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Analysis of the evidence to support the definition of Specific Protection Goals for bumble bees and solitary bees

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Abstract

In the context of the definition of Specific Protection Goals for bees, risk managers asked EFSA to provide the scientific background to support the decision-making process about what needs to be protected and to what extent. In this document, information, data and tools have been investigated to support the risk managers in deciding on the Specific Protection Goals for bumble bees and solitary bees. In particular, EFSA summarised the relevant information on biology and ecology and investigated the possibility of performing an analysis of background variability to support the definition of a threshold of acceptable effects. Accounting for the current level of knowledge, EFSA concluded that this analysis is complex, and it would require information that is not yet available and tools that have not yet been fully evaluated. Nevertheless, based on the limited data available, represented by the control groups of few experimental field studies, an analysis of variability of different relevant endpoints was provided. By taking into account the results of this analysis, the biology and ecology of bumble bees and solitary bees, as well as the impact for risk assessment, EFSA illustrated possible approaches that could be followed by risk managers.

Key words: bumble bees, solitary bees; background variability; colony dynamics; Specific Protection Goals

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Summary

Following the decision on the Specific Protection Goal (SPG) on honey bees, the European Commission asked EFSA to support risk managers for their decision making regarding the SPGs for bumble bees and solitary bees to be implemented for the regulatory risk assessment of pesticides under the Regulation 1107/2009.

In this document, EFSA summarised the basics of the biology and ecology of bumble bees and solitary bees, the current state of knowledge on their background variability and other relevant information that can be used for decision making based on a weight of evidence approach.

The analysis of the background variability performed for honey bee colony strength and the method and model used for this analysis cannot be directly transferred to bumble bees and solitary bees, due to their different biology and ecology. However, in principle, it may be possible to apply a similar concept by using a combination of mechanistic modelling and data from field studies, if available. Therefore, available models to simulate the population dynamics for bumble bees and solitary bees and data from field studies have been investigated in view of their potential to provide information on the normal operating range or background variability for these bees.

Some potentially useful models have been identified. However, as they require further in-depth analysis and consideration, EFSA cannot recommend a specific model for performing the analysis of background variability of bumble bees and solitary bees on the basis of the current knowledge and within the timeframe of the mandate.

As an alternative to model simulations, the variability in colony strength and population abundance observed in the control replicates of field studies could represent a source of information for the normal operating range (NOR), although it cannot be considered exhaustive due to the limitation of the experimental field studies in isolation. The available field studies (control data) for bumble bees and solitary bees were assessed, and an analysis of the background variability, quantified as coefficient of variation (CV), was performed. In total, seven datasets were identified as suitable for bumble bees (*Bombus terrestris*), and eight datasets for solitary bees (*Osmia bicornis*). Taking into account the low number of studies, the available datasets are considered too limited to give a comprehensive estimate of the NOR. Nevertheless, our results indicate that the background variability for the endpoint colony weight of *B. terrestris* could be comparable with that for honey bee colony strength. Regarding the variability observed in the available field studies with *O. bicornis*, for a rough comparison with SPG agreed for honey bees, it can only be noted that it is greater than 10%.

As possible other sources of information, approaches in other areas of ecotoxicology were considered such as for insect pollinators in the EFSA PPR Panel (2015b) opinion on Non-Target Arthropods and for aquatic invertebrates in the aquatic guidance (EFSA PPR Panel, 2013).

Based on the information presented in this document, the Working Group (WG) provides suggestions that could be considered by risk managers to define the SPGs for bumble bees and solitary bees. In particular, the WG considers that there are two potential options that could be followed for defining the magnitude dimension of the SPGs: (1) an *a priori* defined threshold option; and (2) an undefined threshold option. Both options can be supported by the evidence presented, i.e. the level of knowledge, general considerations and potential impact for the risk assessment. It is highlighted that any decision relying on the current knowledge may require revision in the future when new data and tools become available.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

In the first supporting document[1], published at the end of July 2020, EFSA described a set of four approaches that represents a combination of possible scientific and pragmatic processes for risk managers to determine Specific Protection Goals (SPGs). These approaches were developed by considering the request of the European Commission mandate to 'take into account planned and ongoing discussions initiated by the Commission on defining specific environmental protection goals and review the risk assessment guidance based on the Specific Protection Goals agreed during this process' (ToR6).

EFSA took into consideration the preliminary outcome of the action initiated in 2019 by the European Commission involving Member States (MS) and stakeholders, in particular the positive opinion conveyed by stakeholders and MS on the use of the EFSA framework for identifying SPGs[2]. In addition, EFSA has considered the feedback from MS[3] on this aspect of the 2013 guidance document.

The four approaches and the scientific concepts underlying each approach were discussed with MS and the European Commission. As a result of the discussion, a large majority of the MS expressed a preference for the approach based on the analysis of the background variability of colony size for honey bees. The selected approach was implemented by EFSA and the outcome supported the decision to establish an SPG for honey bees (*Apis mellifera*) for the entire EU corresponding to a value of 10% as the maximum permitted level of honey bee colony size reduction following pesticide exposure[4].

Following the decision on the SPG on honey bees, the European Commission asked EFSA to support risk managers in their decision making regarding the SPGs for bumble bees and solitary bees to be implemented for the regulatory risk assessment of pesticides under the Regulation (EC) No 1107/2009.

1.2. Scope of this document

With this document, EFSA will summarise the biology and ecology of bumble bees and solitary bees and the current state of knowledge to investigate their background variability. An overview of the available data and tools, as well as of knowledge gaps, will be provided. The overall aim is to consolidate the body of evidence to support risk managers in their decision making for bumble bees and solitary bees. Possible approaches based on the available information, including a consideration of existing approaches in the area of ecotoxicology, will be highlighted and implications for risk assessment will be described.

2. Wild bees

2.1. Biology and ecology

Wild bees and other pollinators are a key part of European biodiversity and provide a wide range of benefits to crops, wild plants, and food production (Nieto et al., 2014). Multiple recent studies have reported declines around the world in insect abundance and diversity, including pollinators (Sánchez-Bayo and Wyckhuys, 2019; Wagner, 2020; Wagner et al., 2021) and the major drivers of their decline have been identified (Dicks et al., 2021).

For pollinators, declines for some taxa, particularly in north-west Europe and North America, have been well documented (IPBES, 2016). Although some authors suggest caution in the interpretation of these declines (Didham et al., 2020) or report limitation of the data showing such declines because they are restricted to some geographical areas and to few taxa (Saunders et al., 2020), all concur on the overall knowledge gaps and the need for systematic and long-term monitoring.

In Europe, wild bees represent a highly diverse group among pollinators, and have the highest species

[1] https://www.efsa.europa.eu/sites/default/files/topic/EFSA-Supporting-document-for-RMs-in-defining-SPGs.pdf
[2] EFSA Scientific Committee (2016) and EFSA PPR Panel (2010).
[3] https://ec.europa.eu/food/plants/pesticides/protection-bees_en
[4] https://ec.europa.eu/food/plants/pesticides/protection-bees_en

richness in southern Europe, particularly in the Mediterranean area (Potts et al., 2021). The wild bee species in Europe belong to six families[5]: Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae and Melittidae.

Although honey bees (in Europe represented by one species, *Apis mellifera*, and several sub-species) can form unmanaged, feral colonies, most wild bees belong to the non-*Apis* group and can be split into bumble bees (the only eusocial[6] wild bee species) and solitary bees (the larger group of wild bee species), which are considered in this document.

Bumble bees belong to the genus *Bombus*, which in Europe includes 68 species (Nieto et al., 2014). They live in small (tens to hundreds of individuals) annual colonies with a single egg-laying queen. Some bumble bee species, in Europe primarily *B. terrestris*, are used as managed pollinators in agriculture, but most species and colonies are wild. Bumble bees construct their nests in cavities above or below ground and use wax for their larval and food store cells but lack the regular structure and appearance of honey bee nests. Unlike in honey bee colonies, each queen is responsible for establishing a colony in spring, laying eggs, tending to the brood and foraging for pollen and nectar until the first generations of workers develop. After the first generation of larvae become adults, the queen remains in the nest to lay eggs and care for the brood, while the workers divide their time between in-hive tasks such as brood care and outside foraging. Unlike in honey bees, workers switch between in-hive tasks and foraging so they cannot be readily classified into these two groups. Bumble bee colonies store much less food than honey bees, usually only enough food to allow the colony to persist through short periods of poor weather. They collect mostly pollen from different plants (polylectic) and therefore visit a wide range of flowers. As the colony is the reproductive unit, successful reproduction means that new queens and drones are produced towards the end of the colony cycle. For most species the new queens hibernate during the winter and start new colonies the following spring while the workers and drones from the original colony will die before winter. The bumble bee queen behaves as a 'solitary bee' when overwintering and at the time of emergence until the establishment of a new colony. Any effect on the queen at this stage (e.g. on overwintering success, nest initiation) will impair the colony itself (Straub et al., 2015).

Solitary bees are a taxonomically diverse group (in Europe approximately 1900 species according to Nieto et al., 2014), therefore some generalisations may not apply to all species. The species in this group are not eusocial, which means that there are only females and males and neither worker caste nor colonies. Most of the solitary bee species have one generation of offspring per year (univoltine) (Brittain and Potts, 2011). Only small numbers of solitary bee species are managed and commercially available as pollinators (*Osmia* spp. and other species from the Megachilidae family) so the vast majority of the solitary bee species are wild. Most are specialised in collecting pollen from one genus or one family of plants (oligolectic species) and are therefore limited to areas where their food sources grow and are susceptible there in their reaction to environmental stress (Williams et al., 2010, De Palma et al., 2015; Forrest et al., 2015). Solitary bees vary considerably in size, appearance and use a wide variety of nesting substrates (mud, wood, masonry, leaf and other plant materials) and generally provision each offspring only once (i.e. each cell in the nest is provisioned with enough pollen to feed one larvae through the summer). They produce a relatively small (approximately 10) number of offspring per female.

These two non-*Apis* groups contain a variety of different feeding, nesting and breeding behaviours (EFSA PPR Panel, 2012). For example, solitary bees tend to have a small foraging range and therefore are more vulnerable to issues associated with habitat fragmentation in agricultural landscapes (Gathmann and Tscharntke, 2002; Zurbuchen et al., 2010), while bumble bee queens can move over much larger distances and therefore bumble bee population dynamics occur on a much larger scale (Fijen, 2021). Their responses to stressors, including pesticides, can diverge (Rundlöf et al., 2015; Wood et al., 2020) and in nature they compete with honey bees (Lindström et al., 2016; Mallinger et al., 2017; Henry and Rodet, 2018, Herrera, 2020; Angelella et al., 2021; Meeus et al., 2021). These differences indicate the need for a risk assessment methodology for bumble bees and solitary bees tailored to each group, which also would imply specific SPGs.

[5] https://ec.europa.eu/environment/nature/conservation/species/redlist/bees/introduction.htm

[6] Showing an advanced level of social organization, in which a single female or caste produces the offspring and non-reproductive individuals cooperate in caring for the young.

2.2. Definition of the SPGs for bumble bees and solitary bees in 2013

SPGs for bumble bees and solitary bees have been defined in the EFSA PPR Panel (2012) opinion and implemented in the EFSA (2013) guidance document, as summarised in Table 1. The definition of these SPGs is in line with EFSA methods (EFSA PPR Panel, 2010; EFSA Scientific Committee, 2016) and therefore, the WG for the revision of EFSA (2013) considered that a full revision of this definition is outside the scope of this mandate.

Table 1: Specific Protection Goals implemented in EFSA (2013)

Dimensions	Bumble bees	Solitary bees
Ecological entities	Colony	Population
Attribute	Colony strength	Population abundance
Magnitude	Negligible effect (percentage of colony size reduction as for honey bees)	Negligible effect (percentage of population abundance reduction as for honey bees)
Temporal scale	Not relevant, i.e. any time	Not relevant, i.e. any time
Spatial scale	Edge of field	Edge of field

The WG clarified that the spatial dimension 'edge of field' refers to the location of the colonies/populations, i.e. directly adjacent to the treated field. The different exposure scenarios considered in the risk assessment, however, refer to foraging in the treated field (e.g. treated crop and in-field flowering weeds scenarios) and in the areas surrounding the field (e.g. the field margin and the adjacent crop scenarios) (see Section 5.1).

EFSA (2013) highlighted that this spatial scale definition is a worst-case situation compared with colonies or populations located further away from the treated field.

The WG also recognised that bumble bee and solitary bee nests located in the field may be exposed to pesticides, e.g. via direct exposure of larvae and adults to soil residues. However insufficient information is available to address this exposure (Gradish et al., 2019; Sgolastra et al., 2019).

Overall, based on the available state of knowledge, the WG suggested using the definition of SPGs for bumble bees and solitary bees in Table 1 and at the moment focus only on the review of the 'Magnitude' dimension (see Section 6). The WG, however, acknowledged that further future considerations may be needed, e.g. in relation to the annual colony dynamics of bumble bee colonies and related population consequences, possibly including research development.

3. Background variability of bumble bees and solitary bees

The normal operating range (NOR) provides an indication of the range of typical 'natural variability' that can be used as a baseline to understand what magnitude of effects can be tolerated following exposure to a pesticide.

EFSA used the concept of a NOR to inform the definition of the magnitude dimension for honey bees (EFSA et al., 2021).

Also in this document the term 'natural' is replaced by the term 'background' as it is more appropriate when referring to the anthropogenically impacted agricultural landscapes of Europe (Kruse-Plaß et al., 2021). However, the term 'background' reflects that the analysis considers no exposure to pesticides (e.g. such as 'controls' in experimental field studies).

In EFSA et al. (2021), the NOR was defined by simulating the background variability of honey bee colony strength. The underlying principle was that the relative difference between the mean colony size and the lower limit of the operating range informs the maximum colony size reduction due to background variability. This difference informed the definition of the magnitude of the acceptable effect, by assuming that effects (e.g. percentage of colony size reduction) are acceptable when they remain in a range defined on the basis of NOR.

The analysis of the background variability performed for honey bee colony strength and the method and model used for this analysis cannot be directly transferred to bumble bees and solitary bees, due to their different biology and ecology (see Section 2). However, in principle, it may be possible to apply a similar concept by using a combination of mechanistic modelling and data from field studies, if available.

Population dynamics are complex and mechanistic models are often used to simplify such complexity and concentrate on reproducing specific population processes (Oro, 2013).

In this section, the available models to simulate the population dynamics for bumble bees and solitary bees and data (not linked to models) from field studies are presented in view of their potential to provide information on the NOR or background variability.

It is anticipated that little information is known about the abundance of many wild bee species, and even less about their dynamics and background variability mainly due to their large diversity and variation in life histories (Potts et al., 2015, 2021; Cameron and Sadd, 2020; Wood et al., 2020). A recent and comprehensive review of the status and trends of insect pollinators, including bees, pointed towards remaining major knowledge gaps, for example on how abundance and biomass of pollinators are changing and how to estimate changes in population sizes, in particular for wild bees (Potts et al., 2021). McArdle et al. (1990) concluded that, without proper information on population variation under field conditions, progress in all these areas will be seriously hindered.

It is also noted that the analysis of the background variability for bumble bees and solitary bees by using both models and field data should be complemented by additional considerations. Usually models or experimental data are based on an individual species used as a 'model species' (e.g. *B. terrestris*, *O. bicornis*). The identification of the most appropriate 'surrogate' species that may be the most representative 'service providing unit' in terms of vulnerability and sensitivity would be an important step (EFSA PPR Panel, 2010; Sgolastra et al., 2019) along with considerations on whether the NOR of such 'surrogate' species could be considered as representative for the related bee groups. The presence of 68 bumble bee species in the EU and approximately 1900 solitary bee species (see Section 2) makes such analysis complex and would require comprehensive data on bee biology and ecology that are currently not available (Wood et al., 2020) (see also Section 3.3). Conversely, test species need to be easily available and be able to be reared under laboratory conditions, and this also needs to be considered when choosing 'model species'.

EFSA recently performed a comprehensive literature review of studies investigating bee background mortality levels (EFSA et al., 2020), which included bumble bees and solitary bees as well. In principle, this kind of data could be used to inform theoretical models that describe population dynamics. In this specific case, data found for the two groups of wild bees were limited in number and scattered across a handful of species. However, even if these data had been comprehensive enough to give an accurate picture of the spatiotemporal variability of background mortality levels, this would not have been enough to address the variability in colony strength and/or population abundance. This is because background mortality levels express only the fraction of bees in a colony/population that die in a certain time interval (usually daily). However, to estimate the strength and population abundance, both the births and deaths in that population/colony would need to be known. Furthermore, especially for populations, also other inputs/outputs are important, such as immigration and emigration. For appropriate estimation about variability of colony strength and population abundance, variability of all these aspects would need to be known in space and time.

3.1. Models

The WG attempted to systematically identify any suitable mathematical model available in the open literature. Potentially relevant publications were identified by carrying out a literature search, similar to that performed for honey bee models in EFSA et al. (2021). Of the 158 studies identified, 134 were removed as they were clearly unrelated to either colony size in bumble bees or population abundance in solitary bees. The remaining 24 studies were assessed in more detail, and four of them were excluded as the models described had been presented in previous studies (assessed during the exercise), and they did not provide any additional information about the original model. Therefore, the full assessment was carried out on 20 studies, which were classified based on the following criteria: (i) if the study contained a model that could estimate either colony size or population abundance, (ii) the type of model, and (iii) the type of bee (bumble bee or solitary bee). The models were identified as being potentially useful if they provided an estimate of either colony size (bumble bees only) or population abundance (bumble bees and/or solitary bees). We retained only mechanistic models, which use mathematical relations to describe the underlying processes in the systems, and excluded purely statistical models for which predictions beyond the study population would not be appropriate.

The WG identified 10 potentially useful models (seven exclusively for bumble bees, one for solitary bees, and two that dealt with bumble bees and solitary bees). The full process is detailed in Appendix A of this document.

However, for a firm conclusion on their suitability to analyse and predict the background variability in different environmental conditions and over time, these models (and those still in development, see Section 3.3) should be evaluated according to EFSA good modelling practices opinion (EFSA PPR Panel, 2014), which is a prerequisite to appreciate which model(s) are fit for purpose, to understand their sensitivity, uncertainties, data needed for the calibration, available documentation and accessibility to the model code. Such an evaluation is not feasible within the timeframe of the current mandate.

In addition, the WG highlighted that any analysis of the background variability for bumble bees and solitary bees using models would require individual models to cover the numerous species within these two groups to incorporate species-specific ecological processes (e.g. competition for resources, predation or migration), which are likely to affect their colony or population dynamics.

Overall, on the basis of the current knowledge, the WG cannot recommend any specific model for performing the analysis of background variability of bumble bees or solitary bees, as those identified as potentially useful require further in-depth analysis and consideration. Therefore, a comprehensive analysis with the support of modelling cannot be performed. Nevertheless, the WG acknowledges that modelling is a powerful tool and the release of newer colony and population dynamic models for honey bees, bumble bees and solitary bees may be possible in the near future (see Section 3.3).

3.2. Field studies

As an alternative to model simulations, the variability in colony strength and population abundance observed in the control replicates of field studies could represent a source of information for the NOR, even if exploring the background variability based on field experimental studies cannot be considered exhaustive due to limitation of the experimental approaches in isolation. In fact, exploring the background variability using field data is in principle possible, but studies carried out with this scope are not readily available. The possibility to analyse variability in different settings is subject to a substantial experimental effort, which requires significant investment in terms of time and economic resources.

The studies available in the literature reporting effects of pesticides on bumble bees and solitary bees are scarce; some information on bumble bees and neonicotinoids is available in Camp and Lehmann (2021), who performed a scoping literature review searched from 1980 until 2019. Wood et al. (2020) reported that the bee genera with less species diversity, i.e. *Apis* and *Bombus*, are those with the highest number of publications. Lehmann and Camp (2021) conducted a systematic literature review on effects of pesticides on solitary bees and noted that very few field studies are available for most species of solitary bees. The available published field studies were considered for this document.

According to the legal data requirements (Regulation (EU) No 283/2013, Annex Part A, Section 8.3.1 and Regulation (EU) No 284/2013, Annex Part A, Section 10.3.1), data should be submitted to address the risk to bees. Although the term 'bees' is clearly not limited to honey bees, these legal requirements do not explicitly mention bumble bees and solitary bees and also agreed standard guidelines or protocols are not fully available. This, in combination with the fact that EFSA (2013) was not implemented in the regulatory process, has as a consequence that the availability of field studies with bumble bees and solitary bees in pesticide dossiers submitted to EFSA is very limited. Some studies are available in the dataset assembled for the review of the three neonicotinoid substances (EFSA, 2018a,b,c,d). These studies are also considered for this document.

Following the information session held on 23 November 2021[3] for MS and stakeholders, one additional dataset (for a field study with bumble bees and solitary bees performed in 2018) was submitted by the Julius Kuhn Institute (Germany).

The available field study control data for bumble bees and solitary bees were assessed, and an analysis of the background variability, quantified as coefficient of variation (CV), was performed. Details on this analysis are reported in Appendix B.

In total, seven datasets were identified as suitable for bumble bees and eight datasets for solitary bees. The available studies were conducted in Germany, France, Hungary, Sweden and the UK, therefore representing mainly the Central and Northern Zone. Especially from the Southern Zone, studies are not available. However, it should be noted that the results for the modelling exercise for honey bees, presented in EFSA et al. (2021), indicated that the range of variability was similar for the different regulatory zones within the EU (e.g. refer to Table 10 in EFSA et al., 2021). In addition, the dataset of 33 field studies with honey bees, considered in EFSA et al. (2021), contained both studies from the Central and Southern Zone. The variability in the control replicates from these studies (quantified as the CV) was in the same range for studies performed in both zones (refer to figure 22B in EFSA et al., 2021). In the information session, a concern was raised that the background variability in the Southern Zone could be higher than in other zones. While no data were submitted to underpin this concern, it is noted that assuming a lower background variability than present in reality would be conservative in the current exercise. Taking this into account, it is assumed that the results for the variability in the control data from field studies with bumble bees and solitary bees performed in the Northern and Central Zone may be representative also for the Southern Zone.

Data are available for only one bumble bee species (*B. terrestris*) and one solitary bee species (*O. bicornis*).

The key question for the bumble bee SPG that was considered was, 'Can colonies grow strong enough to provide pollination services AND produce new queens to be able to establish new colonies the following season?' In the current document, results are presented for the endpoints 'number of workers', 'number of adults' and 'colony weight'. The WG considers the number and weight of reproductive drones and/or queens also as relevant endpoints. Analyses for these are not presented for the sake of brevity, but may be considered when drafting guidance for bumble bee field studies. Note that preliminary analysis indicated that for the number of queens and drones the variability is likely be higher compared with the number of workers and adults.

For solitary bees, the key question for the SPG is, 'Can the (starting) population replace itself?' From this question, it follows that informative and operable endpoints would be those that quantify reproductive output in relation to the starting population. It is considered most relevant to compare reproductive output related to the same type of variable for the starting population, e.g. number of cocoons produced in relation to the number of female cocoons in the starting population, or number of females in the next generation in relation to the number of females in the starting population. Taking into account the data availability and considering the relevance of the endpoints for the SPG, four endpoints were considered for further data analysis: 'number of females emerged in the next generation per number of females emerged in the starting population', 'number of female cocoons per introduced female cocoon', 'number of cocoons (both sexes) per introduced female cocoon' and 'number of brood cells per introduced female cocoon'. It should be noted that there are important differences in study setup between the different datasets. Experience with solitary bee field testing is limited and well established test protocols are not available yet. The variation in test methods reflects the attempts of the scientific community to investigate how field testing could be done. It should be kept in mind that this variation in study setup is a source of variability in itself.

A summary of the available data for bumble bees and solitary bees, in comparison with honey bees is shown in Table 2. It is clear that data from field studies that could be used to investigate the NOR for bumble bees and solitary bees are scarce compared with honey bees, and that model simulations are not available (see Section 3.1). The limited availability of field study data generally prevents a comprehensive analysis.

Table 2: Summary of the available data from model simulations and field studies and results for coefficient of variation (CV) for honey bees, bumble bees and solitary bees

	Honey bees[(a)]		Bumble bees	Solitary bees[(b)]
Species	*Apis mellifera* (one species)		Data available on *B. terrestris* (68 species in Europe)	Data available on *O. bicornis* (1900 species in Europe)
Available data	Model simulations: 19 scenarios, 500 replicate colonies/scenario	Field data: 33 field studies (52 fields overall, 1–16 replicate colonies/field)	Field data: 7 field studies (33 fields overall, 2–25 replicate colonies/field)	Field data: 8 field studies (35 fields overall, 1 population/field)
Colony size CV	CV: 5–20%	CV: 0–50% (n workers ≈ n adults)	CV workers: 0–135% CV adults: 0–95% (n workers ≠ n adults)	Not relevant
Colony weight CV			CV: 5–60%	Not relevant

(a): See EFSA et al. (2021).
(b): CV values calculated are for between-site variability, and cannot be compared with those for bumble bees or honey bees (which represent within-site variability).

There is variation in the calculated CV values between studies. This might be partly explained by important methodological differences between studies, which are not surprising as harmonised protocols are not yet available, and experience is relatively limited. Therefore, the observed variability should be interpreted with caution.

For bumble bees (*B. terrestris*), the CV values for number of workers and number of adults range from 0–135% and from 0–95%. For these endpoints, the variability is higher compared with colony weight, for which the CV values ranged from 5–60%. For the latter endpoint, the variability is comparable with that observed for colony strength in honey bee field studies (CV values ranging from 0–50%). Taking into account the low number of studies, the available dataset is considered too limited to give a quantitative estimate of the NOR or background variability. Nevertheless, our results indicate that the background variability for the endpoint bumble bee colony weight of *B. terrestris* could be comparable with that for honey bee colony strength.

For solitary bees (*O. bicornis*), the CV values for the four endpoints considered [number of females emerged in the next generation per number of females emerged in the starting population, number of female cocoons per introduced female cocoon, number of cocoons (both sexes) per introduced female cocoon, and number of brood cells per introduced female cocoon] generally ranged from 40 to 70%, with one study having CV values exceeding 100%. Note that for these bees, the CV values represent between-site variability, and cannot be compared with the results for bumble bees or honey bees. Taking into account the low number of studies, and the methodological differences between studies, the available dataset is considered too limited to give any reasonable indications for the NOR or background variability for *O. bicornis*. Nevertheless, for a rough comparison with SPG agreed for honey bees. it is noted that the variability observed in the available field studies with *O. bicornis* is greater than 10%.

In addition to the limitations of the experimental approaches in isolation to explore the background variability, it should be noted that the above conclusions for bumble bees and solitary bees are characterised by a relatively high level of uncertainty. This is due to the following sources of uncertainties:

- The number of studies is very limited (seven datasets for bumble bees and eight for solitary bees), and not all EU Regulatory Zones are equally well represented.
- There are no well established test guidelines for bumble bee and solitary bee testing, and therefore there are important methodological differences between studies, which is likely to be an additional source of variability.
- There are data available for only one species of each group (*B. terrestris* for bumble bees and

O. bicornis for solitary bees).

These uncertainties should be taken into account when any decisions are made based on these conclusions.

3.3. Future developments

Several projects and activities are ongoing aimed at improving knowledge, providing data and tools on bees. For example, the Horizon 2020 project PoshBee (Brown et al., 2021)[7] is carrying out several sub-projects and will make available new models for bumble bee and solitary bees by mid-2023.

The B-GOOD project and other sources will provide landscape data from various EU regions (EFSA Scientific Committee, 2021) to increase the models' representativeness.

In 2021, EFSA initiated activities to advance the risk assessment methodologies for insect pollinators. This includes a call for developing a roadmap for actions that will provide a comprehensive overview of related EU activities, knowledge gaps and societal interests. The roadmap will also indicate the research areas that should be prioritised and in which significant financial resources will be invested.

In the meantime, for addressing the well known knowledge gaps, EFSA has initiated a procurement[8] for collecting and generating data (with experimental work) on Non-Target Arthropods, including insect pollinators that will contribute to fulfil data gaps for risk assessment.

The WG recommends a comprehensive evaluation of the most promising models and consideration of new relevant data on species vulnerability and sensitivity as soon as they become available.

4. Reference tier (field studies) design in relation to the magnitude of acceptable effects

To complement the information presented in this document, in this section a preliminary theoretical estimation of the requirements for field studies is reported for different potential thresholds of acceptable effects. The definition of the SPG, and particularly the selected 'magnitude' of effect, has a direct impact on the requirements and feasibility of the reference tier testing (i.e. field studies) (see Section 6).

4.1. Preliminary estimation of the requirements for field studies

In EFSA et al. (2021), a preliminary theoretical estimation of the higher tier requirements depending on the selected 'threshold' of acceptable effects was presented for honey bees. These estimations were based on the power of a *t*-test, in which two groups (i.e. one control and one treatment) are compared with each other. The total variability in colony size within each group is assumed to proceed from two components: a variability within one field and a variability between several fields. As in the previous guidance document EFSA (2013), a CV for variability within one field of 15% and a CV for variability between several fields of 5% were assumed. These estimations do not consider the increase in variability in time that colonies are likely to experience in field studies (see section 7.1 of EFSA et al., 2021 for further details).

4.1.1. Theoretical requirements for field studies on bumble bees

Field studies with bumble bees are characterised by a similar design as for field studies with honey bees: in both cases some colonies were placed next to a test field that was either untreated (control) or treated with the tested substance (treatment). Given this similar design, an estimation for the higher tier requirements for bumble bee studies could be performed in a similar way, i.e. using the same assumption used for honey bees in EFSA et al. (2021). Due to lack of specific data for bumble bees, a CV for variability between several fields of 5% is also assumed here. The available field data for bumble bees indicate that the CV for variability within one field is generally between 5 and 25% at the start of the study (see Figures B.2–B.4 in Appendix B). Therefore, it is considered reasonable to assume a CV

[7] https://www.nature.uni-freiburg.de/ressourcen/publikationen-pdfs/preprints-article-72231-en-1poshbee.pdf
[8] https://etendering.ted.europa.eu/cft/cft-display.html?cftId=8625

for variability within one field of 15% when estimating the higher tier requirements for bumble bees, as also done in EFSA et al. (2021). Based on this, the preliminary estimation for the higher tier requirements for bumble bee field studies are the same as reported in EFSA et al. (2021), and are shown in Table 3.

Table 3: Estimated total number of fields (i.e. treated + control fields) and bumble bee colonies required to detect a statistically significant reduction in colony strength at a given level. The calculations are based on assuming a coefficient of variation of 15% within a field and 5% between fields, and a value for alpha of 0.9 and for beta of 0.2. This table assumes that 5–8 bee colonies are monitored per field as an example [note: table also included in EFSA et al. (2021) as Table 15 for honey bees]

	Thresholds of acceptable effect (i.e.% of colony size reduction)													
	1%	**2%**	**3%**	**4%**	**5%**	**6%**	**7%**	**8%**	**9%**	**10%**	**12%**	**15%**	**20%**	**25%**
8 colonies/field														
Fields	944	234	104	58	38	26	20	14	12	10	6	4	2	2
Colonies	7552	1872	832	464	304	208	160	112	96	80	48	24	16	16
7 colonies/field														
Fields	1014	252	112	62	40	28	20	16	12	10	8	4	4	2
Colonies	7098	1764	784	434	280	196	140	112	84	70	56	24	28	14
6 colonies/field														
Fields	1108	276	122	68	44	30	22	18	14	12	8	6	4	2
Colonies	6648	1656	732	408	264	180	132	108	84	72	48	36	24	12
5 colonies/field														
Fields	1242	308	136	76	48	34	24	20	16	12	8	6	4	2
Colonies	6210	1540	680	380	240	170	120	100	80	60	40	30	20	10

4.1.2. Theoretical requirements for field studies on solitary bees

For solitary bees, the relevant attribute for the SPG is the population abundance, rather than the colony strength. Therefore, the study design for solitary bee field studies differs from that for honey bees and bumble bees. As a population is defined by individuals of the same species co-occurring in space and time that interbreed, all individuals present at a single field site in a certain study would be considered part of the same population. Although some nest units can be placed next to the treated and control fields, the bees nesting at these different nest units will still be part of the same population. Therefore, only the variability between several fields is important. As concluded in Section 3.2, the available dataset is considered too limited to give any reasonable indications of the background variability for *O. bicornis*. Therefore, calculations as in Table 3 cannot be performed for solitary bees.

4.2. Examples of available higher tier studies

As also acknowledged under Section 3.2, field studies with bumble bees and solitary bees are still in a rather 'experimental' phase: there is a lack of established methods and experience (especially for solitary bee studies) and, as a consequence, there are currently no well established guidelines. Nevertheless, some studies are available that are discussed in detail in Appendix B. In Section 3.2, the control data from these studies were analysed in an attempt to derive the NOR. In this section, the effect that could be detected in these studies (i.e. the difference between the control and the treatment data based on the actual variability in the studies) is considered.

4.2.1. Bumble bees

A summary of the study design for the available bumble bee field studies (i.e. number of fields, number of colonies per field) is shown in Table 4.

For some of the studies available, a *post hoc* analysis of the statistical power was performed, i.e. Rundlöf et al. (2015) and Woodcock et al. (2017).

Rundlöf et al. (2015) performed a bumble bee field study using 16 fields (eight treated, eight control) in southern Sweden, with six colonies per field, and therefore a total of 96 colonies deployed in the experiment. With the parametrisation used in Section 4.1 and according to the preliminary estimations in Table 3, this study design should be able to detect as significant effects close to 8%. In the follow-up publication by Wintermantel et al. (2018), a power analysis was performed on the same dataset, to assess the effect size that could potentially be detected given the study design, replication and model choice. The method and parametrisation used were, however, different from the one used in Section 4.1. The outcome of this power analysis for the number of adult workers suggested that effect sizes of approximately 15% could be detected with a statistical power of 80% and a confidence (alpha) of 95%.

Woodcock et al. (2017) tested bumble bees in 33 different fields: 11 fields for the control and 11 for each of the two treatments, as two substances were tested, scattered over three countries (Germany, Hungary and UK). For bumble bees, six colonies per field were used, with a total of 198 colonies deployed. With the parametrisation used in Section 4.1.1 and according to the preliminary estimations in Table 5, this study design for bumble bees should be able to detect as significant effects close to 7%. However, a more complex *post hoc* analysis performed by the same authors, revealed that their study could detect only considerably larger effects. For example, for bumble bee worker number, effect sizes of 50% or higher were detectable with a statistical power of 80%. For bumble bee peak colony weight, effect sizes of 25 to 30% could be detected with the same statistical power. This could be partly explained because of the large observed variability among countries.

Overall, based on the preliminary calculations according to the parametrisation reported in Section 4.1, it can be concluded that current available studies should be able to detect differences between the control and treatment of 8–15% as statistically significant. It should however be noted that these calculations are based on the number of fields per treatment and the number of colonies per field used in the studies, and do not consider other specific features (i.e. actual variability observed, temporal distribution of the replicates, etc.). The *post hoc* power analysis performed in two studies indicates that the actual detectable effects will probably be slightly higher than those presented in Table 4.

Table 4: Overview of the number of fields, number of colonies/field and the total number of colonies used in the different bumble bee field studies considered in Appendix B. The theoretically detectable effect (as determined in Section 4.1) for this combination of fields and colonies is also shown, together with the actual detectable effects determined by a *post hoc* power analysis (if available in the respective study reports). Note that the methodology and parameterisation used to determine the theoretically detectable effect and the actual detectable effect were different, making a direct comparison between these values difficult

<table>
<tr><th>Study reference</th><th>Number of fields</th><th>Number of colonies/fields</th><th>Total number of colonies</th><th colspan="2">Theoretically detectable effect[(a)]</th><th>Actual detectable effect[(b)]</th></tr>
<tr><td>**C.1342 + Sterk et al., 2016**</td><td>12 (6 control + 6 treated)</td><td>9</td><td>108</td><td colspan="2"><9%</td><td>ND</td></tr>
<tr><td>**Rundlöf et al., 2015 + Wintermantel et al., 2018**</td><td>16 (8 control + 8 treated)</td><td>6</td><td>96</td><td colspan="2">8–9%</td><td>For number of adult workers: 15%</td></tr>
<tr><td>**T.1513**</td><td>3 (2 control + 1 treated)</td><td>25</td><td>75</td><td colspan="2">15–20% (probably lower)</td><td>ND</td></tr>
<tr><td>**C+T.2013G + Woodcock et al., 2017**</td><td>9 (3 control + 2 × 3 treated)</td><td>6</td><td>54</td><td>15%[(c)]</td><td rowspan="2">±7%</td><td rowspan="2">For number of adult workers: 50% or higher. For bumble bee peak colony</td></tr>
<tr><td>**C+T.2013H + Woodcock et al.,**</td><td>12 (4 control + 2</td><td>6</td><td>72</td><td>12%[(c)]</td></tr>
</table>

2017	× 4 treated)					weight: 25–30%
C+T.2013U + Woodcock et al., 2017	12 (4 control + 2 × 4 treated)	6	72	12%[(c)]		
JKI, 2018	8 (4 control + 4 treated)	5	40	12%		ND

ND: not determined.

(a): Theoretical mean percentage of reduction in colony strength in the treatment compared with the control, which can be detected as statistically significant. This value was derived from Table 5 (i.e. estimated using the method and parametrisation described in Section 4.1). Note that in some cases the actual combination of number of colonies/field and number of fields for the study was not included in Table 5. In that case, the value presented here is an approximation.

(b): Percentage reduction in the treatment compared with the control that could be detected as statistically significant with a power of 80%, based on a *post hoc* power analysis performed by the study authors. The method and parametrisation used for this *post hoc* power analysis was different than the one used to derive the values from Table 5. Therefore, a direct comparison with the theoretically detectable effect shown in this table is not straightforward.

(c): In each of these datasets, there were two different treatments. Consequently, for the theoretically detectable effect only the number of fields and colonies for the control in combination with a single treatment were considered. In addition, in the study by Woodcock et al. (2017), these three datasets were considered as a single study, resulting in a total of 22 fields considering a single treatment (i.e. 11 control and 11 treated). This would then result in a theoretically measurable effect of close to 7%.

4.2.2. Solitary bees

A summary of the study design (i.e. number of fields, number of female cocoons introduced/field) for the available solitary bee studies is shown in Table 5.

For some of the studies available, a *post hoc* analysis of the statistical power was performed, i.e. Ruddle et al. (2018) and Woodcock et al. (2017).

Ruddle et al. (2018) performed a study for solitary bees using six field sites, each with one treated and one control field. The authors performed a *post hoc* power analysis to determine the effect size detectable with 80% power. The estimated relative minimum detectable differences varied between endpoints from 15 to 20% for weight of cocoons or adults, to 75 to ~100% for endpoints related to reproduction such as total nests, total cells, total nests/female released, and total cell/female released. It should however be noted that the endpoints for which this power analysis was performed were different from those considered as most informative for the SPG (see Section 3.2 and Appendix B).

Woodcock et al. (2017) tested solitary bees in 33 different fields: 11 fields for the control and 11 for each of the two treatments, as two substances were tested, scattered over three countries (Germany, Hungary and UK). For solitary bees, there were as many 'populations' considered as there were fields. The *post hoc* power analysis performed by the same authors, revealed that their study could detect only large effects. For solitary bee (*O. bicornis*) cell production, only effect sizes well exceeding 50% were detectable with a statistical power of 80%. This could be partly explained because of the large observed variability among countries.

As explained in Section 4.1.2, it is currently not possible to make an *a priori* estimate of the detectable effect in solitary bee field studies based on the number of fields used in a study alone. There are two examples from studies in which a *post hoc* power analysis was performed. Both these examples indicate that, for endpoints related to reproduction, only large effects of above 50% or higher would be statistically detectable. As shown in Appendix B, the variability between fields is lower in studies in which a large number of female cocoons (hundreds) is introduced compared with studies that include smaller numbers (tens). Therefore, it seems that one study design aspect that influences the power of the study, is the number of female cocoons introduced. Further research is needed to investigate the study design that would be needed to enable the detection of lower effect levels (e.g. approximately 10–15%).

Table 5: Overview of the number of fields and number of female cocoons/field in the different solitary bee field studies considered in Appendix B. A theoretically detectable effect (as

determined for bumble bees in Section 4.1) for this number of fields could not be estimated. The actual detectable effects, determined by a *post hoc* power analysis, are also shown if available in the respective study reports

Study reference	Number of fields	Number of populations	Number of female cocoons/field or population	Theoretically detectable effect[(a)]	Actual detectable effect[(b)]
T.721,722, 723 + Ruddle et al., 2018 (trials from 2014)	6 (3 control + 3 treated)	6	1200	ND	For weight of cocoons or adults: 15–20%. For endpoints related to reproduction: 75–100%
Ruddle et al., 2018 (trials from 2015)	6 (3 control + 3 treated)	6	480	ND	
C.1039 + Peters et al., 2016	12 (6 control + 6 treated)	12	682	ND	ND
Rundlöf et al., 2015	16 (8 control + 8 treated)	16	12	ND	ND
C+T.2013G + Woodcock et al., 2017	9 (3 control + 2 × 3 treated)[(c)]	9	25	ND	For cell production: greater than 50%
C+T.2013H + Woodcock et al., 2017	12 (4 control + 2 × 4 treated)[(c)]	12	25		
C+T.2013U + Woodcock et al., 2017	12 (4 control + 2 × 4 treated)[(c)]	12	25		
JKI, 2018	8 (4 control + 4 treated)	8	200	ND	ND

ND: not determined.

(a): A theoretical detectable effect as determined for bumble bees in Section 4.1 could not be estimated for solitary bee studies.

(b): Percentage reduction in the treatment compared to the control that could be detected as statistically significant with a power of 80%, based on a *post hoc* power analysis performed by the study authors.

(c): In each of these datasets, there were two different treatments. Consequently, for any kind of power analysis, only the number of fields and colonies for the control in combination with a single treatment should be considered. In addition, in the study by Woodcock et al. (2017), these three datasets were considered as a single study, resulting in a total of 22 fields considering a single treatment (i.e. 11 control and 11 treated).

5. Existing approaches for SPGs in the area ecotoxicology

In this section the approaches proposed for insect pollinators of the EFSA PPR Panel (2015b) opinion on Non-Target Arthropods (NTA) is included as well as the approach given in the aquatic guidance (EFSA PPR Panel, 2013). Both documents have applied the EFSA method for defining SPG, notably the EFSA PPR Panel opinion of 2010.

5.1. Definition of SPG for insect pollinators in the Non-Target Arthropods opinion (EFSA PPR Panel, 2015b)

EFSA PPR Panel opinion on NTA (EFSA PPR Panel, 2015b) proposed SPGs options by considering various ecosystem services, including for NTAs as providers of pollination in agricultural landscapes. The latter are summarised in Table 6.

Table 6: Definition of SPGs for insect pollinators in the opinion on Non-Target Arthropods (EFSA PPR Panel, 2015b)

Dimensions	In-field	Off-field
Ecological entities	Functional group	Population
Attribute	Abundance	Abundance/biomass
Magnitude, Temporal Special scale	Local scale: Small effect up to months at local scale: 10% < effects < 35% during crop flowering. Medium effects up to weeks at local scale: 35% < effects < 65 % up to 4 weeks outside flowering period Landscape scale: to be defined	Negligible effects Local scale: ≤10 % or comparable to non-detectable effects on the abundance of NTA populations that are directly caused by exposure in the off-field habitat (at any time) Landscape scale: negligible effects on abundance and spatial occupancy of NTA pollinator species (at any time)

The magnitude of effects is based on the partitioning of effects derived from general effect classes in ecotoxicology:

- large effects: pronounced reduction above 65%;
- medium effects: reduction between 35% and 65%;
- small effects: reduction above 10% and below 35%;
- negligible effects: reduction up to 10% (comparable with non-detectable effects).

The opinion says that these effect classes are deemed to be pertinent for the assessment of effects on NTAs at local scale, defined in this opinion as 'the treated field and the immediate surroundings'. Based on this definition, the options proposed by the EFSA PPR Panel (2015b) at local scale appear compliant with EFSA (2013) in which the spatial scale for colonies/population is the edge of field. Note that in this case 'edge of field' mainly refers to the location of the colonies/populations, i.e. directly adjacent to the treated field. The different exposure scenarios considered in the risk assessment, however, refer to foraging in the treated field (e.g. treated crop and flowering weeds scenario) and in the areas surrounding the field (e.g. the field margin and the adjacent crop scenario).

The proposed scaling of the magnitude of effects in EFSA PPR Panel (2015b) is based on expert judgement and not based on data. The expert judgement was based on the typical dose–response curves (i.e. EC_{10}, EC_{50}, etc.). No data, e.g. on the normal operating range, are available to further substantiate the proposed effect classification.

It is also noted that the options proposed in the opinion were not yet discussed in a dialogue with risk managers; therefore, since no final decision is available, none of them is implemented in the risk assessment.

The current protection goal implemented in the guidance document for Non-Target Arthropods (European Commission, 2002) relies on the regulatory general protection goal, i.e. maintenance of function in the in-field areas and of the biodiversity in the off-field areas. This is assessed in (higher tier) risk assessment on a case-by-case basis and requires expert judgement to interpret field study results. The general criteria, questioned by the MS experts (EFSA, 2019), considered are: (1) the potential for re-colonisation after a toxic effect should be demonstrated within one year for in-field habitats; and (2) within an ecologically relevant time for off-field habitats.

5.2. Definition of SPG for aquatic invertebrate organisms (EFSA PPR Panel, 2013)

The guidance document (EFSA PPR Panel, 2013) includes SPG for aquatic organisms living in the water column of edge-of-field surface water bodies. The SPGs proposed for aquatic invertebrates, and currently implemented in the risk assessment, are reported in the Table 7.

Table 7: Definition of SPGs for aquatic invertebrates in the guidance document (EFSA PPR, 2013)

Dimensions	Without recovery	With recovery
Ecological entities	Population	
Attribute	Abundance/biomass	
Magnitude/ Temporal scale	Negligible effect (at any time)	Small effect: Months Medium effect: Weeks Large effect: Days
Special scale	Edge-of-field surface water	

The magnitude of the effects is not quantitatively defined by a specific threshold; 'negligible effects' are equivalent to effect class 1 or effect class 2 responses for the most sensitive populations in appropriately designed and conducted micro-/mesocosm experiments:

- **Effect class 1** (no treatment-related effects demonstrated for the most sensitive endpoints). No (statistically and/or ecologically significant) effects observed as a result of the treatment. Observed differences between treatment and controls show no clear causal relationship.
- **Effect class 2** (slight effects). Effects concern short-term and quantitatively restricted responses usually observed at individual samplings only.

Due to the nature of the higher tier studies, i.e. microcosm/mesocosm studies, aimed at defining the toxicity endpoint (i.e. NOEC, ECx), the robustness of the higher tier endpoints is further evaluated by the minimal detectable difference (MDD) concept. For this purpose, the guidance proposes classes of MDD due to treatment-related decline in abundance/biomass.

The MDD is a statistical indicator used in ecotoxicological risk assessment. It calculates a 'detectable' effect based on the variance of the data, independent of the actual estimated effect. This means in practice that no thresholds of effects are defined. Instead, a study is supposed to have the power to detect statistically significant treatment-related 'negligible' or 'small' effects as defined in the SPG for aquatic invertebrates, if the MDD is below the defined class values. It is, however, noted that assessment factors (i.e. from 2 to 4), arbitrarily selected based on expert judgement, are always applied to account for the uncertainties relative to microcosm/mesocosm studies as a surrogate reference tier to assess whether the SPGs are addressed.

The WG noted that the concept of using an approach based on statistical power and the determination of the significance of possible effects, instead of defining thresholds, could in principle be applied to field studies on bees, specifically for solitary bees (see Section 6.2).

However, this approach would require further consideration and analysis of data that are currently scarce. In addition, the current approach for MDD calculation was challenged in recent studies related to its apparently limited power control, potentially leading to false negatives (type II errors) (Duquesne et al., 2020; Mair et al., 2020). The use of confidence interval (CI)-based methods was in statistical simulation experiments found to be superior to the MDD in terms of controlling statistical power and type II error rates (Mair et al., 2020). It appears therefore useful to consider the use of CI-based methods for the detection of possible effects for bees, for which however further considerations are needed.

6. Possible approaches for setting the SPGs for bumble bee and solitary bees based on the available evidence

Based on the information presented in this document, the WG provides suggestions for defining the magnitude dimension of the SPGs, accounting for the level of knowledge, general considerations and potential impact for the risk assessment. The information presented could be considered as lines of evidence to support a weight of evidence approach for decision making. It is, however, recognised that any decision relying on the current knowledge may require revision in the future when new data and tools become available.

It is worth anticipating that within the review of EFSA (2013) the WG analysed data to set extrapolation factors for the risk assessment to address some aspects of the different vulnerability between the three bee groups. This is the case for example for:

- ecotoxicological sensitivity;
- level of exposure (dietary, contact), e.g. in relation to food consumption, feed provision to larvae.

However, there are several ecological factors (see Section 2 and Figure 1) that could influence the vulnerability of bumble bees and solitary bees to pesticides compared with honey bees (EFSA PPR Panel, 2012, tables 2.3 and 2.4). In general, biology and ecology of bumble bees and, especially of solitary bees, suggest a lower resilience and higher vulnerability to stressors relative to honey bees. Although it is unclear to what extent each ecological factor contributes to its vulnerability, it is important to highlight that this is a remaining source of uncertainty in the risk assessment that is difficult to quantify due to the lack of data. Therefore, even if on the one hand the WG will identify extrapolation factors to be included in the risk assessment schemes of bumble bees and solitary bees, a conservative approach for the SPGs setting within the Regulation (EC) No 1107/2009 could be warranted, as also recommended by the opinion (EFSA PPR Panel, 2012). On the other hand, it is recognised that more data on biology and ecology, particularly for solitary bees, are needed for a more accurate evidence-based decision-making approach.

Overall, based on the available information, the WG considers that there are two potential options that could be followed (see Figure 1):

- An *a priori* defined threshold option: which requires the definition of a threshold of acceptable effects.
- An undefined threshold option: which relies on statistical power to determine the significance of possible effects.

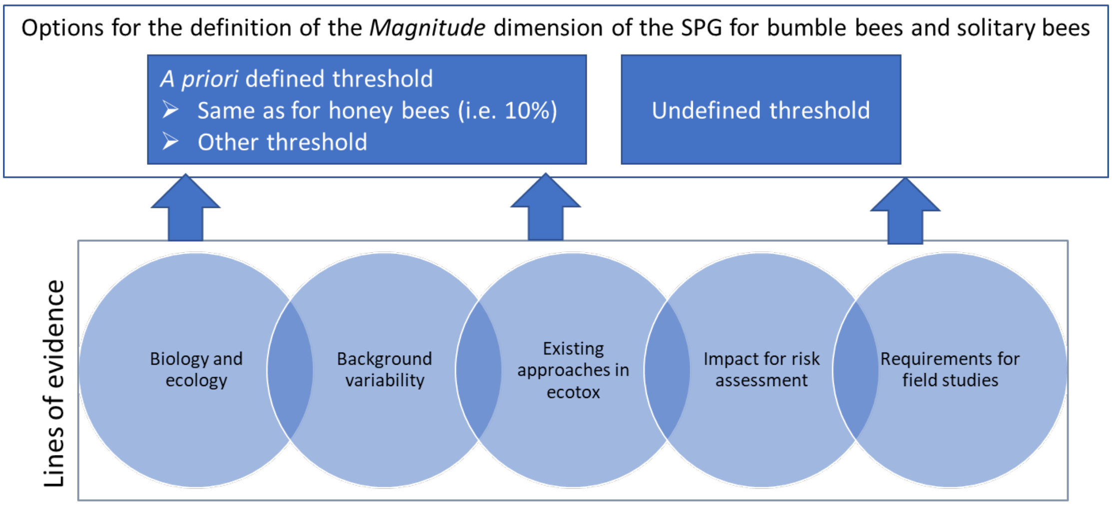

Figure 1: Lines of evidence contributing to the definition of the magnitude dimension for the SPGs of bumble bees or solitary bees

6.1. An *a priori* defined threshold option

The term 'threshold of acceptable effects' (e.g. percentage of colony size reduction) was introduced in EFSA et al. (2021) because it is difficult to establish consensus on an undisputable scientific definition of qualitative class effects such as 'negligible', 'small', and 'medium'. Furthermore, the term 'acceptable' is also in accordance with Art. 4 and Annex II, point 3.8.3 of Regulation (EC) 1107/2009. Therefore, the concept of 'acceptable effect' is considered as more suited in this context than any qualitative definition of effect class.

Two potential strategies are illustrated in the sections below to define a threshold of acceptable effects for bumble bees and solitary bees.

6.1.1. Same threshold as agreed for honey bees (i.e. 10%)

Risk managers could decide to set the same threshold as agreed for honey bees. This approach was followed in EFSA (2013) for both bumble bees and solitary bees; it was suggested as a pragmatic approach, in the absence of data, by EFSA PPR Panel (2012) in combination with additional assessment factors for the lower tier risk assessment.

If risk managers were to follow the same strategy as in EFSA (2013) for the revised guidance document, the maximum permitted effect size threshold for bumble bees or solitary bees would be set at 10% reduction in colony size or population size, respectively.

The lines of evidence that can support this approach are:

- analysis of the background variability for bumble bees and solitary bees based on the available data included in Section 3.2 and Appendix B, and the related highlighted uncertainties (see the next paragraph for differences among bumble bees and solitary bees);
- impact for risk assessment (Section 6.1.3);
- theoretical requirements for higher tier studies and power of existing field studies (for bumble bees only, as no further information is available for solitary bees) (Section 4).

Regarding the background variability:

- For bumble bees, the information available (see Section 3.2) indicates that variability in colony weight (as a proxy for the NOR) in field studies is comparable with the variability for the number of bees observed in honey bee field studies. In addition, it is noted that variability in honey bee field studies can be higher than the agreed threshold value, which is based on a modelling exercise. By acknowledging all the uncertainties of the analysis performed (e.g. limited dataset, one bumble bee species tested), it is possible to conclude that the 10% value may still be considered in the lower end of the range of variability observed in the bumble bee field studies.
- For solitary bees, it is not possible to compare the available data with honey bee field studies (see Section 3.2). Nevertheless, for a rough comparison with SPG agreed for honey bees, it is noted that the variability observed in the field studies with *O. bicornis* is greater than 10%. Therefore, the argument that by selecting 10% as the threshold for an acceptable effect on the population abundance reduction, it is likely to be below the variability of endpoints used as a proxy for population abundance observed in field studies, may also be valid (see Section 3.2). However, also for solitary bees the uncertainties of the analysis performed should be considered, e.g. limited dataset and with different methodologies, one solitary bee species tested.

The impact for risk assessment is reported in Section 6.1.3, whereas the field study design relative to the magnitude of acceptable effects is reported in Section 4.

6.1.2. Other specific threshold(s)

Risk managers could decide to set a percentage value of size reduction specific for both bumble bee colonies and a percentage value of size reduction specific for solitary bee population abundance, independently of the magnitude of acceptable effects agreed for honey bees. Unlike the strategy described above in Section 6.1.1, this approach could allow the biology and ecology of these two bee groups to be specifically addressed (see Section 2.1).

The lines of evidence that can be used for this approach are:

- biology and ecology of bumble bees and solitary bees (Section 2.1);
- analysis of the background variability based on the available data included in Section 3.2 and Appendix B, and the related highlighted uncertainties;
- impact for risk assessment (Section 6.1.3);
- theoretical requirements for higher tier studies and power of existing field studies for bumble bees only, no further information is available for solitary bees (Section 4).

The biology and ecology of bumble bees and solitary bees (see Section 2.1) could be considered as a general line of evidence as no data are available, apart from those considered for the background variability analysis and the general information provided, to empirically support the selection of a specific threshold.

The WG has considered the definition of the SPG option given in the PPR opinion on NTAs to explore possible suggestions (see Section 5.1). In this opinion, the PPR suggests more elaborate SPG options for pollinators and indicates a possible magnitude of acceptable effects up to 10% in the off-field and up to 35% in the in-field. These options were neither discussed with risk managers nor implemented in any risk assessment scheme. Furthermore, they do not comply with the definition of SPG for bumble bees and solitary bees available in EFSA (2013), e.g. the PPR panel suggested different definitions for ecological entity, magnitude, temporal and spatial scales. The in-field concept in the NTA opinion is defined for the functional group. However, in the scope of this document the ecological entity for bumble bees is the colony and for solitary bees it is the population. Even if bee colonies/populations are 'located' at the edge of the field, both in-field and off-field exposure applies to these ecological entities (see Section 2.2). In a functional group the ecosystem service 'pollination' can be provided by different species and therefore the PPR opinion suggests that a 'small to medium effect' can be tolerated for the group for a certain timeframe without affecting the ecosystem service. However, this is not applicable to the definition of the ecological entity for the bees: for example small or medium effects on univoltine or oligolectic bees species may be detrimental for the population or may impact the ecosystem service (e.g. no recovery for univoltine species, or some plants requiring specific species may not be pollinated). Furthermore, although the proposed effect class for the NTAs in the off-field (i.e. ≤10%), based on expert judgement, corresponds to the percentage magnitude of acceptable effects agreed for honey bees, for NTA this is only relevant for effects directly caused by exposure in the off-field habitat. Therefore, the suggestions in the PPR NTA opinion for pollinators would require further developments and data before they can be considered (see Section 3.3) and they cannot be followed up for bumble bees and solitary bees in the timeframe of this mandate.

Regarding the background variability:

- Although the dataset was considered too limited to give a quantitative estimate of the NOR or background variability for bumble bees (see Section 3.2), for the definition of any other specific threshold of acceptable effects, it can be noted that the analyses of the available data indicate that the background variability for the endpoint colony weight of *B. terrestris* could be comparable with that for honey bee colony strength. Therefore, any other specific threshold in the range of the value agreed for honey bees appears supported.
- Also for solitary bees, the available dataset was considered too limited to give any reasonable indications for the NOR or background variability (see Section 3.2). As reported in Section 6.1.1, for a rough comparison with SPG agreed for honey bees, it can be noted that the variability observed in the available field studies with *O. bicornis* was greater than 10%, but in general the analysis is considered inconclusive for this bee group to support the definition of any other specific threshold.

The impact for risk assessment is reported in Section 6.1.3, whereas the field study design relative to the magnitude of acceptable effects is reported in Section 4.

6.1.3. Impact for risk assessment for any *a priori* defined threshold option

Any '*a priori* defined threshold of acceptable effect option' can be implemented in the lower tier risk assessment by using the same approach for the three groups of bees, i.e. the predicted effects on the colony/population following the exposure to a pesticide will be compared directly with the agreed SPG. This would ensure harmonisation between honey bees, bumble bees and solitary bees, as similar risk assessment schemes can be proposed and the overall evaluation of risk of pesticides to bees would be generally less complex.

At a higher tier level, the threshold of acceptable effect can be directly measured in the field studies by comparing the mean colony sizes (for bumble bees) or the population abundance (for solitary bees) of the treatment and control groups. Effects are considered acceptable only if the mean of the treatment group is not decreased by more than the defined threshold, which is calculated relative to the mean of the control group. The field studies should be designed with a sufficient number of replicates to statistically detect differences between treatment and control smaller or equal to the selected threshold (see Section 4). As regards field study there are no well established test guidelines for bumble bee and solitary bee testing (see Section 3.2).

6.2. Undefined threshold option

Risk managers could decide not to define a threshold of acceptable effects, until better data become available. This approach could be useful to move forwards the implementation of a risk assessment scheme in the absence of data, particularly for solitary bees. However, it has some disadvantages that are described below.

The lines of evidence that can be used for this approach are:

- existing approaches in the area of ecotoxicology: this is the strategy currently used in e.g. for aquatic organisms;
- uncertainties regarding the available data used for the analysis of the background variability in Section 3.2 and Appendix B and general knowledge gaps;
- impact for risk assessment (Section 6.2.1);
- theoretical requirements for higher tier studies and power of existing field studies (for bumble bees only, as no further information is available for solitary bees) (Section 4).

As, unlike other groups of non-target organisms (e.g. aquatic organisms), trigger values for bumble bees and solitary bees are not defined in Regulation (EC) No 546/2011, this approach would require the pragmatic and empirical definition of assessment factors for the lower tier risk assessment, in addition to the factors that the WG will identify based on inter-species ecotoxicology sensitivity and biology. This is because, in the absence of a threshold of acceptable effects, it is necessary to ensure that the risk assessment schemes predict no 'unacceptable' effects with high certainty and, due to the lack of a robust reference tier for bumble bees and solitary bees, the lower tier risk assessment cannot be calibrated based on higher tier data.

In addition, this approach would allow only the development of a general definition of the requirements for field studies because, in the absence of a defined threshold, they cannot be designed to detect a specific magnitude of effects (see Section 4.1). Study design and interpretation of the results should be discussed on a case-by-case basis as approaches for evaluating the robustness of the higher tier studies (e.g. similar to the MDD for aquatic organisms in Section 5.2) are not yet readily available. The WG noted that the concept of using an approach based on statistical power and the determination of the significance of possible effects instead of defining thresholds could in principle be applied to field studies on bees, specifically for solitary bees, to account for the difficulty in defining at the moment acceptable effect thresholds due to the lack of data. However, the possibility to develop approaches for evaluating the robustness of higher tier studies on bees would require further consideration and developments (see Section 5.2) that cannot be addressed within the timeframe of this mandate.

Regarding the uncertainties of the background variability analysis presented in Section 3:

- For bumbles bees the data were limited and only based on the species *B. terrestris.*
- For solitary bees, data were limited and only based on the species *O. bicornis* and they varied in different methodological approaches.

The impact for risk assessment is reported in Section 6.2.1, whereas the field study design relative to the magnitude of acceptable effects is reported in Section 4.

6.2.1. Impact for risk assessment for an undefined threshold option

An 'undefined threshold of acceptable effects' can be implemented in lower tier risk assessment by relying on standard laboratory studies and by applying assessment factors that would need to be pragmatically and empirically defined as mentioned above.

The definition of SPGs, based on this qualitative approach will result in a different lower tier risk assessment approach than the one the WG will propose for honey bees, adding complexity to the risk assessment and to the decision making. In addition, the pragmatic and empirical definition of trigger values may lead to low performance of the lower tier risk assessment (e.g. to identify the substances of low concern), which as consequence makes the tiered approach less effective and requires higher tier studies in most of the cases.

At higher tier level, as the specific level of protection is unknown, the risk assessment may be possibly pending on the robustness of the higher tier studies available for the case under evaluation. Only a (qualitatively) general protection goal could be implemented (see Section 6.2). This means that harmonised evaluation between different substances may be more challenging as field studies will not be designed to detect a specific magnitude of effects.

7. Concluding remarks

The definition of the 'ecological entity, attribute, spatial and temporal scale' dimensions included in EFSA (2013) is still considered the most appropriate option based on current knowledge.

For the 'magnitude' dimension the 2013 guidance definition of 'negligible effect' requires a proper quantitative classification. This might be achieved by determining a threshold of acceptable effects based on the background variability ('defined threshold option'), as was performed for honey bees. Although a comprehensive and structured analysis of the background variability for bumble bees and solitary bees is hampered by data that are not yet available and tools that have not yet been fully evaluated, risk managers could select this option and define a threshold of acceptable effects for these bees based on the information and considerations presented in this document.

Alternatively, risk managers could decide not to define any threshold by selecting the undefined threshold option, also based on the evidence, level of knowledge and considerations provided. An overall summary of the possible approaches, evidence and consideration is reported in Table 8.

Table 8: Overview of the possible approaches that could be followed to define the SPGs for bumble bees and solitary bees and related considerations

Lines of evidence	**Defined threshold**	**Undefined threshold**	**Defined threshold**	**Undefined threshold**
	Bumble bees		**Solitary bees**	
Biology and Ecology	Biology and ecology cannot be fully covered in the risk assessment due to lack of data. Bumble bees and solitary bees have different biology and ecology. Extrapolation factors between species (e.g. from honey bees to bumble bees and solitary bees) based on some aspects of their biology can be considered in the risk assessment			
Background variability	Comprehensive data on NOR not available. Available data may support a threshold as agreed for honey bees or any other threshold in that range	Not applicable	Comprehensive data on NOR not available. Available data give inconclusive results	Not applicable
Impact for lower tier risk assessment	Risk assessment schemes will be developed to be compliant with the defined threshold. It will result in more harmonisation and less complexity as a similar risk assessment among honey bees, bumble and solitary bees will be implemented	It will require definition of assessment factors (trigger values) that may reduce the effectiveness of the lower tier risk assessment. It will lead to less harmonisation and more complexity due to the implementation of different risk assessment schemes among honey bees, bumble bees and solitary bees	Risk assessment schemes will be developed to be compliant with the defined threshold. It will result in more harmonisation and less complexity as a similar risk assessment among honey bees, bumble and solitary bees will be implemented	It will require definition of assessment factors (trigger values) that may reduce the effectiveness of the lower tier risk assessment. It will lead to less harmonisation and more complexity due to the implementation of different risk assessment schemes among honey bees, bumble bees and solitary bees
Requirements	Available studies	Case-by-case. In the	Not possible to give	Case-by-case. In

for field studies vs power of available field studies	indicate feasibility to detect ~10% (based on colony weight)	future, a definition of MDD or CI values may support more harmonised evaluation	indication based on the available data (the studies for which a power analysis is available indicate that 10% may not be feasible with the current design)	future, definition of MDD or CI values may support more harmonised evaluation

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Appendix A – Results of the analysis of the available models for bumble bee and solitary bees

A.1. Introduction

In a recent exercise, EFSA et al. (2021) used an agent-based model to estimate the levels of background variability of adults in honey bee colonies, these data were then used to inform the development of the SPG for honeybees. Therefore, our aim here is to identify models published after the last guidance document in 2013 that might give insights into the background variability of bumble bee and solitary bee populations. If suitable models are available, it may be possible to carry out a similar process as the one completed for honeybees to assess the background variability within bumble bee and solitary bee populations to set their SPGs.

A.2. Assessment of the studies

An initial string search (see Section A.4 for details) identified 158 studies. The studies were subjected to a preliminary screening of the titles and abstracts and any studies that were clearly unsuitable were discarded, this included: studies on the 'bumble bee model' for black holes; studies containing the word 'model' used in a different context, (e.g. model species); studies on bumble bees and solitary bees with no model included (e.g. pathology, evolution, biogeography); studies on bumble bees and solitary bees containing models unrelated to population size (e.g. flight mechanic models, spatial distribution models). In addition to removing studies that were not relevant for our purposes we excluded an additional four studies as these were simply implementations of models for which the paper describing the original model was already included in this exercise. Therefore, the full assessment of the text was carried out on 20 studies.

A similar exercise was carried out to assess the suitability of a range of models for honeybees (EFSA et al., 2021). However, the scope of the previous exercise was to assess the number of individuals within a colony in one species, *Apis mellifera*, rather than identifying models for multiple species at either a colony or population level. Therefore, the same task cannot simply be repeated, although a similar table format was used to arrange the studies and compare them based on a set of predefined criteria.

The following steps were followed for evaluating the available studies:

1) Identify the type/types of bee the study deals with (bumble bee, solitary bee or both?)
2) What type of model is it (e.g. differential equations, agent-based model, matrix model)?
3) Does the model produce an estimate of the adult population size (yes/no)?
4) Does the model produce an estimate of the colony size (yes/no)?
5) What is the name of the model (if available)?
6) Is the code/script/program available (yes/no)?
7) Could the model be potentially useful (yes/no)?

A.3. Results and Conclusion

The output of this process can be viewed in **Table A.1.**, where 10 studies were identified as being potentially suitable to predict either the number of adult bumble bees in a colony or the population size of bumble bees or solitary bees. It is important to note that this is a preliminary step to finding potentially useful models. Any suitable models still need to be assessed According to EFSA PPR Panel (2014) before they can be recommended for use in either setting the SPGs for bumble bee or solitary bee populations or be used in the risk assessment process.

Table A.1.: Overview of the models for bumble bees and solitary bees

Abbreviated authors	Year of publication	Type of bee considered	Type of model	Estimate of population size/abundance	Estimate of colony size	Name of the model	Access to the code	Potentially useful
Banks et al.	2020	Bumble bee	Delay differential equation model	No	Yes	NA	No	Yes
Banks et al.	2017	Bumble bee	Delay differential equations	Yes	No	NA	No	Yes
Becher et al.	2018	Bumble bee	Agent-based model	Yes	Yes	Bumble-BEEHAVE	Yes	Yes
Bennett et al.	2014	Both	Statistical model	Yes	No	NA	No	No
Blasi et al.	2021	Both	Statistical model	Yes	No	NA	Yes	No
Crone and Williams	2016	Bumble bee	Mechanistic	No	No	NA	No	Yes
Everaars and Dormann	2014	Solitary bee	Agent-based model	Yes	Unclear	SOLBEE	Yes	Yes
Ford Versypt et al.	2018	Bumble bee	Agent-based model	No	No	BeeNestABM	Yes	No
Gegear et al.	2021	Bumble bee	Agent-based model	Unclear	Unclear	SimBee	Yes	Yes
Groff et al.	2016	Solitary bee	Statistical model	Yes	No	InVEST	Yes	No
Häussler et al.	2017	Both	Spatially explicit, process-based ecological model?	Yes	No	Poll4pop	Yes	Yes
Iles et al.	2018	Bumble bee	Spatial matrix model	No	Unclear	NA	Yes	Yes
Kerr et al.	2021	Bumble bee	Statistical model	No	Yes	NA	Yes	No
Malfi et al.	2018	Bumble bee	Stochastic, partially individual-based simulation model of bumble bee colony development	No	Unclear	NA	Yes	Yes
Matechou et al.	2018	Bumble bee	Statistical model	No	No	NA	No	No
Newton et al.	2018	Virtual species	Agent-based model	No	No	NA	Yes	No

Abbreviated authors	Year of publication	Type of bee considered	Type of model	Estimate of population size/abundance	Estimate of colony size	Name of the model	Access to the code	Potentially useful
Nicholson et al.	2019	Unclear	Unclear	Unclear	Unclear	Lonsdorf et al. model (LEM) (Lonsdorf et al., 2009); central place foraging model (CPF) (Olsson and Bolin, 2014)	No	No
Riedinger et al.	2015	Both	Mechanistic model	Yes	No	NA	No	Yes
Stewart et al.	2021	Bumble bee	Statistical model	No	Yes	NA	No	No
Thomson	2021	Bumble bee	Statistical model	Yes	No	*Bombus* abundance model	No	No

NA: not applicable.

Glossary

Type of bee

Bumble bee: any member of the genus *Bombus*.

Solitary bee: any species of non-eusocial bee.

Model classifications

Agent-based model: Usually complex, mechanistic models that consider and simulate individual behaviour, dynamics at higher organisation levels such as population emerges from individual decisions and actions. Spatially explicit, often operating on maps.

Differential equation model: Relatively simple models describing average individuals, can infer major drivers, can give mechanistic explanation based on the complexity and parameters involved. No consideration of spatial aspects (in ordinary DE).

Statistical model: Empirical models that can pull out parameter estimates to infer how various factors can influence the abundance of bees but do not provide any mechanistic explanation. Would not apply generally.

A.4. Sources of Information and summary of results

Source	Platform	Results
Biosis	Web of Science	106
CAB Abstracts	Web of Science	91
Scopus	Scopus.com (Elsevier)	135
Web of Science Core Collection (SCI-EXPANDED, CPCI-S, BKCI-S, ESCI)	Web of Science	128
After de-duplication		158

A.4.1. Search structure and limits

- Search 1: (wild/bumble bees NEAR/15 population AND models) in Title, Abstract and Keywords.
- Search 2: (Wild/bumble bees AND Model) in Title.
- Limits: Articles published from 2013.

A.4.2. Search string

Biosis

Date of the search: 10 August 2021

Set	Query	Results
#12	#11 AND PY=(2013 OR 2014 OR 2015 OR 2016 OR 2017 OR 2018 OR 2019 OR 2020 OR 2021)	106
#11	#10 OR #5	161
#10	#9 AND #8	98
#9	TI=(model* OR simulat*)	751,234
#8	#6 OR #7	14,188
#7	TI=(pollinator*) AND #1	1,540
#6	TI=(((social OR solitary OR wild) AND (bee OR bees OR pollinator*)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE OR HALICTINAE OR	13,017

	Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada NEAR/10 (bee OR bees)) OR NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus")	
#5	#3 AND #4	70
#4	TS =(model* OR simulat*)	3,294,798
#3	TS=((((social OR solitary OR wild) NEAR/5 (bee OR bees OR pollinator*)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE OR HALICTINAE OR Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada NEAR/10 (bee OR bees)) OR NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus") NEAR/15 ((population* NEAR/5 (abundan* OR development* OR declin* OR decreas* OR dynamics OR richness OR structure OR variabi*)) OR ((colon* OR intracolon* OR hive OR hives) NEAR/5 (collapse	558

	OR development OR growth OR size OR strength))))	
#2	TS = ((population* NEAR/5 (abundan* OR development* OR declin* OR decreas* OR dynamics OR richness OR structure OR variabi*)) OR ((colon* OR intracolon* OR hive OR hives) NEAR/5 (collapse OR development OR growth OR size OR strength)))	240,503
#1	TS=(((social OR solitary OR wild) NEAR/5 (bee OR bees OR pollinator*)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE OR HALICTINAE OR Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada NEAR/10 (bee OR bees)) OR NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus")	23,690

CAB Abstracts

Date of the search: 10 August 2021

Set	Query	Results
#12	#11 AND PY=(2013 OR 2014 OR 2015 OR 2016 OR 2017 OR 2018 OR 2019 OR 2020 OR 2021)	91
#11	#10 OR #5	141
#10	#9 AND #8	81
#9	TI=(model* OR simulat*)	276,243
#8	#6 OR #7	14,913
#7	TI=(pollinator*) AND #1	2,777
#6	TI=(((social OR solitary OR wild) AND (bee OR bees OR pollinator*)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE OR HALICTINAE OR Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada NEAR/10 (bee OR bees)) OR	12,611

	NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus")	
#5	#3 AND #4	69
#4	TS =(model* OR simulat*)	1,581,940
#3	TS=((((social OR solitary OR wild) NEAR/5 (bee OR bees OR pollinator*)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE OR HALICTINAE OR Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada NEAR/10 (bee OR bees)) OR NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus") NEAR/15 ((population* NEAR/5 (abundan* OR development* OR declin* OR decreas* OR dynamics OR richness OR structure OR variabi*)) OR ((colon* OR intracolon* OR hive OR hives) NEAR/5 (collapse OR development OR growth OR size OR strength))))	592
#2	TS = ((population* NEAR/5 (abundan* OR development* OR declin* OR decreas* OR dynamics OR richness OR structure OR variabi*)) OR ((colon* OR intracolon* OR hive	160,888

	OR hives) NEAR/5 (collapse OR development OR growth OR size OR strength)))	
#1	TS=(((social OR solitary OR wild) NEAR/5 (bee OR bees OR pollinator*)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE OR HALICTINAE OR Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada NEAR/10 (bee OR bees)) OR NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus")	67,971

Scopus

Date of the search 10 August 2021

Set	Query	Results
#13	#11 AND #12	135
#12	PUBYEAR AFT 2012	27,333,807
#11	#10 OR #4	199
#10	#9 AND #8	124
#9	TITLE(model* OR simulat*)	3,639,942
#8	#6 OR #7	10,757
#7	TITLE(pollinator*) AND #1	1,438
#6	TITLE(pollinator*)	4,280
#5	TITLE (((social OR solitary OR wild) AND (bee OR bees OR pollinator*)) OR afranthidium OR aglaoapis OR amegilla OR ammobates OR ammobatoides OR ancyla OR andrena OR andrenidae OR andreninae OR anthidiellum OR anthidium OR anthophora OR apidae OR apinae OR biastes OR camptopoeum OR ceratina OR ceylalictus OR chelostoma OR chiasmognathus OR clavipanurgus OR coelioxys OR colletes OR colletidae OR colletinae OR cubitalia OR dasypoda OR dasypodainae OR dioxys OR dufourea OR ensliniana OR eoanthidium OR epeoloides OR epeolus OR eucera OR flavipanurgus OR habropoda OR haetosmia OR halictidae OR halictinae OR halictus OR heriades OR hofferia OR hoplitis OR hylaeniae OR hylaeus OR icteranthidium OR lasioglossum OR lithurgus OR macropis OR megachile OR megachilidae OR megachilinae OR melecta OR melitta OR melittidae OR melittinae OR melitturga OR metadioxys OR (nomada AND (bee OR bees)) OR nomadinae OR nomiinae OR nomiapis OR nomioidinae OR nomioides OR osmia OR panurginus OR panurgus OR paradioxys OR parammobatodes OR pasites OR panurginae OR protosmia OR pseudoanthidium OR rhodanthidium OR rhophitinae OR rhophitoides	9,633

	OR rophites OR schmiedeknechtia OR simpanurgus OR sphecodes OR stelis OR stenoheriades OR systropha OR tarsalia OR tetralonia OR tetraloniella OR thrincohalictus OR thyreus OR trachusa OR triepeolus OR xylocopa OR xylocopinae OR bombus OR bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum" OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus")	
#4	#3 AND #2	85
#3	TITLE-ABS-KEY (model* OR simulat*)	16,363,397
#2	TITLE-ABS-KEY((((social OR solitary OR wild) W/5 (bee OR bees)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE OR HALICTINAE OR Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada W/10 (bee OR bees)) OR NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus") W/15 ((population* W/5 (abundan* OR development* OR declin* OR decreas* OR dynamics OR richness OR structure OR variabi*)) OR ((colon* OR intracolon* OR hive OR hives) W/5 (collapse OR development OR growth OR size OR strength))))	530
#1	TITLE-ABS-KEY (((social OR solitary OR wild) W/5 (bee OR bees OR pollinator*)) OR afranthidium OR aglaoapis OR amegilla OR ammobates OR ammobatoides OR ancyla OR andrena OR andrenidae OR andreninae OR anthidiellum OR anthidium OR anthophora OR apidae OR apinae OR biastes OR camptopoeum OR ceratina OR ceylalictus OR chelostoma OR chiasmognathus OR clavipanurgus OR coelioxys OR colletes OR colletidae	19,303

	OR colletinae OR cubitalia OR dasypoda OR dasypodainae OR dioxys OR dufourea OR ensliniana OR eoanthidium OR epeoloides OR epeolus OR eucera OR flavipanurgus OR habropoda OR haetosmia OR halictidae OR halictinae OR halictus OR heriades OR hofferia OR hoplitis OR hylaeniae OR hylaeus OR icteranthidium OR lasioglossum OR lithurgus OR macropis OR megachile OR megachilidae OR megachilinae OR melecta OR melitta OR melittidae OR melittinae OR melitturga OR metadioxys OR (nomada W/10 (bee OR bees)) OR nomadinae OR nomiinae OR nomiapis OR nomioidinae OR nomioides OR osmia OR panurginus OR panurgus OR paradioxys OR parammobatodes OR pasites OR panurginae OR protosmia OR pseudoanthidium OR rhodanthidium OR rhophitinae OR rhophitoides OR rophites OR schmiedeknechtia OR simpanurgus OR sphecodes OR stelis OR stenoheriades OR systropha OR tarsalia OR tetralonia OR tetraloniella OR thrincohalictus OR thyreus OR trachusa OR triepeolus OR xylocopa OR xylocopinae OR bombus OR bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus")	

Web of Science Core Collection (Indexes=SCI, CPI-S, BKCI-S, ESCI)

Date of the search: 10 August 2021

Set	Query	Results
#12	#11 AND PY=(2013 OR 2014 OR 2015 OR 2016 OR 2017 OR 2018 OR 2019 OR 2020 OR 2021)	128
#11	#10 OR #5	187
#10	#9 AND #8	116
#9	TI=(model* OR simulat*)	2,937,860
#8	#6 OR #7	10,575
#7	TI=(pollinator*) AND #1	1,499
#6	TI=(((social OR solitary OR wild) AND (bee OR bees OR pollinator*)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE OR HALICTINAE OR Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada NEAR/10 (bee OR bees)) OR NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B.	9,428

	consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus")	
#5	#3 AND #4	80
#4	TS =(model* OR simulat*)	3,294,798
#3	TS=((((social OR solitary OR wild) NEAR/5 (bee OR bees OR pollinator*)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE OR HALICTINAE OR Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada NEAR/10 (bee OR bees)) OR NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus") NEAR/15 ((population* NEAR/5 (abundan* OR development* OR declin* OR decreas* OR dynamics OR richness OR structure OR variabi*)) OR ((colon* OR intracolon* OR hive OR hives) NEAR/5 (collapse OR development OR growth OR size OR strength))))	533
#2	TS = ((population* NEAR/5 (abundan* OR development* OR declin* OR decreas* OR dynamics OR richness OR structure OR variabi*)) OR ((colon* OR intracolon* OR hive OR hives) NEAR/5 (collapse OR development OR growth OR size OR strength)))	265,008
#1	TS=(((social OR solitary OR wild) NEAR/5 (bee OR bees OR pollinator*)) OR Afranthidium OR Aglaoapis OR Amegilla OR Ammobates OR Ammobatoides OR Ancyla OR Andrena OR ANDRENIDAE OR ANDRENINAE OR Anthidiellum OR Anthidium OR Anthophora OR APIDAE OR APINAE OR Biastes OR Camptopoeum OR Ceratina OR Ceylalictus OR Chelostoma OR Chiasmognathus OR Clavipanurgus OR Coelioxys OR Colletes OR COLLETIDAE OR COLLETINAE OR Cubitalia OR Dasypoda OR DASYPODAINAE OR Dioxys OR Dufourea OR Ensliniana OR Eoanthidium OR Epeoloides OR Epeolus OR Eucera OR Flavipanurgus OR Habropoda OR Haetosmia OR HALICTIDAE	18,339

	OR HALICTINAE OR Halictus OR Heriades OR Hofferia OR Hoplitis OR HYLAENIAE OR Hylaeus OR Icteranthidium OR Lasioglossum OR Lithurgus OR Macropis OR Megachile OR MEGACHILIDAE OR MEGACHILINAE OR Melecta OR Melitta OR MELITTIDAE OR MELITTINAE OR Melitturga OR Metadioxys OR (Nomada NEAR/10 (bee OR bees)) OR NOMADINAE OR NOMIINAE OR Nomiapis OR NOMIOIDINAE OR Nomioides OR Osmia OR Panurginus OR Panurgus OR Paradioxys OR Parammobatodes OR Pasites OR PANURGINAE OR Protosmia OR Pseudoanthidium OR Rhodanthidium OR RHOPHITINAE OR Rhophitoides OR Rophites OR Schmiedeknechtia OR Simpanurgus OR Sphecodes OR Stelis OR Stenoheriades OR Systropha OR Tarsalia OR Tetralonia OR Tetraloniella OR Thrincohalictus OR Thyreus OR Trachusa OR Triepeolus OR Xylocopa OR XYLOCOPINAE OR Bombus OR Bumblebee* OR "Bumble bee*" OR "B. alpinus " OR "B. argillaceus " OR "B. armeniacus " OR "B. balteatus " OR "B. barbutellus " OR "B. bohemicus " OR "B. brodmannicus " OR "B. campestris" OR "B. cingulatus " OR "B. confusus " OR "B. consobrinus" OR "B. cryptarum" OR "B. cullumanus " OR "B. deuteronymus" OR "B. distinguendus" OR "B. flavidus " OR "B. fragrans " OR "B. gerstaeckeri " OR "B. glacialis " OR "B. haematurus " OR "B. hortorum " OR "B. humilis " OR "B. hyperboreus " OR "B. hypnorum " OR "B. inexspectatus " OR "B. jonellus" OR "B. laesus" OR "B. lapidarius" OR "B. lapponicus" OR "B. lucorum" OR "B. magnus" OR "B. mendax" OR "B. mesomelas" OR "B. mlokosievitzii" OR "B. mocsaryi" OR "B. modestus" OR "B. monticola" OR "B. mucidus" OR "B. muscorum" OR "B. niveatus" OR "B. norvegicus" OR "B. pascuorum" OR "B. patagiatus" OR "B. perezi" OR "B. pereziellus" OR "B. polaris" OR "B. pomorum" OR "B. pratorum" OR "B. pyrenaeus" OR "B. quadricolor" OR "B. reinigiellus" OR "B. ruderarius" OR "B. ruderatus" OR "B. rupestris" OR "B. saltuarius" OR "B. schrencki" OR "B. semenoviellus" OR "B. sichelii" OR "B. soroeensis" OR "B. sporadicus" OR "B. subterraneus" OR "B. sylvarum" OR "B. sylvestris" OR "B. terrestris" OR "B. vestalis" OR "B. veteranus" OR "B. wurflenii" OR "B. zonatus")	

Appendix B – Analysis of variability in bumble bee and solitary bee field study control data

B.1. Introduction

The normal operating range (NOR) provides an indication of the range of 'background variability' of colony strength (for bumble bees) and population abundances (for solitary bees), which can be used as a baseline to understand what magnitude of effects following exposure to a pesticide would be relevant.

The NOR was used to inform the Specific Protection Goal (SPG) for honey bees (EFSA et al., 2021). The honey bee SPG (i.e. threshold of acceptable effects) is based on the background variability in colony strength (measured as the number of adult bees in the colony), calculated as the distance between the mean and lower end of the operating range. Model simulations were performed for 19 different scenarios (i.e. different locations in the EU). The variability in size between replicate colonies (i.e. the colonies at each EU scenario) at every measurement time point has been quantified as the coefficient of variation (CV). CVs of model simulations and field data for honey bees have been compared, indicating that field data are more variable than model simulations.

Similarly, the concept of the NOR could also be used to inform the SPG for bumble bees and solitary bees. As explained in Section 3.1 of the main document, there are currently no models available for bumble bees and solitary bees that can be recommended for use in a similar way as was done for honey bees. However, data from field studies are available, which could potentially provide information on the variability of colony strength and population abundance, as an indicator for the NOR.

This appendix describes the analysis the Working Group undertook on the available field data, and that aimed at investigating the background variability in those data. As a basis for this analysis, the following methodological approach was adopted:

1) Identify (measurable) parameters related to the attributes of the Specific Protection Goals (SPGs) for bumble bees (colony strength) and solitary bees (population abundance).
2) Screen the field studies with bumble bees and solitary bees available in the dataset from the assessment EFSA carried out for three neonicotinoid active substances (EFSA, 2018a,b,c), from this point forwards the EFSA neonicotinoid dataset, and published literature for data on these parameters that could be used to inform on the variability.
3) Extract useful data.
4) Calculate the variability of the parameters: between different colonies in the same field for bumble bees; between different fields for solitary bees. Variability is expressed in terms of the coefficient of variation (CV), i.e. the standard deviation divided by the mean.

Below, the outcome of this analysis is presented for bumble bees and solitary bees separately.

B.2. Bumble bees

B.2.1. Endpoints considered as informative for the SPG for bumble bees

As described in Section 2.2 of this document, the current ecological entity dimension as defined for the SPG for bumble bees is the bumble bee colony, and the attribute considered is the colony strength (defined as the number of adults, analogue to the honey bee SPG). The current exercise only focuses on the review of the magnitude dimension, to define a threshold of acceptable effects.

In the context of the current exercise, the key question for the bumble bee SPG that was considered was the following, 'Can colonies grow strong enough to provide pollination services AND produce new queens to be able to establish new colonies the following season?'

From the above question, it follows that informative and operable endpoints would be those that relate to colony strength (i.e. number of workers, number of adult bees, colony weight, number of intact cocoons, number of emerged cocoons), colony reproduction (number of new queens, number of new males, number of intact queen cocoons, number of emerged queen cocoons), queen overwintering survival and queen nest initiation success the following season.

This is generally in line with the recommendations from Cabrera et al. (2016), who also suggested the changes in colony weight over time and the mean number of queens produced per colony as two of the most meaningful biological parameters for assessing the risk of pesticides to bumble bees. In addition, these authors also suggested the mean mass of new queens produced per colony, the proportion of colonies producing new queens and the timing of foundress queen mortality as meaningful parameters.

B.2.2. Data and literature identification and screening

Relevant data would be obtained from bumble bee colonies or queens in a field setting, i.e. colonies or bees reared throughout their life cycle in controlled laboratory conditions would not be relevant, to capture the performance of colonies and bees under environmentally realistic conditions.

The dataset assembled for the review of the neonicotinoids (EFSA, 2018a,b,c) through an open call for data and systematic literature search, was used for the present analysis. This EFSA neonicotinoid dataset (EFSA, 2018a,b,c) contained 13 potentially useful references, which in some cases encompassed studies on two bee species and in multiple locations. In total, 24 experiments were available, 13 of which were conducted with bumble bees.

In addition, a literature screening was performed (using the search string "Bombus AND (queen production OR colony development)"), which yielded some papers on bumble bees. None of these papers contained any additional useful data. Five of the identified studies were publications based on the references/experiments listed in the EFSA neonicotinoid dataset. A more extensive and systematic literature search on bumble bee field studies could not be performed due to time constraints.

Following the information session held on 23 November 2021 for Member States and stakeholders, one additional dataset was submitted by the Julius Kuhn Institute (JKI) in Germany.

The studies considered were all field experiments testing the effects of a pesticide. These studies contain both control and treated fields. As we are interested in the background variability, only the data for the controls were extracted, as bees in the controls were not exposed to the pesticide under investigation.

The criteria for exclusion as listed in Table B.1. were used to decide whether a study contained useful data for the current exercise. From these exclusion criteria, it follows that only studies that contained raw data were included, and had no indication that bees were contaminated with the test item in the control fields, and contained more than one (multi)hive per site.

Table B.1.: Exclusion criteria applied for the screening of the data and studies performed with bumble bees

A study was excluded when:	Explanation
Raw data not available	The raw data are essential to perform the analysis
Indication of contamination with the test item in the controls	The studies considered were all field experiments testing the effects of a pesticide. These studies contain both control and treated fields. To ensure that the background variability under untreated conditions was captured, studies were excluded when there was an indication that the bees in the control fields had been exposed to the test item. Please note that some studies included bumble bees, solitary bees and honey bees. In some studies, control honey bees were contaminated, and the studies were therefore excluded from the analysis of background variability performed for honey bees (EFSA et al., 2021). However, other control bees were not, and the studies could be retained in the current analysis
Study performed outside Europe	The aim is to inform the SPG for the European risk assessment, i.e. it is necessary to look at the background variability under European conditions. Therefore, studies were excluded if they were performed outside Europe even if they were performed with species also occurring in Europe
Only one colony per site	A colony is the unit of interest. Data on more than one colony at the same site are therefore needed to study variability

Colony feeding during field placement (including monitoring phase) before endpoint measurement	Artificial feeding during the experiment is considered to confound the background variability in colony dynamics. If artificial feeding took place only for a very limited amount of time, such as during transport between the treated and a monitoring area, it was accepted. Artificial feeding under the rearing and before field placement were not reasons for exclusion

Tables B.2, B.3 and B.4 give an overview of the bumble bee studies in the EFSA neonicotinoid dataset, those obtained from the public literature, and the additional dataset submitted by the JKI, respectively, together with the rationale for including or excluding the study.

Finally, four studies from the EFSA neonicotinoid dataset (RefID 1184, 1342, 1513 and 1525) were identified as containing useful data for the current exercise. These four studies in total contained six suitable datasets (ExperimentID C.2065, C.1342, T.1513, C+T.2013G, C+T.2013H, C+T.2013U). In addition, four studies from the public literature were identified as useful. The data in these studies were mostly those from ExperimentID C.2065, RefID1525 and RefID1342 from the EFSA neonicotinoid dataset and therefore they were considered together. The additional dataset submitted by the JKI met the inclusion criteria. In total, there were therefore seven datasets for bumble bees. All of them used *B. terrestris* as the test species. The studies were conducted in Germany, Hungary, Sweden and UK. Datasets contained 2–8 fields, with 2–25 colonies or multicolony units per field.

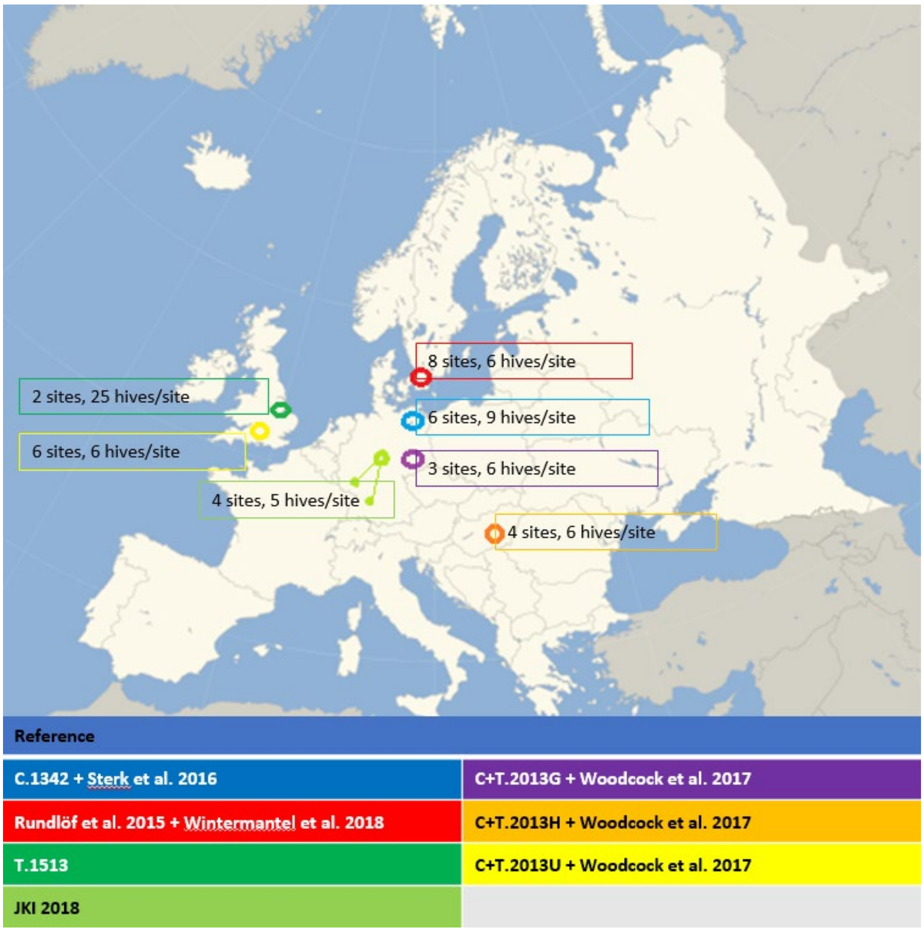


Figure B.1: Overview of the location of the bumble bee field studies considered, the number of sites and the number of hives/site for each study

Table B.2.: Overview of the field studies on bumble bees available in the EFSA neonicotinoid dataset (details on RefID, ExperimentID and Evaluation note file can be found in EFSA, 2018a,b,c). All studies were performed with *B. terrestris*

RefID	ExperimentID	Evaluation note file	Country	Exclude or include	Reasons for exclusion	Sites	Colonies per site
1342	C.1342	C.Field.pdf	Germany	Include	NA	6	9 (3 multihives with 3 single hives each)
357	C.2033	C.Field.pdf	Germany	Exclude	Colonies kept in the laboratory in the post-exposure phase; no raw data per replicate, only box-and-whisker plots; contamination with the target substance in the control		
357	C.2032	C.Field.pdf	Germany	Exclude	Colony feeding in the post-exposure phase; no raw data per replicate, only box-and-whisker plots; contamination with the target substance in the control		
357	C.2034	C.Field.pdf	Germany	Exclude	No raw data per replicate, only box-and-whisker plots; contamination with the target substance in the control		
1184	C.2065	C.Field.pdf	Sweden	Include	NA	8	6 (4 dissection)
311	C.311	C.Monitoring.pdf	Canada	Exclude	One multihive per field; no raw data per hive; bees were given sugar solution (test crop maize)		
1388	I.1388	I.Field.pdf	France	Exclude	Hives were in the field from 9–17 July and were then moved to the laboratory where they received syrup and pollen paste, effect measurements were taken after 17 days in the laboratory		
724	I.724	I.Field.pdf	Germany	Exclude	Bees were provided with sugar solution (test crop potato)		
725	I.725	I.Field.pdf	Germany	Exclude	Bees were provided with sugar solution (test crop potato)		
1513	T.1513	T.field.pdf	UK	Include	NA	2	25
1525	C+T.2013G	C+T.Field	Germany	Include	NA	3	6 (2 multihives with 3 single hives each)
1525	C+T.2013H	C+T.Field	Hungary	Include	NA	4	6 (2 multihives with 3 single hives each)
1525	C+T.2013U	C+T.Field	UK	Include	NA	6	6 (2 multihives with 3 single hives each)

C.: clothianidin; I.: imidacloprid; T.: thiamethoxam. NA: not applicable.

Table B.3.: Overview of the field studies on bumble bees obtained from the public literature following a quick search (using the search string "Bombus AND (queen production OR colony development)"). All studies were performed with *B. terrestris*

Author	Year	Title	Journal	Vol.	Pages	Comment	Species	Exclude or include	Reasons for exclusion
Sterk G, Peters B, Gao Z, Zumkier U	2016	Large-scale monitoring of effects of clothianidin-dressed OSR seeds on pollinating insects in Northern Germany: effects on large earth bumble bees (*B. terrestris*)	Ecotoxicology	25	1666–1678	Follow-up paper of RefID 1342	*Bombus terrestris*	Include	NA
Wintermantel D, Locke B, Andersson GKS, Semberg E, Forsgren E, Osterman J, Rahbek Pedersen T, Bommarco R, Smith HG, Rundlöf M, de Miranda J	2018	Field-level clothianidin exposure affects bumble bees but generally not their pathogens	Nature Communications	9	5446	Follow-up analysis of partly the same data as in RefID 1184, ExperimentID C.2065	*Bombus terrestris*	Include	NA
Hermann JD, Haddad NMN Levey DJ	2018	Mean body size predicts colony performance in the common eastern bumble bee (*Bombus impatiens*)	Ecological Entomology	43	458–462	-	*Bombus terrestris*	Exclude	N-American study
Rundlöf M, Andersson GKS, Bommarco R, Fries I, Hederström V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR, Yourstone J, Smith HG	2015	Seed coating with a neonicotinoid insecticide negatively affects wild bees	Nature	521	77-U162	Are RefID 1184 and ExperimentID C.1184 and C.2065, already evaluated by EFSA	*Bombus terrestris*	Include	NA
Woodcock BA, Bullock JM, Shore RF, Heard MS, Pereira MG, Redhead J, Ridding L, Dean H, Sleep D, Henrys P, Peyton J, Hulmes S, Hulmes L, Sárospataki M, Saure C, Edwards M, Genersch E, Knäbe S, Pywell RF	2017	Country-specific effects of neonicotinoid pesticides on honey bees and wild bees	Science	356	1393–1395	Follow-up paper of RefID 1525	*Bombus terrestris*	Include	NA

NA: not applicable.

Table B.4.: Overview of the field study on bumble bees (*B. terrestris*) submitted by the Julius Kuhn Institute following the info session held on 23 November 2021 for Member States and stakeholders

Country	Year	Exclude or include	Reasons for exclusion	Species	Colony groups	Colonies per group
Germany	2018	Include (in part)	Following the field exposure phase, the colonies were transferred to the laboratory for approximately 25 days to ensure that all available brood will be hatched and then all colonies were frozen for dissection. During the post-exposure phase in the laboratory, the bees were fed with sugar water and pollen. Therefore, the data on the number of bees, counted at the end of the study (after the laboratory phase) cannot be used. However, the data on colony weight obtained during the field exposure phase, during which no feeding occurred, are suitable, and are therefore included.	*Bombus terrestris*	4	5

B.2.3. Data analysis

B.2.3.1. Included endpoints

From the seven datasets that were identified as useful, data were available for the following endpoints: colony weight, number of workers, number of queens, number of drones, total number of adults (workers + queens + drones). Note that for one study (JKI, 2018), only the data on colony weight could be included (see Table B.4)

The number of workers is the endpoint that is most closely related to that used to estimate the colony strength for honey bees. However, for bumble bees, the total number of adults (i.e. the sum of workers, queens and males) is probably more relevant to define the colony strength than the number of workers alone, as bumble bees rely on annual reproduction for establishing new colonies in the next year.

In the current document, results are presented for the endpoints number of workers, number of adults and colony weight. The WG considers the number and weight of reproductive (drones and/or queens) also relevant endpoints. Analyses for these may be considered for the requirements of bumble bee field studies. Note that preliminary calculations indicated that for the number of queens and drones the variability is likely to be higher compared with the number of workers and adults.

Data on the number of bees (workers, drones and queens; separately or summed) is generally available for the start of the experiment, and for the final sampling at the end of the experiment, only. At the study end, the colonies are generally terminated by freezing, and subsequently dissected for a detailed count on the number of bees and cells. Only for C.1342, the number of bees was also counted on multiple occasions during the study. It should however be noted that in this case, a photograph of the colony was taken on each sampling occasion, and the number of bees was estimated based on a categorisation. Therefore, these intermediate values for the number of bees are less accurate than those for the end of the experiment.

Colony weight was the only parameter that was measured on multiple occasions throughout the study in all datasets. This is because it is an easy parameter to measure, and a non-invasive measurement (i.e. colony disturbance is limited). In addition, Lefebvre and Pierre (2006) investigated the correlation between the weight of a bumble bee colony and the number of bees. These authors weighed and counted the number of adult bees in nine *B. terrestris* colonies from GIE La Croix (Brittany, France) every other day for 30 days in spring, starting at 4 weeks of colony age. The colonies were not food supplemented and foraged freely in an experimental area at the INRA Centre of Le Rheu (Brittany, France). Lefebvre and Pierre (2006) state that '4-week-old "standard commercial hives"' contained on average 42.3 ± 1.9 (SE) bumblebees' and that the weight of such hives was markedly 'homogeneous (1104.4g ± 6.4), but variation was larger at the end of the monitoring period'. Correlation between weight and bees was on average 0.82 ± 0.06 (Spearman rank correlation coefficient, range 0.49–0.97). Based on these findings, Lefebvre and Pierre (2006) concluded that 'when colonies are not supplemented with syrup fed inside hives, the weight is a useful indicator of the population growth of bumble bee colonies'. Therefore, the WG considers colony weight to be a relevant endpoint to investigate the colony strength and its variability in bumble bee colonies. Colony weight is especially useful to investigate the variability over time.

It should be noted that, generally, the bumble bee colonies were kept in plastic nest boxes, and were also weighed as such. Therefore, in most studies, the weight reported is the sum of the actual colony (i.e. the cells, larvae, eggs, food stores and adults) and the plastic box. Therefore, when this was the case, the weight data were corrected for the plastic box weight. In most studies, the weight of the plastic nest boxes used was not reported. However, experience within the WG has shown that, generally, the weight of the plastic boxes used is relatively constant. The average weight from a batch of 94 cleaned plastic nest boxes from BioBest used in an experiment in 2019 was 382 g (±3.7 g SD; M. Rundlöf, pers. comm.). This value of 382 g was used to correct the weight data, when necessary.

It should also be noted that in some studies (e.g. RefID 1342 and RefID 1525) three bumble bee colonies (each in an individual plastic box) were placed together in a larger polystyrene or plastic box, a so-called 'multihive'. The three colonies in a multihive are not fully independent units, as there might be some transfer of the worker bees between the colonies in the multihive during the course of the test. However, each individual colony was assessed separately (e.g. weighed separately, separate counts of the number of bees) in both RefID 1342 and RefID 1525. Therefore, as data for each colony separately are available, the assessment of the variability (see Section B.2.3.3) was also performed using the individual colony data.

B.2.3.2. Estimation of the colony age

The intention was to investigate the variability in colony strength over time. Given that bumble bee colonies have a typical developmental pattern (i.e. starting from the colony initiation by the queen, up until the moment that the colony dies and the newly produced queens overwinter), the relevant time unit is considered to be the colony age, rather than the calendar date. However, for most available studies, the colony age at the start of the study was not reported as such. Instead, the number of worker bees in the colony at the start of the study was given. Therefore, as a first step, the age of the colonies at the start of the respective studies was estimated based on the relationship between the number of bees and the colony age empirically derived by Duchateau and Velthuis (1988).

Duchateau and Velthuis (1988) studied the number of workers in 21 *Bombus terrestris terrestris* colonies until the competition point [i.e. the day on which at least one of the following characteristics was seen for the first time: (1) the presence of more than one open eggcup at a time, indicating that next to the queen a worker is preparing for oviposition; (2) the oviposition by a worker; (3) the eating of eggs; and (4) aggression between the queen and some of the workers or among workers]. Their results were presented in figure 1 in their paper. Colonies were kept in wooden nest boxes in a climate room. All colonies were provided with pollen in their nest boxes. Six colonies also received diluted honey in their nest boxes, six other colonies were allowed to collect diluted honey in flight cages. Nine other colonies could forage for nectar and pollen in the field, and were no longer fed as soon as the first workers foraged outside. Every day, the colony development (egg cells, larval groups, cocoons and adults) was recorded. Newly emerged bees were marked with identity tags. The data from this study are considered useful to estimate colony age or colony worker number for other *B. terrestris* studies. The numbers in Table B.5 were estimated by the Working Group from figure 1 in the paper by Duchateau and Velthuis (1988).

Table B.5.: Number of workers for *B. terrestris* colony age 0–55 days, estimated from figure 1 of Duchateau and Velthuis (1988)

Colony age (days)	No. of workers
0–21	0
22	2
23	4
24	6
25	7
26	8
27	9
28–35	10
36	12
37	15
38	18
39	21
40	26
41	29
42	33
43	37
44	40
45	43
46	47
47	51
48	58
49	63
50	69
51	78

52	86
53	95
54	105
55	116

B.2.3.3. Assessment of the variability

The variability between replicate colonies within a field site, for each of the suitable studies was quantified as the CV, i.e. the standard deviation divided by the mean. The CV values were calculated for each endpoint, and for every measurement time point for which data were available.

B.2.4. Results and discussion

The variability, quantified as CV, in relation to the colony age (days since colony initiation, i.e. first egg laid by the founding queen) is shown in the Figures B.1–B.3 for number of workers, number of adults and colony weight, respectively.

For a first assessment, the CV values were plotted for each study separately. This is because there were important differences between the studies regarding the age of the colonies at the start of the test. Generally, colonies are standardised as much as possible at the start of the study and therefore, e.g. the number of adult bees in the colonies, will be comparable. As a consequence, the variability at the start of the study is relatively low compared with any timepoint later in the study. Therefore, the variability at a colony age of e.g. 70 days will be higher in a study that was initiated with 40-day-old colonies compared with a study that was initiated with 70-day-old colonies. Consequently, the colony age at test start should be taken into account when comparing the variability between studies.

It is noted that in some cases (e.g. the last time point of study C.1342 in Figure B.1) some of the CV values are close to or above 100%. This is an indication of an underlying skewed distribution; indeed, examination of the data for some of those cases shows that the CV values would be more correctly calculated based on an underlying lognormal distribution (instead of a normal distribution as was done here). As there are only a few such cases, however, the CV values shown were all calculated with the same underlying assumptions to preserve consistency in the overview.

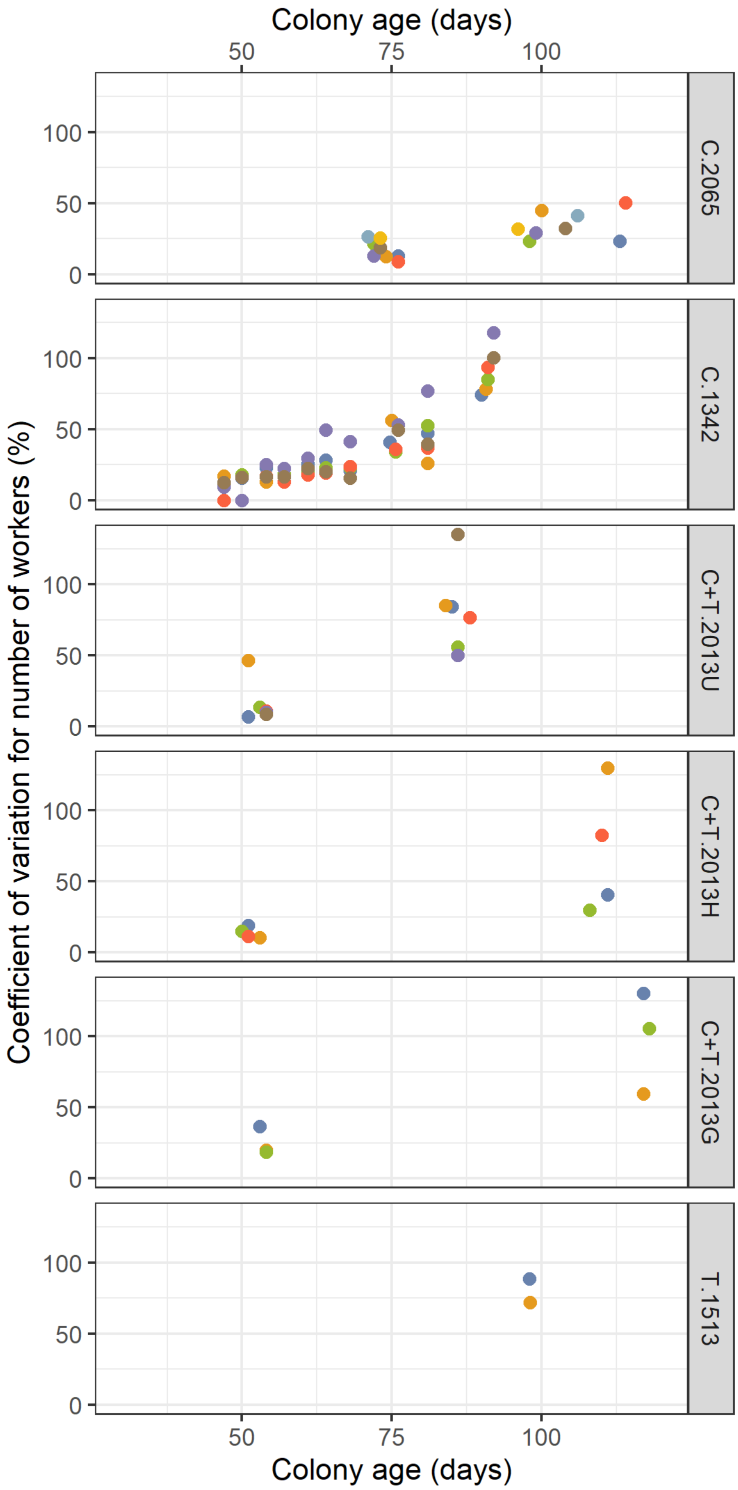
Figures B.2–B.4 show that different studies start in different parts of the colony cycle and have a different length and frequency of assessment. The plots further give some indications on the data richness (or poorness) of the different endpoints measured.

As stated above, colonies are standardised as much as possible at the start of the study. In most of the available studies, this was done using colonies originating from sister queens, of the same age and with approximately the same number of bees at the start of the test. For the study by JKI (2018), however, the colonies used were standard colonies obtained from a commercial supplier. While the colony age was approximately the same for all colonies used, not all queens were sister queens. Consequently, there was a higher variability in the number of workers at the start of the test (data not shown) compared with the other studies in the dataset. This also translated in a higher variability in the colony weight at the test start compared with the other studies.

From the results for the CV for the colony weight, two general trends can be observed:

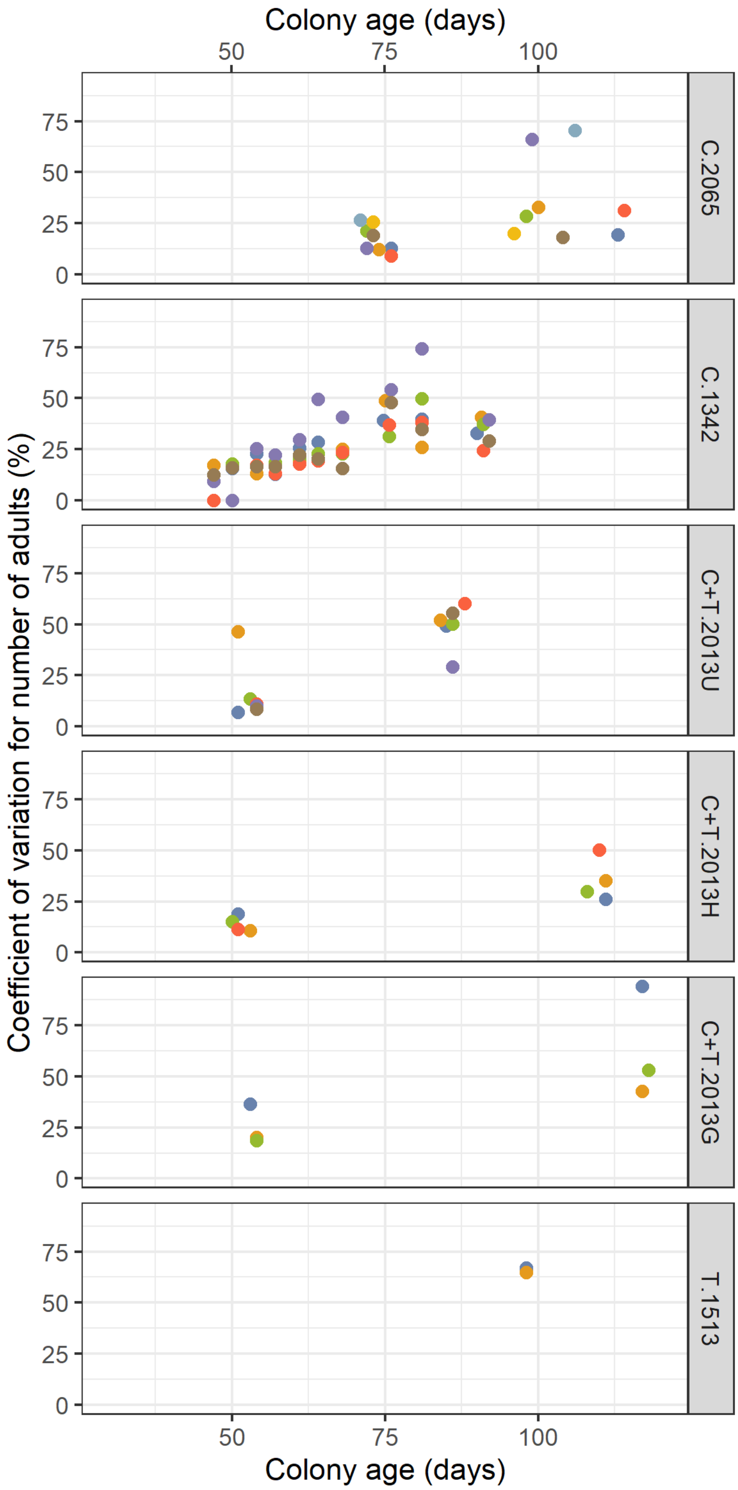
1) The CV among endpoints and studies vary. While in some studies the colony weight was relatively similar among replicates, in other studies this was not the case.
2) The CV tends to increase with time. This is likely to happen because at the beginning of field studies, the colonies are standardised as much as possible, to have more meaningful comparisons.

The first trend is also observed for the number of workers/adults, although for these endpoints, the variability tends to be higher compared with the variability for colony weight. Although the number of studies with data on the number of bees for multiple assessment points is limited, the second trend of increasing CV with time can also be observed for the available data for the number of workers/adults.



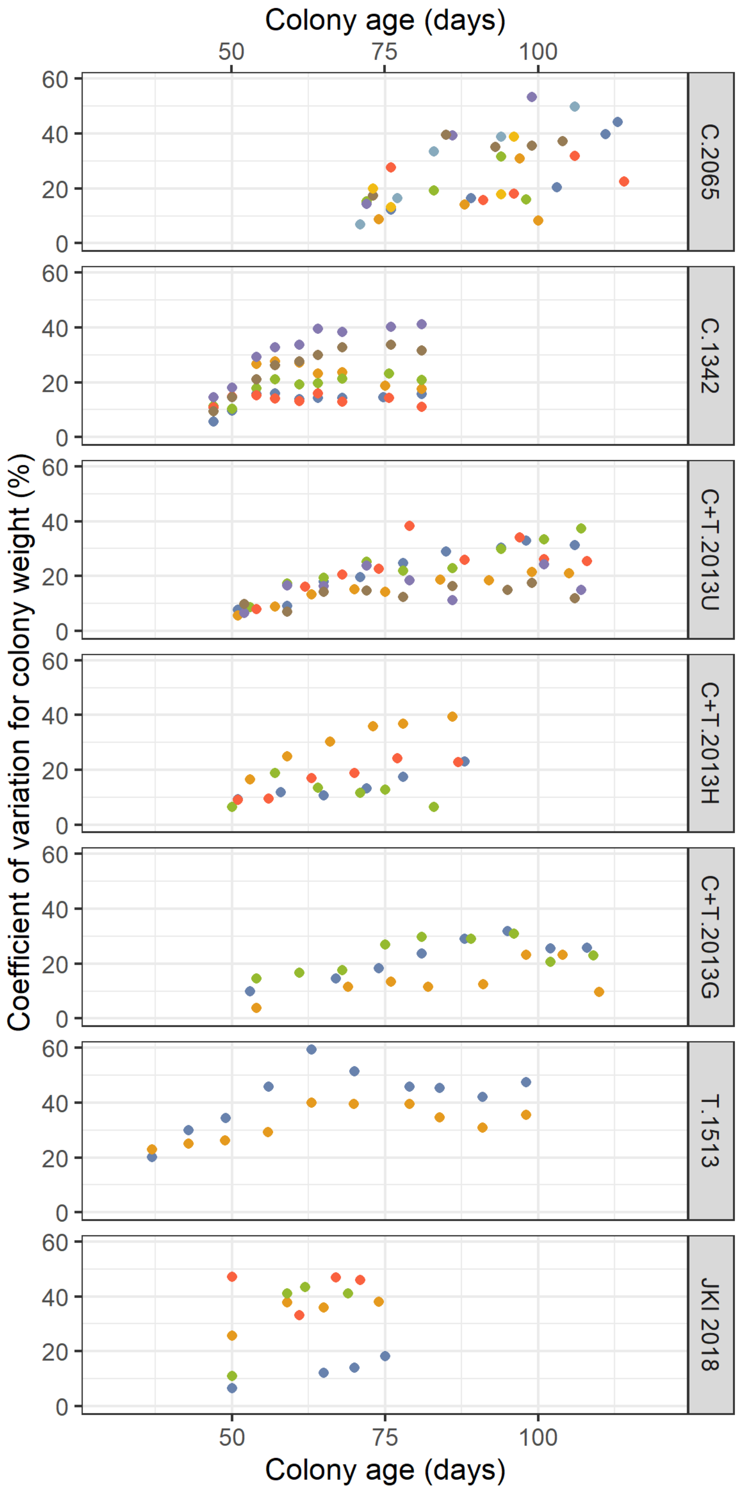
Each panel shows the results of a different study (study ID on the right); multiple fields in the same study are indicated by different colours.

Figure B.2: Coefficient of variation (%) for the number of workers in the different available bumble bee studies



Each panel shows the results of a different study (study ID on the right); multiple fields in the same study are indicated by different colours.

Figure B.3.: Coefficient of variation (%) for the number of adults in the different available bumble bee studies

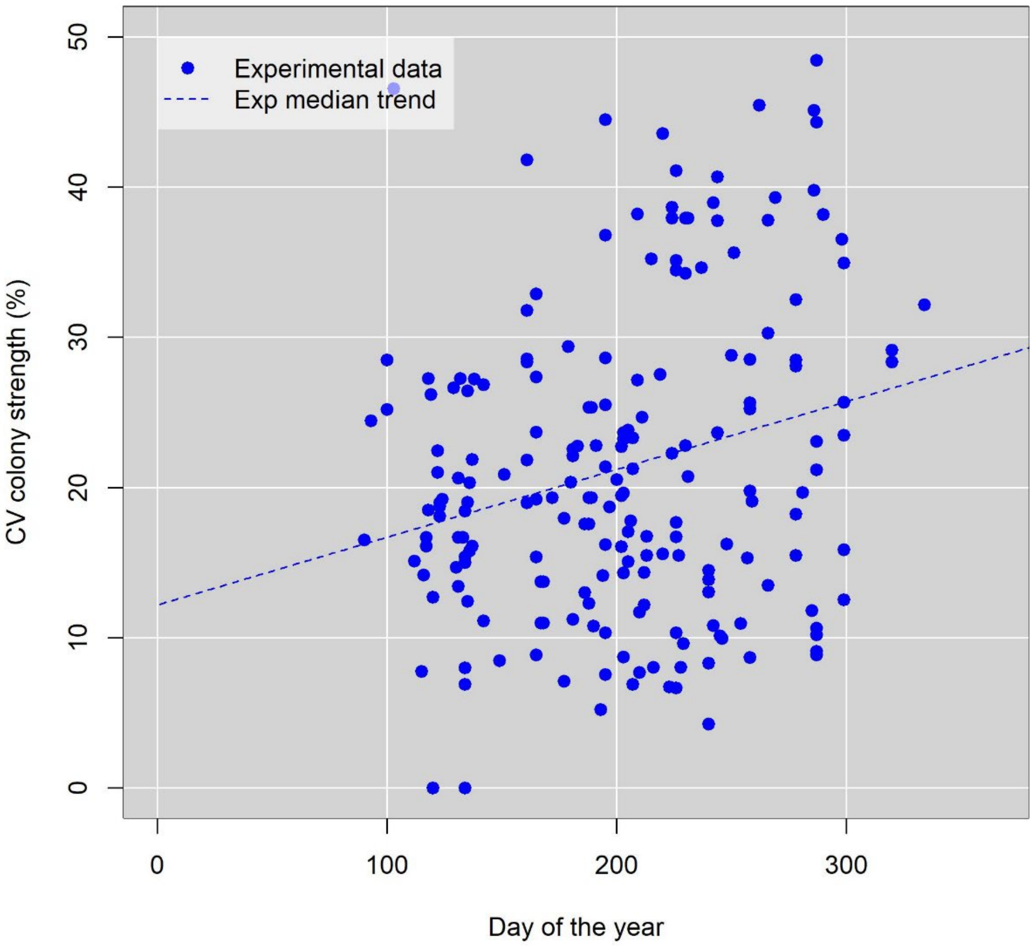


Each panel shows the results of a different study (study ID on the right); multiple fields in the same study are indicated by different colours.

Figure B.4.: Coefficient of variation (%) for colony weight in the different available bumble bee studies

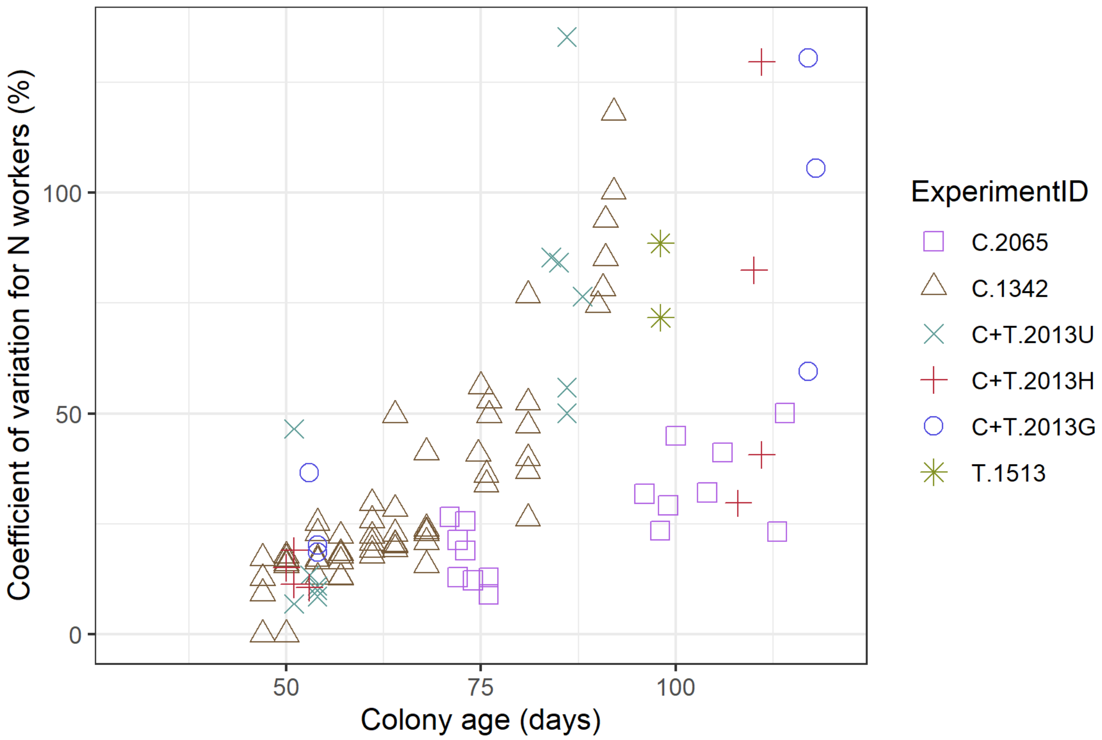
These two general trends were also observed based on field study data with honey bees (as reported in the Technical report on the analysis of background variability of honey bee colony size; EFSA et al., 2021). Comparing the CV values for colony size obtained from honey bee field data (see Figure B.5, which corresponds to figure 22 from EFSA, 2021) with the CV values for bumble bee colony weight and number of workers for all available datasets combined (see Figures B.6 and B.7, respectively), indicates that at least the CV values for bumble bee colony weight are in the same range of the CV values for honey bee colony size. This could give some indication that the background variability for bumble bee colony weight is comparable with that for honey bee colony strength.

It should be noted that experience with bumble bee field testing is still relatively limited and harmonised test protocols are not available yet. Consequently, there are differences in study setup between the different datasets. The variation in test methods reflects the attempts of the scientific community to investigate how field testing could be done. It should be kept in mind that this variation in study setup is a source of variability in itself, and could explain differences in CV values between different studies.



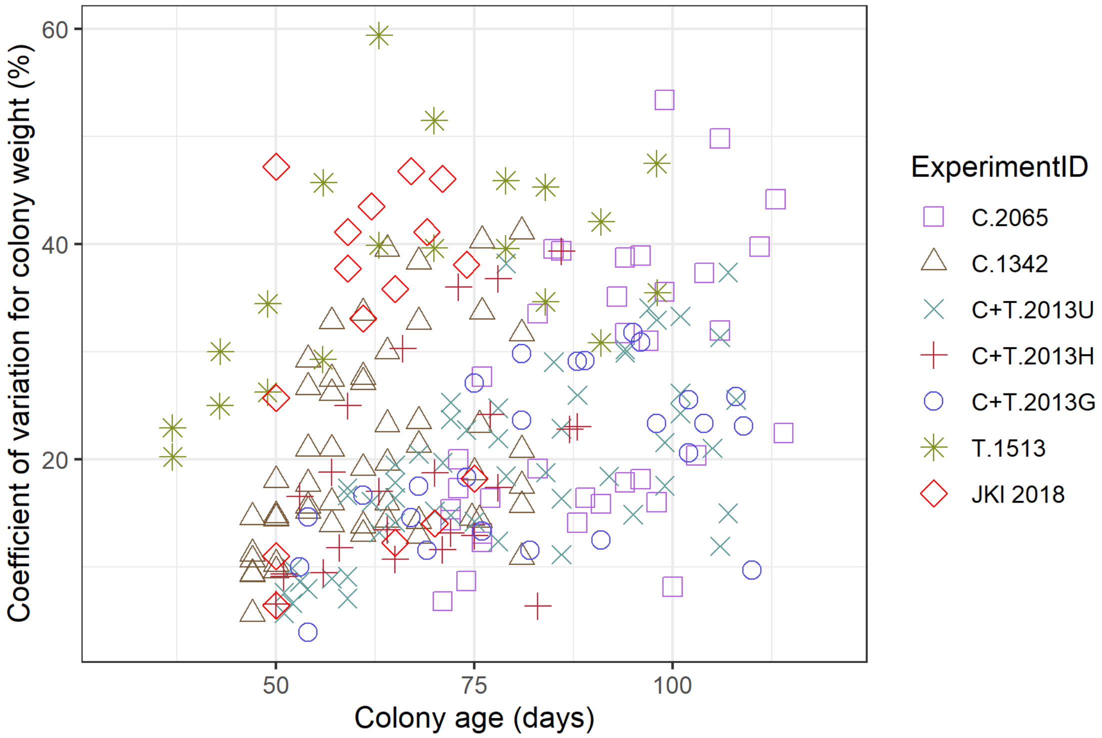
The variability is quantified as coefficient of variation (CV) across all the available replicates. The dashed lines illustrate the median tendency for the experimental variability in time.

Figure B.5.: Variability in honey bee colony strength (as total number of adult bees) based on data from field studies (circles) (data presented in figure 22 in EFSA et al., 2021).



The data are the same as in Figure B.1.

Figure B.6.: Variability in bumble bee colony strength (as total number of workers) based on data from all available field studies



The data are the same as in Figure B.3.

Figure B.7.: Variability in bumble bee colony strength (as colony weight) based on data from all available field studies

B.2.5. Conclusions for bumble bees

Although the number of suitable studies available is limited, and data are only available for one bumble bee species (*B. terrestris*), the present exercise indicates the following:

- The number of workers or adults would be the closest to the SPG attribute of colony strength, but the colony weight is a highly correlated endpoint that can be measured accurately and non-invasively over time and throughout the colony dynamics.
- The results for the CV for bumble bee colony weight seem to be generally in line (in terms of range of the values) with those for colony strength (number of adult bees) for honey bee field studies. Furthermore, the same two general trends are observed:
    - The CV among endpoints and studies vary.
    - The CV tends to increase with time.

This could give some indication that the background variability for the endpoint bumble bee colony weight is comparable with the honey bee colony strength.

B.3. Solitary bees

B.3.1. Endpoints considered as informative for the SPG for solitary bees

As described in Section 2.2 of the main document, the current ecological entity dimension as defined for the SPG for solitary bees is the population, and the attribute considered is the population abundance. A population is simply defined by individuals of the same species co-occurring in space and time that interbreed. The current exercise only focuses on the review of the magnitude dimension, to define a threshold of acceptable effects.

In the context of the current exercise, the key question for the solitary bee SPG that was considered is the following: Can the (starting) population replace itself? For example, if the starting population consists of 10 female cocoons, will there be at least 10 female cocoons the following season?

From the above question, it follows that informative and operable endpoints would be those that quantify reproductive output in relation to the starting population. The starting population can be quantified as the number of initial female cocoons, emerged females or nesting females. The reproductive output can be quantified as the number of brood cells, number of produced cocoons, number of female cocoons, number of adults (females + males) in the next generation and number of females in the next generation. It is considered most relevant to compare reproductive output related with the same type of variable for the starting population, i.e. number of cocoons produced in relation to the number of female cocoons in the starting population or number of females in the next generation in relation to the number of females in the starting population.

B.3.2. Data and literature identification

Relevant data would be obtained from solitary bee nesting places in a field setting, i.e. bees reared throughout their life cycle in a controlled laboratory condition would not be relevant, to capture the performance of colonies and bees under environmental realistic conditions.

The dataset assembled for the review of the neonicotinoids (EFSA, 2018ab,c) through an open call for data and a systematic literature search, was used for the present analysis. This EFSA neonicotinoid dataset (EFSA, 2018a,b,c) contained 13 potentially useful references, which in some cases encompassed studies on two bee species and in multiple locations. In total, 24 experiments were available, 11 of which were conducted with solitary bees.

In addition, 12 potentially useful studies were identified through Lehmann and Camp (2021), who performed a systematic literature search on solitary bee pesticide exposure-effects investigations. Most of these studies were however publications based on the references/experiments listed in the EFSA neonicotinoid dataset.

Following the information session held on 23 November 2021 for Member States and stakeholders, one additional dataset was submitted by the Julius Kuhn Institute from Germany.

The studies considered were all field experiments testing the effects of a pesticide. These studies contain both control and treated fields. As we are interested in the background variability, only the data for the controls were extracted, as these were not exposed to the target pesticide.

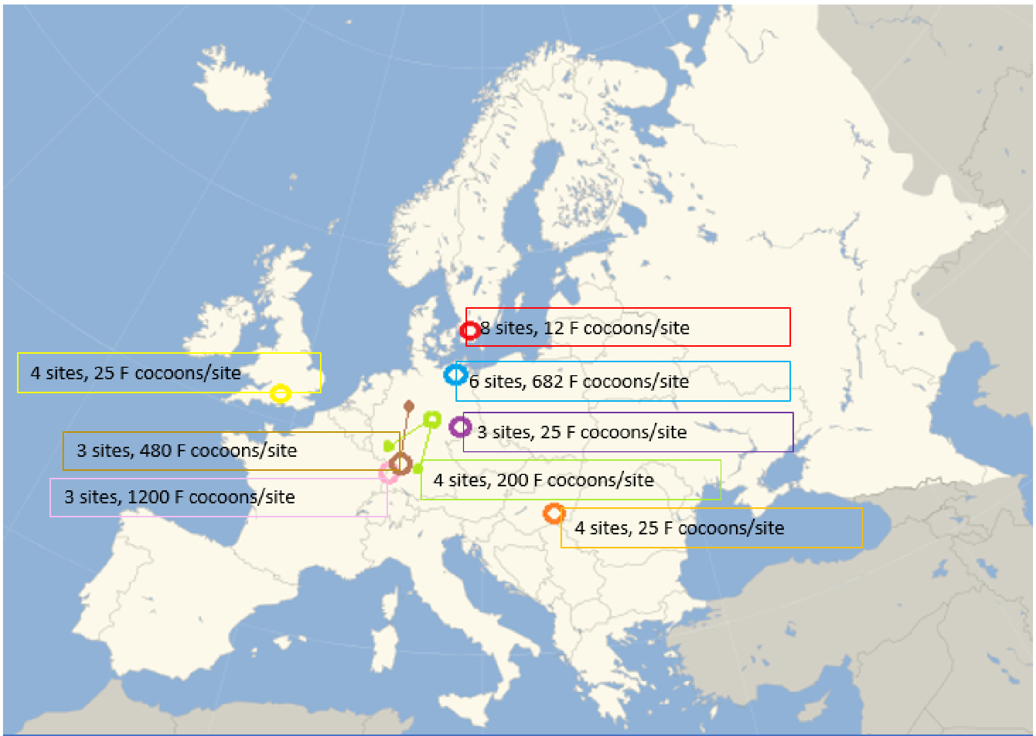
The criteria for exclusion as listed in Table B.6 below were used to decide whether a study contained useful data for the current exercise. From these exclusion criteria it follows that only studies were included that contained the raw data, had no indication that bees were contaminated with the test item in the control fields, and contained more than one site.

Table B.6.: Exclusion criteria applied for the screening of the data and studies performed with solitary bees

A study was excluded when:	Explanation
Raw data not available	The raw data are essential to perform the analysis
Indication of contamination with the test item in the controls	The studies considered were all field experiments testing the effects of a pesticide. These studies contain both control and treated fields. To ensure that the background variability under untreated conditions was captured, studies were excluded when there was an indication that the bees in the control fields had been exposed to the test item. Please note that some studies included bumble bees, solitary bees and honey bees. In some studies, control honey bees were contaminated, and the studies were therefore excluded from the analysis of background variability performed for honey bees (EFSA, 2021). However, other control bees were not, and the studies could be retained in the current analysis
Study performed outside Europe	The aim is to inform the SPG for the European risk assessment, i.e. it is necessary to look at the background variability under European conditions. Therefore, studies were excluded if they were performed outside Europe even if they were performed with species also occurring in Europe
Only one control field	In solitary bee field studies, usually several nest units are placed at one field. However, a population of solitary bees is not defined as all bees that nest in a certain nest site. Even if bees are placed (as cocoons) directly inside an artificial nesting unit, after emergence they can move to other nesting units at the same field (although generally they nest to a high degree in the unit they emerged; Torchio, 1984; Bosch et al., 2002). All bees nesting at (the edge of) a field site are considered to be the population. Therefore, more than one control field is needed to see variability between populations
Female starting population not known	The starting population of females is needed to relate any output. Preferably the number of emerged females is known. If not available, the number of introduced female cocoons or nesting females was considered

Tables B.7, B.8 and B.9 give an overview of the solitary bee studies in the EFSA neonicotinoid dataset, those obtained from the public literature, and the additional dataset submitted by the JKI, respectively, together with the rationale for including or excluding the study.

Finally, three studies from the EFSA neonicotinoid dataset (RefID 1039, 1184 and 1525) were identified as containing useful data for the current exercise. These three studies in total contained five suitable datasets (ExperimentID C.1039, C.1184, C+T.2014G, C+T.2014H, C+T.2014U). In addition, RefID 721–723 (with ExperimentID T.721, T.722, T.723) were published in Ruddle et al. (2018) and these were therefore considered as one dataset. Ruddle et al. (2018) also contained an additional dataset from three fields the following year (referred to further in the current document as Ruddle, 2018D,E,F). In case of deviations between data in Ruddle, 2018 and T.721–723, those in T.721–723 were used for the analysis. Other studies from the public domain were based on the same data as C.1039, C.1184 and C+T.2014G, C+T.2014H, C+T.2014U., and therefore they were considered together. The additional dataset submitted by the JKI met the inclusion criteria. In total, there were therefore eight datasets for solitary bees. All of them used *O. bicornis* (formerly known as *Osmia rufa*) as the test species. The studies were conducted in France, Germany, Hungary, Sweden and UK. Datasets contained 3–8 fields, with 1–8 nesting units per field.



Reference	
T.721,722,723 + Ruddle et al. 2018 (trials from 2014)	C+T.2014G + Woodcock et al. 2017
Ruddle et al. 2018 (trials from 2015)	C+T.2014H + Woodcock et al. 2017
C.1039 + Peters et al. 2016	C+T.2014U + Woodcock et al. 2017
C.1184 = Rundlöf et al. 2015	JKI 2018

Figure B.8.: Overview of the location of the solitary bee field studies considered, the number of sites and the number of female cocoons/site for each study

Table B.7.: Overview of the field studies on solitary bees available in the EFSA neonicotinoid dataset (details on RefID, ExperimentID and Evaluation note file can be found in EFSA, 2018a,b,c). All studies were performed with *O. bicornis*

RefID	ExperimentID	Evaluation note file	Country	Exclude or include	Reasons for exclusion	Sites	Nesting units per site	Introduced female cocoons per site
1039	C.1039	C. Field.pdf	Germany	Include	NA	6	3	682
1184	C.1184	C. Field.pdf	Sweden	Include	NA	8	3	12
357	C.2038	C. Field.pdf	Germany	Exclude	Contamination with the target substance in the control			
357	C.2039	C. Field.pdf	Germany	Exclude	Contamination with the target substance in the control			
357	C.2040	C. Field.pdf	Germany	Exclude	Contamination with the target substance in the control			
721	T.721	T.field.pdf	France	Include	NA[(a)]	1	8	1200
722	T.722	T.field.pdf	Germany	Include	NA[(a)]	1	8	1200
723	T.723	T.field.pdf	Germany	Include	NA[(a)]	1	8	1200
1525	C+T.2014G	C+T.Field	Germany	Include	NA[(b)]	3	2 (1 cocoon placement for both)	25
1525	C+T.2014H	C+T.Field	Hungary	Include	NA[(b)]	4	2 (1 cocoon placement for both)	25
1525	C+T.2014U	C+T.Field	UK	Include	NA[(b)]	4	2 (1 cocoon placement for both)	25

C.: clothianidin, I.: imidacloprid; T.: thiamethoxam. NA: not applicable.

(a): Only one control site (with 8 nesting units) per RefID, however T7.21, T.722 and T.723 were performed in the same year with the same methods and can be considered together so that three replicate sites are available, as also done in Ruddle et al. (2018), see Table B.8.

(b): The study report did not contain information on the number of female cocoons. However, the subsequent publication (Woodcock et al., 2017) state that there was an equal sex ratio for the introduced cocoons, i.e. there were 25 female cocoons at each site.

Table B.8.: Overview of the field studies on solitary bees identified through Lehmann and Camp (2021)

Author	Year	Title	Journal	Vol.	Pages	Comment	Species	Exclude or include	Reasons for exclusion
Ruddle N, Elston C, Klein O, Hamberger A, Thompson H	2018	Effects of exposure to winter oilseed rape grown from thiamethoxam-treated seed on the red mason bee *O. bicornis*	Environmental Toxicology and Chemistry	37	1071–1083	Follow-up analysis of partly the same data as in ExperimentID 721, 722, 723 (performed in 2014), plus data on three additional experiments performed in 2015	*Osmia bicornis*	Include	NA
Peters B, Gao Z, Zumkier U	2016	Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on red mason bees (*Osmia bicornis*)	Ecotoxicology	25	1679–1690	Follow-up paper of RefID 1039	*Osmia bicornis*	Include	NA
Dietzsch AC, Kunz N, Wirtz IP, Stähler M, Heimbach U, Pistorius J	2019	Does winter oilseed rape grown from clothianidin-coated seeds affect experimental populations of mason bees and bumble bees? A semi-field and field study	Journal of Consumer Protection and Food Safety	14	223–238	Follow-up analysis of ExperimentID C.2038, C.2039 and C.2040	*Osmia bicornis*	Exclude	Contamination with the target substance in the control
Abbott VA, Nadeau JL, Higo HA, Winston ML	2008	Lethal and sublethal effects of imidacloprid on *Osmia lignaria* and clothianidin on *Megachile rotundata* (Hymenoptera: Megachilidae)	Journal of Economic Entomology	101	784–796	-	*Megachile rotundata*, *Osmia lignaria*	Exclude	N-American study; no raw data per site (only means and SE); number of control replicates unclear
Johansen CA, Mayer DF, Eves JD, Kious CW	1983	Pesticides and bees	Environmental Entomology	12	1513–1518	–	*Nomia melanderi*	Exclude	N-American study; publication not accessible
Ladurner E, Bosch J,	2008	Foraging and nesting	Journal of	101	647–	–	*Osmia*	Exclude	N-American

Author	Year	Title	Journal	Vol.	Pages	Comment	Species	Exclude or include	Reasons for exclusion
Kemp WP, Maini S		behaviour of *Osmia lignaria* (Hymenoptera: Megachilidae) in the presence of fungicides: cage studies	Economic Entomology		653		*lignaria*		study; cage study (Lehmann and Camp erroneously present this as field study)
Mayer DF, Johansen CA, Shanks CH, Pike KS	1987	Effects of fenvalerate insecticide on pollinators	Journal Entomological Society of British Columbia	84	39–45	–	*Megachile rotundata*	Exclude	N-American study; cage study (Lehmann and Camp erroneously present this as field study)
Mayer DF, Kovacs G, Lunden JD	1998	Field and laboratory tests on the effects of cyhalothrin on adults of *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi*	Journal of Apicultural Research	37	33–37	–	*Megachile rotundata, Nomia melanderi*	Exclude	N-American study; field study only on Megachile; only one control replicate
Piccolomini AM, Flenniken ML, O'Neill KM, Peterson RDK	2018	The effects of an ultra-low-volume application of etofenprox for mosquito management on *Megachile rotundata* (Hymenoptera: Megachilidae) larvae and adults in an agricultural setting	Journal of Economic Entomology	111	33–38	–	*Megachile rotundata*	Exclude	N-American study; only box-and-whisker plots and no raw data per control replicate
Rundlöf M, Andersson GKS, Bommarco R, Fries I, Hederström V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR, Yourstone J, Smith HG	2015	Seed coating with a neonicotinoid insecticide negatively affects wild bees	Nature	521	77–80	Is RefID 1184 and ExperimentID C.1184 and C.2065, already evaluated by EFSA	*Osmia bicornis*	Include	NA
Torchio PF	1983	The effects of field applications of naled and trichlorfon on the alfalfa leaf cutting bee, *Megachile rotundata* (Fabricius)	Journal of the Kansas Entomological Society	56	62–68	–	*Megachile rotundata*	Exclude	N-American study; only one control nest shelter
Woodcock BA, Bullock	2017	Country-specific effects of	Science	356	1393–	Follow-up paper	*Osmia*	Include	NA

Author	Year	Title	Journal	Vol.	Pages	Comment	Species	Exclude or include	Reasons for exclusion
JM, Shore RF, Heard MS, Pereira MG, Redhead J, Ridding L, Dean H, Sleep D, Henrys P, Peyton J, Hulmes S, Hulmes L, Sárospataki M, Saure C, Edwards M, Genersch E, Knäbe S, Pywell RF		neonicotinoid pesticides on honey bees and wild bees			1395	of RefID 1525	*bicornis*		

NA: not applicable.

Table B.9.: Overview of the field study on solitary bees (*Osmia bicornis*) submitted by the Julius Kuhn Institute following the info session held on 23 November 2021 for Member States and stakeholders

Country	Year	Exclude or include	Reasons for exclusion	Species	Sites	Nesting units per site	Introduced female cocoons per site
Germany	2018	Include	NA	*Osmia bicornis*	4	5 (4 at one of the sites)	200 (160 at the site with only 4 nesting units)

B.3.3. Data analysis

B.3.3.1. Included endpoints

From the eight datasets that were identified as useful, data were available for the following endpoints related to the starting population: number of female cocoons in the starting population, number of emerged females in the starting population, number of nesting females in the starting population. In relation to the next generation, data for the following endpoints were available: the number of produced cells, the number of produced (female) cocoons, number of produced emerged adults, number of produced emerged adult females.

As discussed in Section B.3.1, the most informative endpoints for the SPG would be those that quantify reproductive output in relation to the starting population. In addition, not for all of the endpoints listed above, data were equally available from the different datasets. Therefore, taking into account the data availability, and considering the relevance of the endpoints for the SPG, the following four endpoints were considered for further data analysis (the number in brackets represents the number of datasets from which data are available for this endpoint):

- Number of females emerged in the next generation per number of females emerged in the starting population (three datasets)
- Number of female cocoons per introduced female cocoon (three datasets)
- Number of cocoons (both sexes) per introduced female cocoon (four datasets)
- Number of brood cells per introduced female cocoon (seven datasets)

It should be noted that expressing the endpoints relative to the number of introduced female cocoons or the number of emerged females has some potential bias, for the following two reasons: (1) not all females that emerge from the introduced cocoons will nest at the nest boxes provided. A variable percentage of these emerged females will just fly away to nest elsewhere in the landscape; (2) there is a potential for females from the naturally occurring population to nest at the nest boxes provided for the study. Counting the number of nesting females (i.e. those actually nesting at the nest boxes provided) is therefore a more accurate parameter to quantify the starting population. However, from the datasets used, information on the number of nesting females was only available for one study. Therefore, this parameter could not be used to compare the variability between studies.

For the cocoons, both from the starting populations as those from the next generation, the sex ratio was always reported in the study reports. Consequently, the actual number of female cocoons could be calculated. The eclosion success was, however, only reported as an overall percentage of the total number of cocoons (for both sexes together) in most study reports. To be able to calculate the number of emerged females, an equal eclosion success between sexes was assumed.

For solitary bees, there are no endpoints for which data are available over a period, as is the case for honey bees and bumble bees. Instead, there are several endpoints measured only at one timepoint, which relate to population strength and can be compared between studies.

Note however that there is one exception: in some of the studies, there were counts of produced cells in the period directly after application until the end of flowering, e.g. for 15 or 22 consecutive days. As this was done only in a limited amount of studies, there is always an increase over time until the final number of produced cells at the end of the period, and the final number is the most useful as indication of population strength, this increase of cells-over-time is not pursued further.

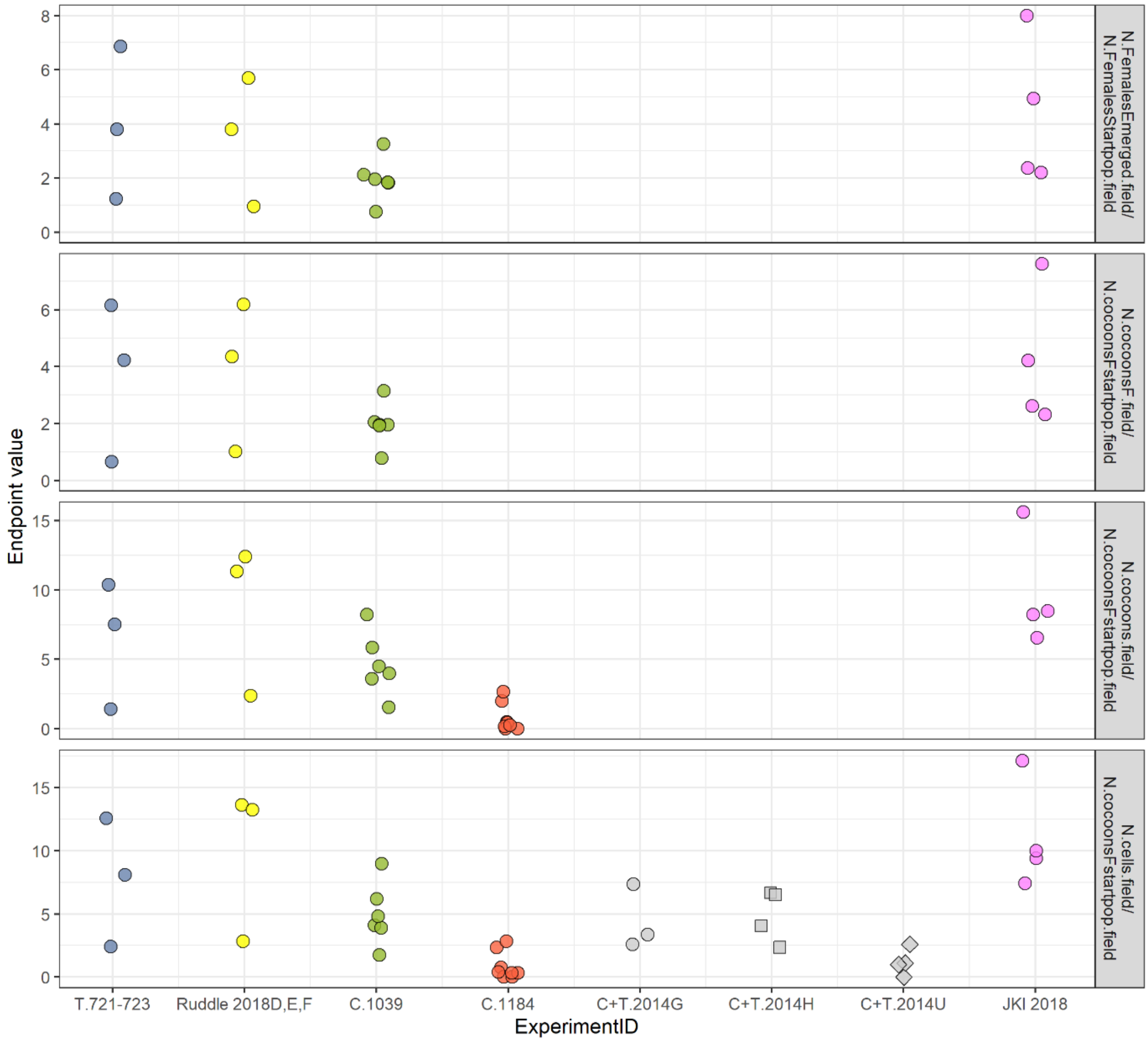
B.3.3.2. Assessment of the variability

The attribute relevant for the solitary bee SPG is the population abundance. A population is defined by individuals of the same species co-occurring in space and time that interbreed. From this definition, it follows that all individuals present at a single field site in a certain study would be considered to be part of the same population. Although in solitary bee field studies usually several nest units are placed at one field, the bees nesting at these different nest units will still be part of the same population. Consequently, to investigate variability between different populations, different field sites have to be compared (i.e. between-site variability).

The variability between replicate field sites within a study was quantified as the CV, i.e. the standard deviation divided by the mean. The CV values were calculated for each dataset, and for each of the four endpoints listed under B.3.3.1. As also stated under B.3.3.1, measurements for the considered endpoints were only performed once, at the end of the study. Consequently, the CV values could only be calculated for a single time point.

B.3.4. Results and Discussion

The actual measured endpoint values for the number of females emerged per female of the starting population, the number of female cocoons per introduced female cocoon, the number of cocoons (both sexes) per introduced female cocoon and the number of brood cells per introduced female cocoon are shown in Figure B.9. The coefficients of variation calculated based on these endpoint values are shown in Figure B.10.



Each panel displays the data for a specific endpoint.

Figure B.9.: Actual measured values for the different endpoints considered for the calculation of the coefficient of variation

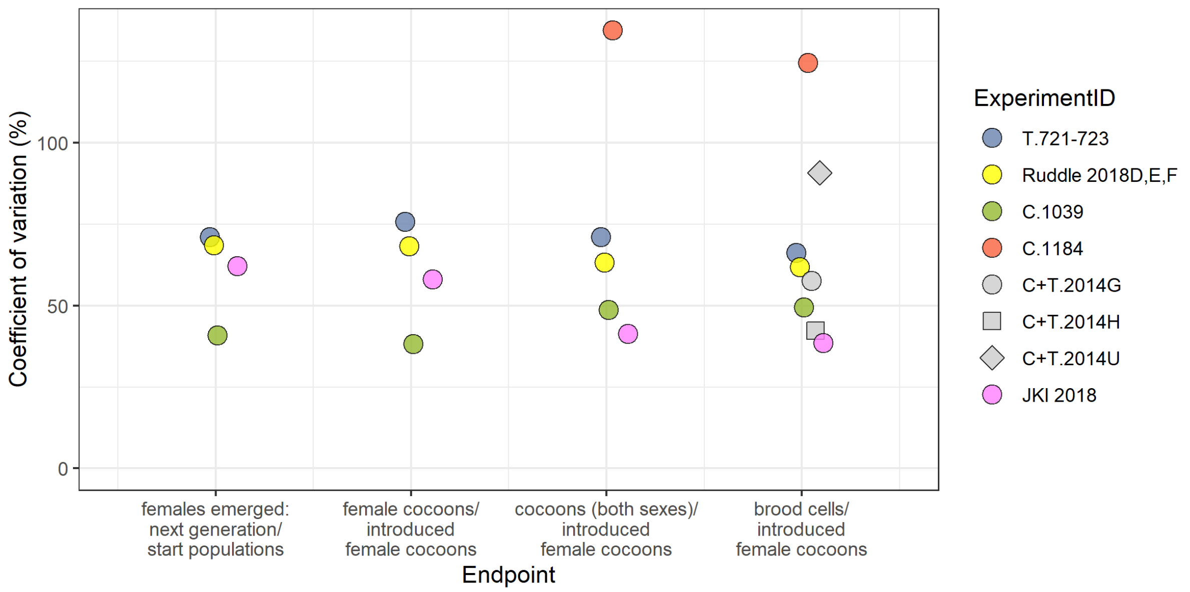


Figure B.10.: Between-field variability of the four selected endpoints, quantified as coefficient of variation (CV) and calculated separately for each of the available datasets (indicated by different colours/shapes)

The number of cocoons in the starting population varied among studies, from as low as 12 or 25 female cocoons per site (RefID 1184 and RefID 1525, respectively) to 682 (RefID 1039) and 1200 female cocoons per site (RefID 721, 722 and 723). The number of cocoons in the starting populations seemed to influence the variability, with fewer cocoons leading to a larger CV value.

It should be noted that under natural conditions, it is however unlikely that a very large number of cocoons will be present on a single location (Steffan-Dewenter and Schiele, 2008). Conversely, in a model system such as a field study, a very large starting population would be needed to reduce the between-site variability.

Taking into account the rather high between-site variability observed in the available field studies, it might be difficult to observe any statistically significant effects between the control and the treatment. Taking the influence of the starting population on the variability into account, the number of introduced cocoons should be carefully considered in relation to the effect size that can/needs to be detected.

This between-site variability could in part be explained by the fact that the endpoints used were expressed relative to the number of introduced female cocoons or emerged females. As also explained in Section B.3.3.1, a part of these introduced females might fly away from the test site and nest elsewhere in the landscape, and this part is likely to be variable from site to site.

The CV values calculated for different studies are variable. This could in part be explained by important differences in study setup between the different datasets. Experience with solitary bee field testing is limited and harmonised test protocols are not available yet. The variation in test methods reflects the attempts of the scientific community to investigate how field testing could be done. It should be kept in mind that this variation in study setup is a source of variability in itself.

It is difficult to compare the CV values calculated for solitary bees with those for bumble bees or honey bees, as for solitary bees only variability between sites could be considered, while for the other two groups we also look at within-site variability.

B.3.5. Conclusions for solitary bees

The number of suitable studies available is limited. In addition, only data for a single species (*Osmia cornuta*) are available. Nevertheless, the present exercise indicates the following:

- The number of cocoons in the starting population may influence the variability (with fewer cocoons leading to a higher variability). Therefore, the number of cocoons introduced is important for the effect size that can be detected in a field study.
- The calculated CV values vary between studies. This is likely to be due to differences in study setup between the different datasets. These differences are to be expected as this is a relatively new field for which experience is still being gained and harmonised test protocols are not available.

As for solitary bees only the between-site variability can be calculated (as all individuals present at one site are part of the same population), the CV values for solitary bees cannot be compared with those for honey bees and bumble bees, for which we can also look at within-site variability.

B.4. Overall conclusions

Table B.10 shows a summary of the available data for bumble bees and solitary bees, in comparison with honey bees. It is clear that data from field studies that could be used to investigate the NOR for bumble bees and solitary bees are scarce compared with honey bees, and that model simulations are not available (see Section 3.1 of the main document). The limited availability of field study data generally prevents a comprehensive analysis.

Table B.10.: Summary of the available data from model simulations and field studies and results for coefficient of variation (CV) for honey bees, bumble bees and solitary bees

	Honey bees(a)		**Bumble bees**	**Solitary bees(b)**
Species	*Apis mellifera* (one species)		Data available on *B. terrestris* (68 species in Europe)	Data available on *O. bicornis* (~1900 species in Europe)
Available data	Model simulations: 19 scenarios, 500 replicate colonies/scenario	Field data: 33 field studies, 52 fields overall, 1–16 replicate colonies/field	Field data: 7 field studies, 33 fields overall, 2–25 replicate colonies/field	Field data: 8 field studies, 3–8 fields/study, 1 population/field
Colony size CV	CV: 5–20%	CV: 0–50% (n workers ≈ n adults)	CV workers: 0–135% CV adults: 0–95% (n workers ≠ n adults)	Not relevant
Colony weight CV			CV: 5–60%	Not relevant

(a): See EFSA et al. (2021).

(b): CV values calculated are for between-site variability, and cannot be compared with those for bumble bees or honey bees (which represent within-site variability).

The available studies were conducted in Germany, France, Hungary, Sweden and the UK, therefore represent mainly the Central and Northern Zone. Especially from the Southern Zone, studies are not available. However, it should be noted that the results for the modelling exercise for honey bees, presented in EFSA et al. (2021), indicated that the range of variability was similar for the different regulatory zones within the EU (e.g. refer to table 10 in EFSA et al., 2021). In addition, the dataset of 33 field studies with honey bees, considered in EFSA et al. (2021), contained both studies from the Central and Southern Zone. The variability in the control replicates from these studies (quantified as the CV) was in the same range as studies performed in both zones (refer to figure 22B in EFSA et al., 2021). In the information session, a concern was raised that the background variability in the Southern Zone could be higher than in other zones. While no data were submitted to underpin this concern, it is noted that assuming a lower background variability than present in reality would be conservative in the current exercise. Taking this into account, it is assumed that the results for the variability in the control data from field studies with bumble bees and solitary bees performed in the Northern and Central Zone will be representative also for the Southern Zone.

Data are available for only one bumble bee species (*B. terrestris*) and one solitary bee species (*O.*

bicornis), while both groups consist of approximately 68 and 1900 species in Europe, respectively. Therefore, any conclusion based on the available data should be considered carefully in terms of representativeness for the whole bee group.

There is variation in the calculated CV values between studies. This might be partly explained by important methodological differences between studies, which are not surprising as harmonised protocols are not yet available and experience is relatively limited. Therefore, the observed variability should be interpreted with caution.

For bumble bees (*B. terrestris*), the CV values for number of workers and number of adults range from 0–135% and from 0–95%. For these endpoints, the variability is higher compared with colony weight, for which the CV values ranged from 5–60%. For the latter endpoint, the variability is comparable with what was observed for colony strength in honey bee field studies (CV values ranging from 0–50%). Taking into account the low number of studies, the available dataset is considered too limited to give a quantitative estimate of the NOR or background variability. Nevertheless, our results indicate that the background variability for the endpoint bumble bee colony weight of *B. terrestris* could be comparable with that for honey bee colony strength.

For solitary bees (*O. bicornis*), the CV values for the four endpoints considered generally ranged from 40 to 70%, with one study having CV values exceeding 100%. Note that for these bees, the CV values represent between-site variability, and cannot be compared with the results for bumble bees or honey bees. Taking into account the low number of studies, and the methodological differences between studies, the available dataset is considered too limited to give any reasonable indications for the NOR or background variability for *O. bicornis*. Nevertheless, for a rough comparison with SPG agreed for honey bees, it is noted that the variability observed in the available field studies with *O. bicornis* is greater than 10%.