whether OSR genuinely is a crop which accumulates a high level of PPP residues in pollen or nectar.

305   For a summary of this section and problems regarding pollinator risk assessments see Figure 1.

306

307   **Application Method, Application Timing, Dose Rate**

308   The application method, timing of the application and the dose rate of an applied PPP are strongly

309   interdependent. For example, by using a seed treatment, the longest possible time period between

310   application and flowering of the plant is attained. In contrast, many fungicides are sprayed directly

311   onto the plant shortly before or during flowering, especially when they have been assessed as non-

312   harmful for bees. Furthermore, seed dressings often contain less active ingredient per hectare and

313   therefore may be considered to be more environmentally friendly. This is reflected in the residue

314   levels of foliar applications and seed dressings reviewed by EFSA (2012, 2013), with residues from seed

315   dressings being substantially lower than from spray applications. Evidence regarding the effect of dose

316   rate on PPP residues in pollen was provided by Bonmatin et al. (2005), who used three different doses

317   of the systemic active ingredient imidacloprid, applied as a seed dressing to sunflower seed. The

318   concentration of imidacloprid in the capitula of several varieties became higher when the dose rate

319   was increased. Furthermore, the ascent of imidacloprid during flowering (see section above) was more

320   pronounced when the doses of the seed dressing were high. However, studies directly comparing the

321   effect on residues in pollen and nectar at different dose rates of foliar applied or non-systemic PPPs

322   are scarce, although it is possible to discern a certain trend from the detailed values provided by EFSA

323   (2012), indicating that higher dose rates cause higher residues in pollen and nectar.

324   Yet, it cannot be concluded that a high application of PPPs naturally results in a high exposure for bees

325   or in high residues in relation to the dose rate. Choudhary and Sharma (2008) applied a range of PPPs

326   to mustard (*Brassica juncea* Czern.) using foliar application, each with a defined dose rate, and showed

327   that PPPs applied at higher rates indeed tend to result in higher residues in pollen and nectar (Table

328   2). Interestingly, RUDs - the residue unit employed in risk assessments to account for different dose

329   rates (RUD = concentration in nectar/ pollen (mg kg^{-1}) at an application rate of 1 kg ha^{-1} or 1 mg seed^{-}

330   ^{1}) - exhibited an opposing trend in this experiment. Lambda-cyhalothrin afforded the highest PPP

331   residues relative to the dose rate and endosulfan, though applied at the highest dose, afforded the

332   lowest residues with respect to the dose. Thus, the ratio of residues from different PPPs relative to

333   the dose rate is not equal for all compounds, it is rather influenced by other factors, for example the

334   different chemical properties of the active ingredients, which are responsible for varying uptake and

335   accumulation in floral parts.

336   Nevertheless, Byrne et al. (2014) observed higher residues in nectar with a doubled dose rate

337   compared to the normal dose rate when treating citrus trees with a soil drench application of

338   imidacloprid. This effect was reinforced at later sampling dates, i.e. with a longer time period between

339   application and flowering. It is assumed that a longer time period between application and flowering

340   results in lower residues because of the dilution, metabolism and dissipation in plants. For

341   imidacloprid, however, to a certain extent the contrary was shown. Whether this effect is similar to

342   that described by Bonmatin et al. (2003) and Laurent and Rathahao (2003) in the above section is not

343   verifiable. It does, however, illustrate that the timing of the application can have a significant impact

344   on residue levels in pollen and nectar. These findings can also be important when comparing varieties

345   and cropping systems. For instance, Pohorecka et al. (2012) found substantially lower residues of

346   thiamethoxam in bee foraging products from winter OSR than spring OSR. It is hypothesised that the

347   longer time period between treatment and bloom of winter OSR led to a higher degradation of the

348   active ingredient.

349   Cowles and Eitzer (2017) also detected late imidacloprid accumulation in sunflower pollen, but under

350   different experimental conditions. Their extensive experimental setup considered three

351   neonicotinoids, applied at different times with different application methods to sunflower and swamp

352   milkweed (*Asclepias incarnata* L.). Again, a low rate imidacloprid soil drench application was the only

353   scenario (application rate, method, and insecticide) found to result in increasing concentrations as the

354   time post-application increased; which meant a soil drench application performed 10 weeks prior to

355   bloom was the only timing for this application scenario that exceeded the designated "toxicity

356   threshold" for bees in pollen concentrations at the lowest dose rate. In contrast, dinotefuran soil

357   drench applications led to higher residues when they were applied closer to the blooming period. The

358   authors concluded that dinotefuran has a better solubility and higher mobility than imidacloprid and

359   therefore the uptake is faster, whereas the uptake of imidacloprid takes longer and so residues

360   accumulate later in pollen. This finding might be especially important for the estimation of residues in

361   crops with a pronounced short or long life cycle and shows that the physicochemical properties of a

362   compound must always be taken into account (see section below for full discussion of physicochemical

363   properties).

364   Cowles and Eitzer (2017) demonstrated that higher application rates resulted in higher residues in

365   pollen and nectar, depending on the chemical applied. However, the method of application had the

366   strongest influence on pollen and nectar residue levels. Soil drench applications resulted overall in

367   higher residues than the foliar applications, even if both were applied only two weeks before bloom.

368   This indicates that, even though the uptake via leaves is good, it cannot be compared with the uptake

369   and transport via the roots and should be considered separately for the assessment of residues in

370   floral matrices. In contrast to these findings, the tables provided by EFSA (2012, 2013) indicate that

371   residue values from foliar applications are higher compared to soil treatments. However, those tables

372   only consider seed dressings, which contain significantly less active ingredient than soil drenches or

373   foliar sprays. Furthermore, many residue values for foliar applications are derived from applications

374   performed during bloom or shortly before, whereas the latest foliar application in Cowles' and Eitzer's

375   experiment was applied two weeks before bloom.

376   Dively and Kamel (2012) showed that foliar-applied neonicotinoids in squash resulted in higher

377   residues in pollen compared to a soil drip and drench application, especially when squash was sprayed

378   at full bloom. In contrast, the PPP residues from foliar applications in nectar were lower after a spray

379   application or did not differ from soil drench and drip irrigation. This leads to the assumption that

380   systemic PPPs are provided over a longer period from the inside of the plant and thus have a greater

381   probability to accumulate and express in nectar. Dively and Kamel found the lowest residue levels

382   from imidacloprid bedding tray soil applications. This was the most distant application method relative

383   to bloom and no increase in residues could be observed. However, the dose rate was very low

384   compared to the other treatment regimes. In total, contrary to Cowles and Eitzer's (2017) experiment,

385   the timing of the application and dose rate seemed to play a more important role than the application

386   method, confirming the assumption that applications closer to bloom result in higher residues. Both

387   Kubik et al. (1999) and Wallner (2009) showed that there is a lag period between the application of

388   fungicides and the maximum residue level in pollen, although the compounds were sprayed directly

389   onto the plant before and during bloom in cherry trees and OSR, respectively. More studies with

390   different PPPs are necessary to confirm these results, especially for foliar applications (Figure 2).

391   Overall, it can be concluded that there is a strong interdependence between the time available for the

392   accumulation of the compound and the time for dissipation, metabolism and translocation in the

393   plant, influenced by the chemical properties of a PPP and the application method.

394   **Physicochemical Properties**

395   A detailed knowledge about the physical and chemical properties of a chemical compound is a

396   necessary prerequisite to understand its general behaviour in metabolism, analytical methods,

397   formulations, and the environment (Tsipi et al. 2015). Therefore, these parameters are usually studied

398   under well-defined conditions and are required for the registration of a PPP. Physicochemical

399   properties determine the uptake of a compound into the plant, its translocation, as well as its

400   dissipation and metabolisation in the plant and the environment.

401   For PPPs applied before bloom, it can be assumed that every parameter influencing the uptake of a

402   compound and its acropetal translocation will influence the residues in floral resources. Some key

403   physicochemical properties include the solubility in water, the partition coefficient octanol/water (log

404   K_{ow}), the dissociation coefficient (pK_a), the molecular size of a compound, the root concentration factor

405   and the transpiration stream concentration factor. These properties can be altered by additives and

406   vary depending on the formulation type (Bonmatin et al. 2015; Farha et al. 2016; Hsu and Kleier 1996;

407   Trapp 2004).

408   Overall, PPPs can be classified according to their behaviour in and on plants. Non-systemic or contact

409   compounds are not distributed in the plant and will probably cause only residues in floral matrices if

410   the flower or pollen comes directly into contact with the PPP. On the contrary, translaminar PPPs are

411   taken up and redistributed from one face of a leaf to the opposite face of a leaf, an important

412   parameter for many fungicides (Klittich et al. 2008), whereas systemic PPPs are distributed within the

413   whole plant, either acropetally via the transpiration stream to older leaves in xylem or in both,

414   acropetal and basipetal directions to new growth in the phloem sap. The most common way for the

415   translocation of (non-ionised) plant systemic insecticides is the unidirectional acropetal translocation

416   in xylem (Wyss and Bolsinger 1997).

417   One key parameter describing PPP translocation for non-ionised compounds in the plant is the

418   partition coefficient octanol/water (log K_{ow}). It describes the compound's lipophilicity and its ability to

419   move through bio membranes; thus it determines the uptake of a PPP through the leaf cuticle and its

420   distribution within the plant (Briggs and Bromilow 1994; Kirkwood 1999; Klittich et al. 2008; Wang and

421   Liu 2007).

422   In general, compounds with a log K_{ow} < 0 are considered to be hydrophilic and compounds with a log

423   K_{ow} > 0 lipophilic (Wang and Liu 2007). Lipophilic compounds tend to cross bio membranes but are

424   partitioned into lipophilic tissue along the symplastic pathway (Sicbaldi et al. 1997). Therefore, the

425   optimum uptake and translocation in xylem occurs for non-ionised PPPs with intermediate log K_{ow}

426   values of 1–3. Translaminar distributed compounds can show log K_{ow} values up to 4.5. Highly polar and

427   highly non-polar compounds are poorly translocated within a plant (Bromilow and Chamberlain 1989;

428   Bromilow and Chamberlain 1995; Sicbaldi et al. 1997; Vryzas 2016).

429   Non-ionised compounds with a lower log K_{ow} can also be distributed in the phloem sap, though

430   entering the symplast is impeded (Bromilow et al. 1987). In contrast, more lipophilic compounds can

431   readily enter the phloem, but also easily move between xylem and phloem. However, as the xylem is

432   moving faster than the phloem, compounds are eventually translocated in the xylem (Peterson and

433   Edgington 1976; Wyss and Bolsinger 1997).

434   In general, most phloem-mobile compounds appear to be weak acids (Trapp 2004) and their

435   translocation is highly dependent on a favourable combination of log K_{ow} and the dissociation

436   coefficient (pK_a) (Wyss and Bolsinger 1997). The pK_a describes the acid strength and ability of a

437   compound to dissociate; it can be regarded as the pH at which a particular acid or base group is 50%

438   ionised (Bromilow and Chamberlain 1995). Plant compartments exhibit different pH values across

439   membranes, ranging from pH 5 in the apoplast to pH 8 in the phloem sap (Chamberlain et al. 1998).

440   Accordingly, a weak acid will appear at low pH in its un-dissociated state, having the ability to easily

441   enter the symplast. Once in the symplast, due to the higher pH, it dissociates and is not able to cross

442   back through the membranes (i.e. the ion trap theory) (Briggs et al. 1987; Bromilow and Chamberlain

443   1995; Chamberlain et al. 1998; Pessarakli 2014; Tyree et al. 1979).

444   It is understood that pollen and nectar, as part of reproductive organs, are a sink for photosynthetic

445   products, even though nectaries can be supplied by phloem and xylem depending on the crop and

446   variety (Heil 2011; Pacini et al. 2016; Wist and Davis 2006). However, many PPPs already found in

447   these matrices, mainly insecticides and fungicides, are considered to be more xylem-mobile according

448   to their physicochemical properties. Thus, an acropetal movement in the plant is conceivable but the

449   exact mechanism as to how these chemicals are transferred into the pollen is not yet understood.

450   Aajoud et al. (2008) demonstrated that the low transpiration stream of different parts of a sunflower

451   head cannot be responsible for all of the fipronil residues found in this tissue. Although fipronil is more

452   likely to move in xylem due to its high log K_{ow} (= 4.0), Aajoud et al. showed under laboratory conditions

453   that fipronil is transported from sources (older leaves) to sinks (growing parts). In general, for non-

454   ionised compounds like fipronil or neonicotinoids the ion trap theory does not apply and the active

455   ingredient can move freely between phloem and xylem according to its bio membrane permeability

456   (Sur and Stork 2003). Aajoud et al. (2008) suggested that both xylem and phloem pathways are

457   involved in the transfer of fipronil to the flower head. Transfer via xylem from the roots to the leaves

458   has been previously demonstrated and depends upon the rate of leaf transpiration, in addition to the

459   compound concentration in the xylem, whereas the phloem pathway seems to be an influencing factor

460   in the translocation to the flower parts and hence to pollen or nectar.

461   Another explanation for unexpected phloem transport is that biotransformation in plants can alter

462   the compounds' properties. For example, due to its physicochemical parameters, imidacloprid is

463   transported in xylem and accumulates in leaves, but some of its metabolites (e.g. 6-chloronicotinic

464   acid) were shown to have properties which are potentially phloem-mobile (Buchholz and Nauen 2002;

465   Chamberlain et al. 1995; Nauen et al. 1999). Furthermore, transformed compounds can form

466   conjugates with glucose, isomaltose and amino acids, which could change the translocation pathway

467   (Jiang et al. 2009; Oliver and Hewitt 2014; Sur and Stork 2003; Wu et al. 2012).

468   These findings could perhaps explain the increase in imidacloprid in upper plant parts, as described in

469   the earlier section about crop-related parameters, and rationalise the presence of PPP residues in

470   physiological sinks like pollen and nectar. Nevertheless, these conclusions might not apply for

471   compounds with other physicochemical properties, especially for PPPs which are considered to be

472   non-systemic.

473   In pollen residue studies, physicochemical properties are often mentioned to describe and explain the

474   reason for differences in residue levels but, to our knowledge, no study has tried to link these PPP

475   characteristics experimentally to the residues found in pollen or other matrices. Kyriakopoulou et al.

476   (2017) found weak correlations between the residue levels in nectar and the solubility in water,

477   although Bromilow and Chamberlain (1995) considered water solubility as a rather poor guide to

478   systemic behaviour. Thorbek and Hyder (2006) used artificial neural networks to examine the

479   relationship between physicochemical properties of different PPPs and residues in food products. In

480   their opinion, the physicochemical properties and the crop type explained up to 50% of the variation.

481   Thorbek and Hyder concluded that these properties control important aspects of the processes

482   leading to residues in food commodities. These findings may be transferred to the residue occurrence

483   in bee-important plant matrices.

484   In general, the uptake of PPPs and their half lives in plants are very well studied, primarily because risk

485   assessments on human exposure or their environmental fate are required for the registration of PPPs,

486   as well as the setting of Maximum Residue Levels (MRLs). However, the process determining the

487   residues in bee-important matrices is not well understood, and more research is required to link the

488   physicochemical properties of a compound to the translocation to pollen or nectar and to the fate and

489   dissipation after the application of a PPP (Figure 3).

490   **Environmental conditions**

491   PPPs applied to a crop enter a complex system, which is greatly influenced by its surrounding

492   environment and underlying manifold interactions. These variations are reflected in the fluctuating

493   PPP residues reported in pollen or nectar, especially in field experiments. Laurent and Rathahao (2003)

494   reported significantly higher variations in pollen residues in a lysimeter experiment compared to

495   greenhouse experiments. Jiang et al. (2018) experienced varying residue fluctuations across the

496   course of one month in field experiments and Rolke et al. (2016) observed high variations even within

497   different sub-areas of one field. Even small-scale weather incidents can change the result of a chemical

498   treatment, for example when the compound is washed off the leaves by rain shortly after application.

499   Additionally, plant growth and physiology are dependent on the surrounding conditions and will

500   influence the behaviour of the compound. This of course makes the comparability of PPP residues in

501   pollen or nectar from different studies difficult, although the environmental fate of all kinds of PPPs

502   are well studied.

503   Key parameters influencing both the chemical fate and the plant are water and temperature.

504   Physicochemical properties are usually assessed under defined laboratory conditions and are

505   therefore likely to change under varying conditions (Hornsby et al. 1995; Tsipi et al. 2015). For

506   example, cuticle permeability was shown to increase rapidly with increasing temperatures (Baur et al.

507   1997; Baur and Schönherr 1995). Degradation processes in soil, vegetation and air are all accelerated

508   at higher temperatures (Bloomfield et al. 2006), whereas colder temperatures limit biological and

509   chemical reaction activities, resulting in longer half-lives and slower dissipation rates (Farha et al.

510   2016). Humidity can increase compound uptake into leaves (Hull 1970; Ramsey et al. 2002), while rain

511   can lead to wash-off and leaching (Hunsche et al. 2007; Radolinski et al. 2018) and water stress was

512   shown to affect the distribution of systemic insecticides in plant leaves (Stamm et al. 2016). Soil

513   conditions are affected by temperature and water availability; organic matter content, microbial life

514   and clay content play a key role in the fate and uptake of soil applied PPPs (Cessna et al. 2017; Di et

515   al. 1998; Gevao et al. 2000; Zhang et al. 2018). PPPs with a long half-life in soil or exposed to conditions

516   that prevent a breakdown in soil are more likely to be taken up during flowering. The transport of

517   xylem-mobile compounds is, inter alia, dependent on the transpiration stream. Therefore,

518   environmental conditions and plant species which enable a high transpiration will enhance acropetal

519   movement and consequently the likelihood of translocation of PPP residues to pollen or nectar.

520   In general, flowers are also exposed to these conditions, however, they may show a different

521   susceptibility to environmental conditions and a different uptake compared to the rest of the plant,

522   due to the different structure and often hydrophilic properties of their surface (Baker and Hunt 1981;

523 Koch et al. 2008). Furthermore, flower opening, the dispersal of pollen, as well as the amount and

524 composition of pollen and nectar produced is dependent on the surrounding environment, especially

525 temperature and humidity (Heil 2011; Pacini et al. 2006; Vidal et al. 2006). PPPs applied during or

526 shortly before bloom will contact the flowers directly and the presence of residues is therefore likely

527 at least in pollen, even for compounds with a short half-life. All factors favouring a fast dissipation or

528 degradation can thus decrease the residues in pollen or nectar. Choudhary and Sharma (2008)

529 recorded a general faster dissipation of PPP residues in pollen than in nectar depending on the active

530 ingredient. They attributed this faster degradation to the fact that pollen is more exposed to

531 environmental conditions than the nectaries, which are typically deeply embedded within the flower.

532 Consequently, the presentation of pollen, the arrangement of anthers and nectaries within the flower

533 and their protection by flower petals could influence the impact of environmental conditions on

534 residue behaviour in pollen and nectar. For example, compounds with a relatively low photo stability,

535 such as pyrethroids, might dissipate faster in pollen grains presented openly to pollinators, compared

536 to residues in nectar. None of the available studies considered or compared the influence of

537 environmental conditions on PPP residues in pollen or nectar (Fig. 4). However, a field study conducted

538 in consecutive years detected correlations in PPP residues from one year to another, despite varying

539 environmental conditions (Dively and Kamel 2012). Nevertheless, the factors acting in different

540 environments on PPPs availability in floral resources are complex and not well understood. Different

541 climates and soils, for example across Europe, are currently accounted for in risk assessments for bees

542 by conducting residue trials at multiple sites across broad geographic regions. However,

543 environmental influences are not understood well enough to allow an extrapolation or comparison

544 between different sites and may require further attention depending on the mode of application and

545 properties of the active ingredient (e.g. soil uses with systemic compounds, UV stability). Controlled-

546 environment studies looking at the effect of for example temperature on residues could provide

547 further insights.

548

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.

23

573   It would be extremely difficult to assess the fate in pollen or nectar for all active ingredients, in all bee-

574   important plants and under different climates. Therefore, methods are needed which enable an

575   accurate estimation and extrapolation of PPP residues in these ecologically important matrices, which

576   are also able to consider the numerous influences they are exposed to. In order to enable this,

577   comparable results are required, which do not just reflect a snapshot of a single randomly selected

578   field area and environmental conditions, but also reveal a broader knowledge which can be

579   transferred to further situations.

580   This can only be achieved by improving the understanding of residue behaviour and their dynamics in

581   these complex tissues. A fundamental challenge for future research will be to quantify the effects of

582   different dynamics and interacting factors on PPP residue levels. Future research should aim to

583   investigate relationships, interdependences and common features amongst various PPP applications,

584   which may allow conclusions to be drawn on residues in pollen and nectar and, as a result, permit

585   suitable systems to be identified which can act as model scenarios or be consulted for worst-case

586   estimations, enabling all other scenarios to be adequately covered.

587   Achievement of this will permit risk assessments to be conducted with considerably less effort and

588   expenditure, whilst simultaneously enabling rapid and accurate assessment of the risks for pollinators

589   posed by PPPs.

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594   **Conflict of interest**

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934 **Tables**

935 **Table 1** Summary of selected PPP residues in pollen expressed as Residue Unit Dose (RUD) (mg a.i. kg^{-1} pollen at an

936 application rate of 1 kg a.i. ha^{-1}) derived from two different sources. The minimum, maximum and mean values demonstrate

937 the high variability of residues found in pollen from spray applications. Some calculations were not derived from a single

938 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.*	a
Spirotetramat	2.2	63.5	83.1	

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

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

945 **Table 2:** Relationship between dose rate, residues in pollen (ppm) and Residue Unit Dose (RUD) for three active

946 ingredients. Data from Choudhary and Sharma (2007). Application of 750 L ha^{-1} water in mustard (*Brassica juncea* Czern.)

947 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