Annex to:

EFSA (European Food Safety Authority), Adriaanse P, Arce A, Focks A, Ingels B, Jölli D, Lambin S, Rundlof M, Süßenbach D, Del Auguila M, Ercolano V, Ferilli F, Ippolito A, Szentes C, Neri F M, Padovani L, Rortais A, Wassenberg J and Auteri D, 2023. Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2023;21(5):7989, 27 pp. doi:10.2903/j.efsa.2023.7989

Annex B – Recommendations for higher tier exposure studies

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Preface

This Annex supersedes the Appendix G reported in EFSA (European Food Safety Authority) (2013) with the intention to provide the regulatory community with more detailed and comprehensive technical recommendations on how to conduct higher tier semi-field and field studies to refine the exposure assessment under realistic conditions. Guidance at a more detailed level has been included to ensure the practicality, reliability and validity of the studies regarding both the field and laboratory analytical phases. In particular, in order to ensure that no major concerns on the validity of the study are likely to be raised during the evaluation of the marketing authorisation application, the following technical and experimental aspects have now been tackled:

- test principles
- experimental design
- field phase (pesticide application, trial site, sampling methods, analysis of residues and sugar content in floral nectar)
- analytical phase (procedures and quality criteria)
- assessment of pollen and nectar residue decline
- assessment of residue dissipation in/on plant foliage
- general considerations for exposure refinement for the succeeding crop and field margins scenarios
- recommendations for studies with non-*Apis* bees

Additionally, clear instructions have been provided on the selection process for RUD values available from the higher tier studies to be used in the exposure assessment refinement (see Appendix A and also par. 5.5.8 of the Guidance Document)

In revising the protocol proposed in EFSA 2013, the EFSA Working Group took into account the principles provided in earlier guidance documents (e.g. European Commission (1997); OEPP/EPPO (European and Mediterranean Plant Protection Organization/Organisation européenne et méditerranéenne pour la protection des plantes) (2010); OECD (Organisation for Economic Co-operation and Development) (2016); European Commission (2018); European Commission (2019)) and publications on related subjects, as well as the reliability criteria set in the literature evaluation protocol developed by EFSA for the updated risk assessment on clothianidin, imidacloprid and thiamethoxam (EFSA (European Food Safety Authority), 2018a).

1. Introduction

This Annex proposes guidance for conducting semi-field and field studies to refine the exposure assessment for bees in the EU regulatory context. The main objectives of these studies are:

- refine the dietary intake by measuring residue levels in nectar and pollen entering the hive following treatment according to the proposed good agricultural practices (GAP)
- refine the acute contact exposure to adult bees by quantifying residue levels on bees foraging during or immediately after spray or solid application (i.e. originating from spray droplets or dust particles).

These studies can also be designed to provide specific information on: i) the dissipation rates of pesticides in pollen and nectar, ii) the residue levels of pesticide "carryover" in pollen and nectar on a permanent crop or on a succeeding crop for annual crops (i.e. potential exposure through soil), iii) the residue levels in other matrices (e.g., leaves), and iv) crop-specific sugar content of nectar.

The exposure refinement can be applied to the active substance and, where relevant, degradation product(s) and to all the exposure scenarios.

For the purposes of this Annex, the following key terminology is used:

Location or site: one geographically defined area where a specific experiment is carried out within a country/region/state. Sites/locations are characterised by homogenous landscape composition, topography, agronomic, and geo-climatic conditions. No clear boundaries can be set for site identification/separation. However, two sites should be considered independent if those have sufficient geographical distance to allow some difference in the general environmental conditions. SANTE/2019/12752 (European Commission, 2019) mentions that different sites must be at least 20 km away from one another. On the contrary, EFSA (European Food Safety Authority) (2014), for soil DegT50, mentions that spatial variation in daily rainfall may be considerable on a scale of 100 km^2. For the present guidance, as rule of thumb, ≈ 100 km is considered a sufficient distance, but in case of very diverse landscape and agro-climatic conditions, smaller geographical distances may still be appropriate. If the distance between sites is within 20-100 km, it must be demonstrated that the conditions are sufficiently different between sites regarding factors influencing residues decline (e.g., weather patterns) to constitute separate sites. If this is not shown, the sites may still be considered to be one site.

Field: a spatially well-defined unit on which the test plant is grown.

Trial: one semi-field or field exposure experiment characterised by a unique location, timing, target, sampling strategy and application (in terms of rate and pattern). A trial may consist of several plots, including control (if foreseen) and one or more treated plots following a specific experimental protocol.

Plot: a spatially characterised sub-unit within a field used for a trial

It should be noted that the above definitions are not fully consistent with the terms used in Annex C as a result of the possible differences in the study design between the exposure studies and the effects studies (e.g. absence of bee hives/colonies/nests in residue studies where pollen/nectar are collected directly from flowers).

Specific guidance on how to determine exposure in higher tier effects studies can be found in Section 3.5.7 of Annex C to the Guidance Document.

1.1. Test principles

Higher tier field studies can be conducted to refine the exposure assessment when the lower tiers indicate that the risk is not acceptable (see Section 3.3 of the Guidance Document). The principle is to conduct experiments that, under representative actual use conditions of the substance, would lead to more environmentally realistic exposure levels for bees.

In particular, higher tier studies on residue levels in nectar and pollen aim to assess the spatial variation of the peak concentration of a substance in nectar and/or pollen produced by a target crop in the area of use of the substance, following a certain representative use (e.g. two foliar spray applications of a dosage of 0.5 kg/ha per treatment in cherries at BBCH 53 - 87 in Southern EU with a minimum interval

between applications of 7 days). The procedure to refine the dietary exposure is (i) to measure these concentrations at a number of locations in the area of use (see paragraph 1.2), (ii) to use the field measured data as input for the exposure models, replacing the default conservative values used in Tier 1 (see Section 5.1 of the Guidance Document). The exposure refinement for the dietary exposure is performed by calculating new shortcut values (SVs) based on the measured RUDs, and hence Tier 2 PEQ_{di} values (see Appendix A to this Annex), which represent the 90th percentile residue intake per day (adults) or per developmental period (larvae). The procedure for refining the acute contact exposure to adult bees is (i) to measure the mass of the substance to which the population of foragers from hives at edges of treated fields in the area of use of the substance(s) is exposed, in terms of the frequency distribution of mass per bee, and (ii) to use the 90th percentile field-measured data for the exposure estimation, i.e., as a PEQ_{co} value (see Section 5.1 of the Guidance Document).

Both semi-field and field studies are intended to reflect increasingly realistic exposure conditions for Tier 2 assessment. In principle, (semi)field studies represent the highest possible tier of the exposure assessment because they most closely represent a realistic exposure scenario. The choice of a specific study type to measure residues will depend on the objective of the study. There are many factors that collectively influence the concentration of a chemical in pollen and nectar in the field (Gierer et al., 2019). Nevertheless, based on the available information on residue levels in pollen and nectar from a large number of field studies (ref to par 4.2.3 and to Sappington et al., 2020), it is possible to define a spectrum of field exposure depending upon the type of study in question: the complexity of the study design increases with decreasing degrees of conservatism and measured residue levels (Figure 1). Thus, residues measured in pollen and nectar collected directly from flowers and/or collected from pollen traps and/or bees confined to tunnel tents or cages represent a maximum exposure level for dietary risk assessment for bees. When residues are collected from bees or pollen traps in field studies, it is expected that these will be lower than those collected directly from flowers, or from bees or pollen traps in semi-field studies (where the bees can only forage on the treated crop and the experimental conditions are relatively more controlled). Two main categories of field studies can be defined:

1. field studies where no/minimal alternative food sources are available in the landscape within 4 km of the hive/colony/nest during the flowering of the focal crop/plant, which will provide a realistic worst-case exposure assessment (here defined as "minimum alternative forage" field studies). This approach offers a compromise between refined estimates of the exposure levels of bees foraging on the treated crop and the added time and resources needed for the more complex study design.

2. field studies where a more realistic foraging landscape is included (here defined as "randomly selected landscape" field studies). This approach represents field realistic measures of residues concentration entering the hive/colony/nest given the spatiotemporal variability in land cover types, crops and alternative forage resources. In this type of study, the challenge is to select sites at random over the area of use (refer to par. 1.3).

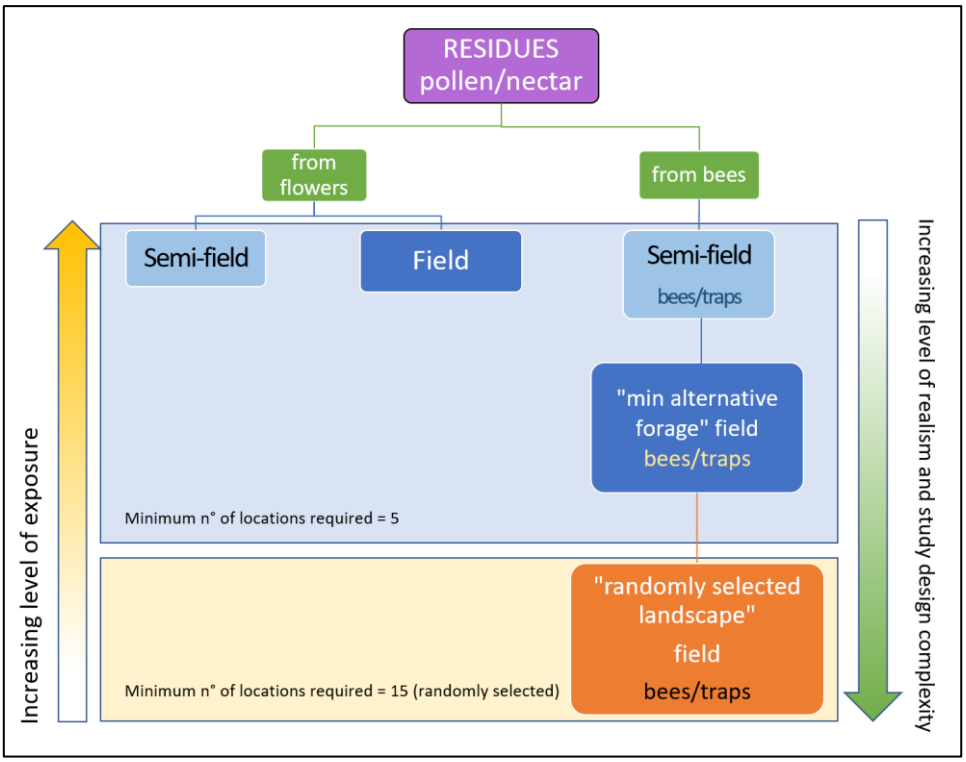

Figure 1: Set of field studies aimed to measure residues in pollen and/or nectar collected directly from flowers and/or collected from bees. The complexity of the study designs and the level of realism increases with the decreasing degree of conservatism and exposure level (refer to text for details).

It should be noted that large-scale surveys such as field monitoring studies of pesticide residues from commercial beekeepers where information on the associated application patterns of the test substance on the relevant crop are not available (in particular the application rate and the application time) cannot be used to quantitatively refine the exposure for regulatory purposes.

The scheme proposed in Figure 1: is applicable to the three main methods of application for pesticide products, i.e. spray application, seed treatment and granules. For other types of application methods, it is the applicant's (or study director's) responsibility to develop a fit-for-purpose study design. It should be considered that the exposure from seed treatments and granular formulations (granules incorporated into the soil or buried with the seed) in the "treated crop" and the "succeeding crop" scenarios derive from residues in pollen and nectar following translocation from below ground (seeds or soil). Otherwise, if the granules are broadcasted after the emergence of the crop, it is expected that the residues behaviour is more similar to that of residues resulting from spray applications. Concerning the surrounding area ('field margin' and 'adjacent crop' scenarios), the most relevant exposure is due to dust drift at the sowing (for treated seeds) or application (for granules). Specific recommendations for field studies aimed to measure residues in pollen and/or nectar in off-field crops/plants can be found in chapter 9.

Due to the complexity and the differing objectives of the residue field studies, the applicants/study directors are recommended to submit the specific protocols to the national competent authority for review prior to initiating any kind of residues study.

The following paragraphs will focus mainly on the scientific and technical aspects for conducting higher tier studies aimed to refine the dietary exposure for honey bees for the treated crop scenario with spray application as these are currently the most common type of trials conducted in the context of regulatory risk assessment. Nevertheless, some recommendations for exposure refinement for non-*Apis* bees, other exposure scenarios and for the contact exposure assessment are also provided.

1.2. Number of locations

Consistently with the lower tiers, the purpose of the higher tier exposure studies is to assess the 90th percentile of the spatiotemporal exposure distribution in the area of use of the substance (refer to Section 3.2 of the Guidance Document). The selection of the locations where to conduct the field experiments and the number of locations has to be tailored to the purpose of the study, i.e. to assess the spatial variation of the RUDs in the area of use of the substance (e.g. if a substance is applied on maize, this area is represented by all the agricultural fields where maize is grown). This spatial variation is used to assess a certain percentile (usually 90th) of the daily residue intake rate (see Section 3.3 of the Guidance Document). This percentile of the intake rate is likely to be related to the corresponding percentile of the RUDs: e.g. the 90th percentile daily residue intake rate of the different bee species can probably only be assessed appropriately if the 90th percentile RUD values of nectar and/or pollen have also been assessed with enough certainty. The number of locations should ensure that the required percentile is assessed with enough certainty. In case of a 90th percentile the EFSA (European Food Safety Authority) (2013) recommended to perform studies for at least five randomly selected locations in the area of use of the substance because the highest of five ranked values of a sample population is the 90th percentile. However, it was already acknowledged that this recommendation should have been further substantiated. Therefore, in the context of the revision of the guidance document a simulation exercise was conducted to assess the relation between the number of randomly selected locations and the chance that, among these locations, the actual 90th percentile of the exposure relevant quantity (i.e. the exposure assessment goal) would be captured. To do so, 10000 "virtual" hives, heterogeneously distributed in a virtual space, were considered, and each one of those was characterised by a certain level of exposure (i.e. the maximum daily residue concentration entering the hive, Figure 2:). Let "*z*" be the entire population of exposure values considered in the exercise.

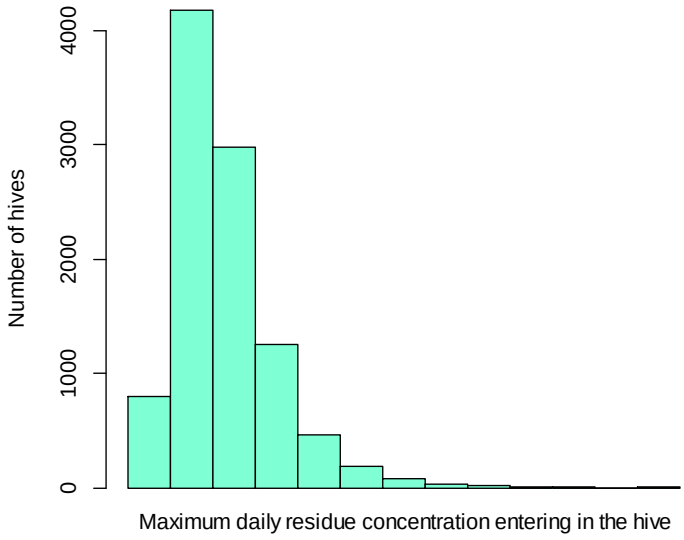

Figure 2: distribution of the level of the exposure in 10000 "virtual" hives used to define the minimum number of locations for field exposure studies

Based on the available information on residues measured in field studies (see Section 5.5.8 of the Guidance Document) it was considered appropriate to assume that "*z*" would follow a log-normal distribution. It must be noted that, for the sake of this exercise, the actual values used in the simulation (i.e. the log-mean and the log-standard deviation that characterise the distribution position and spread of z) did not matter.

After selecting a certain sample size *n*, the exercise consisted in a very simple procedure that was repeated for every sample size from 1 to 30:

1. *n* values were randomly selected from "*z*", forming the sample *s*
2. the maximum value of the sample *s* was identified as max_s
3. max_s was compared with the 90th percentile of *z*
4. steps 1-3 were repeated a large number of times (i.e. 100000 was used in the simulation)

5. check how often out of the 100000 iterations $max_s \geq 90^{th}$ percentile of z

As expected, the chance of having in the sample at least one exposure value that is equal or greater than the actual 90th percentile of z increases with increasing sample sizes. As illustrated in Figure 3: (a), when selecting 5 random locations, the chance of capturing the required level of exposure is quite low (about 40%). Therefore, the Working Group decided to confirm the minimum required number of 5 locations in the agricultural area of use with the caveat that the locations should not be selected randomly but should be appropriately identified in order to reflect worst case exposure conditions, such as in the case of the "minimum alternative forage" field studies (see further details in par. 1,3 for the selection criteria and requirements to be met for this type of field study).

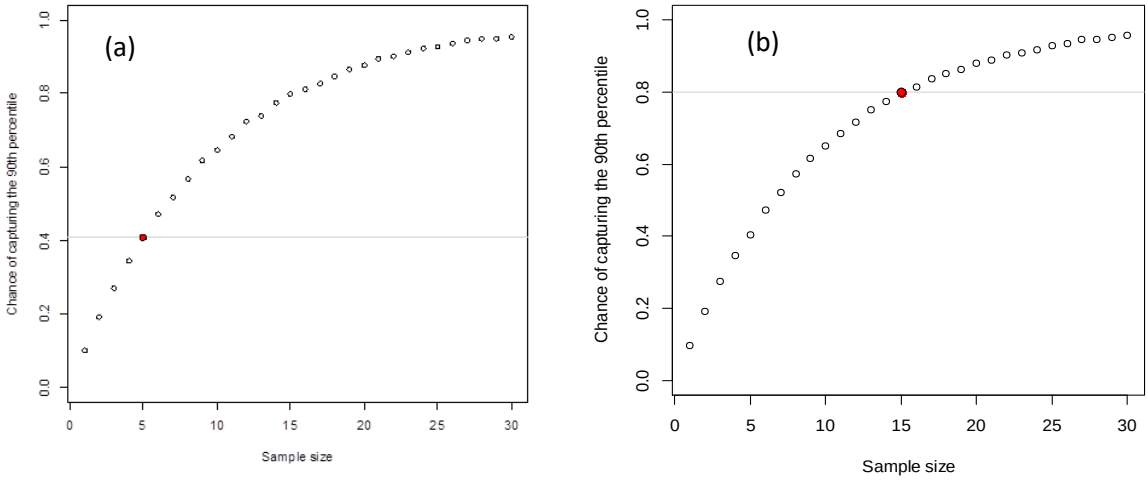

Figure 3: Relation between the sample size (i.e. number of randomly selected hives sampled for exposure) and the chance to capture a hive with an exposure equal or higher than the 90th percentile of the entire exposure distribution. Plots of this relationship are shown for five (a) and fifteen (b) randomly selected locations.

In a "randomly selected landscape" field study with a sample size of 15 randomly selected locations, there is an 80% chance of capturing a value equal or greater than the 90th percentile of "z" (Figure 3: (b)). This is considered as a reasonable compromise, as the same probability is usually also accepted for the power (1-β) of most statistical tests. Therefore, when it is not possible to use a more complex study design to exclude or minimize the alternative forage resources, residue data from a minimum of 15 randomly selected locations over the area of use of the substance are needed to refine the exposure assessment.

Within this exercise, the hives were assumed to be randomly selected, and therefore were considered as completely independent from each other. Hives sampled within the same location would be, by definition, not independent. As such, the sample size described here as number of hives should actually be interpreted as a number of independent locations, as multiple hives considered within the same location do not satisfy the pre-requisite of their independence.

1.3. Selection of locations

In line with the specifications of the exposure assessment goal, the locations should be distributed over the area of use of the substance in question. In order to identify the prospective study areas, as a first step, geospatial information on land cover at the EU level could be used to identify areas where the target crop is grown. In case of annual crops, the CAPRI (Common Agricultural Policy Regional Impacts) database (Leip et al., 2008) could be used to identify proxy areas of potential use of the focal PPP. EU crop maps for CAPRI crops are available at a scale of 1 x 1 km^2. Regarding permanent crops, the working group recommends the CORINE Land Cover database and EUROSTAT data sets (such as LUCAS Land

Use/Cover Area frame statistical survey) as described in Beulke et al. (2015). Another source of data that could be used to identify the location of the target crop is the recent EU-wide crop type map for 2018 at 10-m spatial resolution for 19 crop types developed by the JRC based on Sentinel-1 and LUCAS Copernicus in-situ observations (D'Andrimont et al., 2021).

In addition to the crop cultivation area and the use of the compound, for **"minimum alternative forage"** studies the following criteria should be met:

- a visual and spatially explicit, detailed landscape characterisation in a 1.5 km radius around the hives/colonies/nests at the focal field (defined based on known average foraging distances, see Appendix A in Annex C on higher tier effect studies)
- a maximum of 10% alternative forage resources within 4 km radius around the hives/colonies/nests at the focal field during the experimental exposure phase (i.e. the presence of flowering crops which are potentially foraged by bees, flowering weeds and plants at field margins, forest edges and hedgerows with flowering trees or bushes should not exceed 10% of the total 4 km radius circle area during the flowering period of the target crop). A compilation on the attractiveness of crops to honey bees as food sources of pollen and nectar can be found in Appendix A of the guidance document, while all flowering non-agricultural plants are conservatively considered attractive.

To support the validity of the selected locations for the "minimum alternative forage" studies, ground truthing and field inspections (Westphal et al., 2008; Holzschuh et al., 2016)are necessary to provide a detailed qualitative and quantitative assessment of flower resources within the 1.5 km radius circle surrounding landscape area relevant for bee foraging. The appropriate documentation that should be provided includes:

- topographic map
- satellite/aerial images
- land use/land cover data
- land survey with drone technology
- photographic images

The documentation should enable the characterization of areas of land cover types based on CORINE/CAPRI dataset and areas of co-flowering crops/plants and their growth stages during the exposure period. If the aim of the "minimum alternative forage" study is to refine the exposure with realistic RUD from bee-collected pollen and/or nectar, the assessment of the classification of the landscape composition is required only on the day of samplings (see Section 4.2.2). When effects on bees will also be investigated in the study, taking into consideration the fact that the landscape composition can change over time, the information on alternative flower resources relevant to bees should be recorded ideally at each sampling event of the flowering period of the focal crop, or, at least, at the beginning and at the end of the exposure phase. Within the EU Horizon 2020 PoshBee Project, Dominik and Schweiger (2019) provided a good example of methods that can be used to map habitats and calculate the metrics that describe the compositional heterogeneity of the agricultural landscape surrounding the focal field. As an alternative to the traditional field techniques for mapping flowers, remote sensing and drone technology provide a valuable opportunity for capturing fine-grained information on floral resources at key times of the flowering season (e.g. Gonzales et al. 2022; (Barnsley et al., 2022)).

Before ground truthing the potential selected locations, examination of the 1-km-resolution continuous map of semi natural vegetation in agricultural land in the EU-27 developed by the JRC (Garcia-Feced et al., 2015), is recommended in order to target those ares with a lower abundance of hedgerows, buffer strips, field margins, forest edges or riparian vegetation within agricultural lands.

To ensure a minimal (i.e. max 10%) contribution of other flower resources than the focal field(s) within a 4 km radius, a more general quantification of land cover/land use (LCLU) types and their average flower cover is sufficient (see an example in Box 1, below). (García-Feced et al., 2015); Dominik and Schweiger (2019)It should be noted that the selection of the location should not be the result of the overall combination of worst-case conditions, but rather the picture of a situation which is as realistic as possible, i.e. soil properties and the climate are typical of the intended application area. The worst-

case exposure conditions of the study lie in the fact that the concentration of residues entering the hive derive from the treated crop and the dilution of this concentration due to alternative forage on the landscape should be minimal during the entire flowering period of the target crop.

Box 1. **Standardizing landscape characteristics and flower resource estimation**

An example is given on how to characterise broad land use/land cover (LULC) types and their cover of flower resources to meet the requirement of less than 10% alternative flower resources in the 4 km landscape surrounding the hives/colonies/nests (for "minimum alternative forage" studies). The approach is based on those used in Persson & Smith (2013), Holzschuh et al. (2016) and Dominik and Scheweiger (2019) and include the following steps:

1. Map the area around the hives/colonies/nests at the focal field(s) within a 4 km radius. This can be done using digitalization in a geographical information system (e.g. ArcGIS, ESRI, or QGIS) based on high resolution satellite and aerial images.
2. Classify each major LULC types, for example based on EUNIS, the European Nature Information System, or something similar. The LULC classification used in Dominik and Scheweiger (2019) used EUNIS categories adapted for pollinators: Surface running waters, Waterbodies, Wetlands, Grasslands, Woodlands and heathland, Bare areas, Apple orchards, Crop, Roads, Train tracks and Urban areas. Calculate the area of all the LULC types within the landscape radius.
3. Survey flower resources using a stratified sampling scheme across the LULC types. This means to randomly or systematically select local sampling locations or transects in all the relevant (i.e. containing any flower resources) LULC types and perform floral resource surveys to estimate the average flower cover for each LULC type. This can be done using squares of a certain size (e.g. a number of squares of 0.25 m^2) placed evenly along transects (e.g. a number of transects of 150 x 1 m) and an estimation of the flower cover in these squares.
4. For each LULC type, the average proportion of flower cover should be calculated. This can be used to calculate the area of flower cover in each LULC type. The total area of flower cover can be summed up across LULC types and the total landscape area can finally be used to calculate the total proportion of cover of flowers in the landscape.

It is recommended that the above is done the year before the study during the expected flowering stage of the focal crop and then again during the study year. For bumble bees and solitary bees, the landscape radius may be reduced from 4 km (see Appendix A in Annex C on higher tier effect studies and Kendall et al. (2022) for guidance).

When it is not possible to identify 5 locations addressing the selection criteria listed above to ensure an almost exclusive exposure to bees from the target crop, 15 locations for **"randomly selected landscape" field studies** can be considered. In this case, it is required to exhaustively describe the randomization procedure used to select the locations. In order to ensure that no obvious bias concerning the availability of alternative food resources in the landscape has been introduced in the selection process, a rough description of the landscape characteristics of the different land covers/land uses within the 1.5 km radius circular area around the hives/colonies/nests should be provided. Similarly to what is requested for the 4 km radius area of the "minimum alternative forage" studies, it is needed to roughly estimate the proportion of the landscape areas covered by other crops which are foraged by bees for pollen and/or nectar, and of areas with alternative forage resources during the exposure phase of the study. In this case, however, the requirement of less than 10% alternative flower resources does not apply.

The selected locations may be distributed across relevant geographical and climatic regions, within the MS if the pesticide is to be used in only one MS, within a zone, or, if the pesticide is used (or intended to be used) across a range of MS/zones, then it may be appropriate to have a selection of locations across the MS/zones. Geospatial and statistical analysis should be used to support the non-comparability of different locations if the "100 km distance" rule (as described in the definition of "location") is not followed (e.g. approach adopted in the OECD (Organisation for Economic Co-operation and

Development) (2015) ENASGIPS Crosswalk Tool for matching ecoregions). According to (EC) Regulation 1107/2009, for seed treatments the whole of Europe represents only one "zone".

2. Study plan

Objective(s) and duration of the total experimental period (study initiation date, start and end of the experimental phase, start and end of the analytical phase) should be clearly indicated.

It should be specified if the study is conducted according to a standard protocol/guidance and any modifications and/or deviations from the validity criteria should be clearly indicated.

When defining the study plan, the physico-chemical properties as well as the environmental fate of the compound based upon the available data should be considered. For example, if the compound under evaluation is known i) to be persistent in soil, ii) to be taken up from soil and translocated to the above soil plant matrices (e.g. from the rotational crop residues study) and iii) the product can be used on several crops in rotation, then the accumulation in soil should be considered in the study protocol.

All studies should be carried out under GLP.

3. Experimental design

The following paragraphs define the minimum criteria related to the exposure assessment in (semi-)field studies that must be assessed/reported, and which can be used as validity criteria to ensure that the study is appropriate for regulatory risk assessment of pesticides.

3.1. Test substance

The test item should be clearly identified and characterised by providing: the identity (common name, IUPAC name and CAS number), state or form, source, batch number and date of certificate of analysis of the formulation, purity of the a.s. for test conducted with the technical material, concentration of the active ingredient(s) for studies conducted with formulations and storage conditions of the test substance. For a trial carried out in the context of a (re)authorisation at the EU level for an active substance, the test item should in principle be the representative formulation. If the context is the authorisation of a specific plant protection product, then preferably this should be the test item. If this is not the case, it should be clearly shown that the tested formulation is sufficiently similar to the representative one (i.e., same type of formulation and similar co-formulants). Particular attention should be paid to elements which may alter the environmental fate of the active substance (e.g., encapsulation, solid vs. liquid form, etc.).

3.2. Test crop/plant

The common and scientific name and variety of the tested crop, planting date, seasonal growth cycle of the crop, calendar date and time of the start and the end of flowering period should be specified. The phenological growth stages and BBCH identification should also be recorded during the exposure period and at all sampling dates. It is recommended to select the variety based on their potential for flowering and pollen production.

Highly attractive model crops such as Brassica napus (oilseed rape) and/or Phacelia tanacetifolia may be used for studies aimed to measure initial RUDs following downward spray application during crop flowering with bee-collected residues. However, based on the currently available information (EFSA, 2013; Kyriakopoulou et al., 2017), residue levels measured in pollen and nectar from these highly attractive crops are higher than residue levels in other types of crops for both the plant matrices.

Thus, in general (and in other exposure refinement situations), the test crop/plant to be used in the exposure field study should correspond to the crop reported in the specific GAP under assessment.

In order to reduce the workload for applicants that would need to refine the exposure for different representative uses with application(s) during the flowering period, a "worst-case use pattern" in the GAP can be identified that would cover all other situations where the GAP is less critical or the same (i.e., identify the critical GAP that would lead to the highest possible residues entering the hive). The rationale followed for the identification of the worst-case GAP should be explained and transparently reported, taking into consideration the following:

- As long as the dietary exposure will be refined with the measured residue concentration expressed as initial residue value per unit doses (RUD), the number of applications and the application rate of the GAP is less important for the critical GAP identification. However, a proper application rate should be used to obtain sufficient quantifiable residues of the applied substance. Additionally, if the field study aims also to measure the residue levels of metabolite(s), exaggerated dosages are usually needed for the identification and quantification of the metabolite(s).
- Intended uses with application (or all the applications) during the flowering period (BBCH 60 – 69) will cover the uses with application(s) before flowering
- If the representative use in the GAP is based on multiple applications during both the pre-flowering and the flowering stages, then in the experiment oilseed rape and/or Phacelia cannot be used as target crops unless the PPP is applied during the flowering stage only

In general, for all the other GAP situations the identification of the worst-case GAP is not a straightforward process and is highly dependant on certain parameters that influence the expected exposure level, such as the attractiveness of the crop/plant and the flower architecture. For uses like seed treatments and granular formulations, a "worst-case GAP" approach might not be applicable, although, in most of these cases the variation within the intended application rates and crops is limited (European Commission, 2011). It is therefore crucial that the background information as well as the rational developed in proposing the worst-case GAPs are clearly reported for field studies where the intended crop for the proposed item is not used.

General criteria for the applicability of extrapolation between residue data generated from field studies with different crops can be found in Section 5.5.8 of the Guidance Document.

3.3. Field trials characteristics

Field trials should be accurately described and characterised (ref. to par. 1.3). The following information should be provided for the fields and plots, with the indications of the data sources:

- geographic coordinates (latitude and longitude) and altitude of site
- topography
- field and plot(s) size
- soil type, particle size distribution and physical-chemical properties
- climate
- average crop density of the test crop (plants/m^2)
- row spacing (if relevant)
- plant spacing (if relevant)
- pesticide use history data (for at least the three previous cropping seasons) and management practices (i.e. all cultivation operations, irrigation, fertilisation and applications of maintenance of pesticides)
- closest distance between control (if planned) and treated plot(s)

The environmental conditions at the time of application and during the whole exposure period should be measured at the trial site and recorded (at least daily min/max air temperature, daily rainfall, daily min/max air humidity, wind conditions and directions, daily hours of sunshine and daily total solar radiation). Alternatively, data from weather stations no more than 20 km distance from the experimental field should be used. The conditions in treatments and control plots (if used) should be comparable at the beginning of the experiment. Additionally, it should be clearly documented whether the weather conditions at the trial site are representative of the usual climate in the respective area of use.

The presence of an untreated plot (control) is not essential in the experimental design if the objective of the higher tier study does not include the assessment of the effects on bees. However, it could be useful for collecting different plant matrices to support the analytical method development. For studies where pollen and nectar are collected directly from flowers the size of the focal fields or plots should be such as to guarantee a production of pollen and nectar in amounts that are enough to allow residue analysis over the entire sampling period. This will, in turn, depend on the specific crop/plant's ability to

produce pollen and nectar. In the available database on field residue studies (see Section 5.5.8 of the Guidance Document), the size of the field or plot in the majority of the studies is about 1 ha (range 0.001-15 ha). For field studies in which pollen and nectar are collected by honey bees, the size of the field or plot should fulfil the nutritional requirements of the colony. Depending upon on the ability of the specific crop/plant to produce pollen and nectar, the size of the colony(ies), and taking into consideration the daily dietary requirement of a honey bee, it is possible to estimate the minimum size of the field/plot that is required to conduct the residue study. As an example, Table 1: reports some estimations of the minimum area required for different plants to provide sufficient forage to ensure appropriate nutrition for a colony of 10000 bees with an average lifespan of 27 days, based on data from Van der Steen (In preparation).

Table 1: Examples of minimum field size requirement for different plants to meet the dietary requirement of a colony of 10000 honey bees with an average lifespan of 27 days.

Plant	Hectare/hive
Sunflower	1.422
Field bean	0.578
White clover	3.947
Willow	0.004
Sloe	0.124
Maple	0.002
OSR short	0.285
OSR long	0.285
Dandelion	0.035
White clover	3.947
Ivy	0.266

More generally, EFSA (European Food Safety Authority) (2013) recommended a minimum of 2 ha to provide sufficient flowers and support exclusive foraging and Williams et al. (2013) recommend minimum of 5 ha area of the treated crop to represent a major nutritional source for the colonies of the test during the crop flowering period.

For seed treatment and pre-flowering applications in field studies with bees, we recommend also starting the exposure phase of the bees when there is at least 30% of the plant in field/plot is flowering of the focal crop/plant at least at BBCH 63 in order to ensure sufficient food availability for the bees.

When nectar and pollen are collected from honey bees, in order to avoid cross over issues between plots and problems of foragers visiting flowers of competing crops/plants in the surrounding area, we recommend a minimum distance of 4 km between treatment and control plots.

For semi-field studies, further details on the design and arrangement of the tunnel/cage should be provided: total area covered, dimension of each tunnel (length, width and height), area covered by the crop, material and mesh size of the gauze. Different guidance documents (e.g. OECD (Organisation for Economic Co-operation and Development) (2007); OEPP/EPPO (European and Mediterranean Plant Protection Organization/Organisation européenne et méditerranéenne pour la protection des plantes) (2010)) suggest a minimum size for experimental cages of 40-60 m^2 for semi-field studies with honey bees.

3.4. Hive/colony set setup

Honey bee hive setup and initial colony conditions are mainly based on OEPP/EPPO (European and Mediterranean Plant Protection Organization/Organisation européenne et méditerranéenne pour la protection des plantes) (2010) and Lückmann and Schmitzer (2019). Colonies should be queen-right

and created at least one week before the start of the sampling and be placed at the field sites at least three days before sampling start, to allow acclimatisation to the local conditions (Lückmann and Schmitzer, 2019). For semi-field studies, colonies should be introduced to the cages 2-3 days before spray application though when the application is pre-flowering or a seed treatment, the colonies may be introduced at the same time as flowering and sampling starts (OEPP/EPPO (European and Mediterranean Plant Protection Organization/Organisation européenne et méditerranéenne pour la protection des plantes), 2010). Colonies should not receive any medical treatment within four weeks before the start of the sampling and there should be no visible signs of disease.

Colonies should be as homogeneous in size as possible among the spatial replicates at the start of the study. In both semi-field and field studies, it is important to match the colony size to the available resources. For semi-field studies smaller colonies of around 3000-5000 bees, three full frames containing brood of all stages and a small store of nectar and pollen are sufficient (OEPP/EPPO (European and Mediterranean Plant Protection Organization/Organisation européenne et méditerranéenne pour la protection des plantes), 2010), with one colony per cage or tent and adjustment of their size to provide sufficient resources to the colony throughout the sampling schedule. For field studies, colonies should contain adult bees and brood in all stages, with smaller initial stores of food resources. Colony size should be adapted to what would be appropriate for the season (i.e. smaller colonies in spring and fall and larger during summer) and local beekeeping practices. Also smaller colony sizes could be used, as has been done in many studies exploring honey bee foraging ranges (see Appendix A in Annex C on higher tier effect studies).

In semi-field studies, one colony is placed within the cage or tent and is provided a water source in addition to the plant flowers (OEPP/EPPO (European and Mediterranean Plant Protection Organization/Organisation européenne et méditerranéenne pour la protection des plantes), 2010). In field studies, one or several colonies are placed at the edge of the focal field or plot.

4. Field phase

4.1. Pesticide application

The conditions of the semi-field and field trials should follow the conditions of the specific GAP under evaluation in relation to the method, rate and frequency of use. Except for field studies aimed to measure initial RUDs following spray application during crop flowering with bee-collected residues, the following conditions in line with the proposed GAP for the specific crop should be followed in order to produce a realistic worst-case exposure for the bees for the specific representative use under evaluation:

- maximum application rate (for multiple applications)
- maximum number of applications
- minimum re-treatment interval (for multiple applications)

The application rate should be expressed in terms of amount of product and/or active ingredient per unit area (e.g. kg a.i. per hectare). For three dimensional crops (e.g. orchards, hops, vineyards) with spray applications, it is recommended to also use the "treated leaf wall area" (tLWA) method (OEPP/EPPO (European and Mediterranean Plant Protection Organization/Organisation européenne et méditerranéenne pour la protection des plantes), 2021) to better describe the amount of spray solution reaching the leaves above the ground. For seed treatments the application rate should also be expressed as amount of active ingredient per unit of seed weight (e.g. kg a.s./1000 kg seed) and seeding rate (e.g. kg seed/hectare).

Equipment used for application should be described, including details on calibration. The direction of application and the dust deposition onto bare soil should be reported for spray applications and for solid applications, respectively. For seed treatment applications, the actual seeding rate and seed treatment quality data should be provided, including residue analysis of the active ingredient in the dust as well as the dust abrasiveness (mean Heubach a.s. value and mean Heubach dust at the time of sowing according to the ESA STAT Dust Working Group (2011).

4.2. Sampling and assessment of residues

Residues of the active substance and, where relevant, of metabolite(s) can be measured in:

- pollen collected directly from flowers
- pollen from foraging bees returning to the hive/colony/nest
- pollen from pollen traps
- pollen from solitary bee larval provisions created by foraging bees returing to the nest
- nectar collected directly from flowers
- nectar in the honey stomach from foraging bees or returning to hives
- plant parts
- soil

The selection of the matrix to be analysed, and sampling methodology and frequency will depend on several factors, including the objective of the study, the intended use, the floral size and morphology (i.e. accessibility to pollen from the outside of the flowers) and the physico-chemical properties of the substance. Experimental evidence from field residue trials (see Section 5.5.8 of the Guidance Document; (Byrne et al., 2014; Botías et al., 2015; Căuia et al., 2020)) suggests that sampling pollen and nectar directly from flowers is likely to offer a worst-case exposure scenario with less effort in study design. On the other hand, measured concentrations in pollen and nectar collected from bees provide a better representation of the exposure resulting from actual use conditions but require a more complex and expensive experimental field approach. Analyses of residues in hive matrices, such as stored nectar, bee bread, honey, etc., are considered inadequate to assess the exposure as they may include dilution over time.

The following must be clearly reported:

- the matrix of the samples
- a detailed description of the sampling method
- the type of the sampling area (including the position of sampling locations)
- the time of sampling in relation to the day of application, the time of the day and the development stage of the crop (i.e. BBCH stage).

Good descriptions of the most acknowledged and used **methods for collecting** pollen and nectar from flowers and bees can be found in Human et al. (2013), Corbet (2003), McKenna and Thomson (1988), Morrant et al. (2009), Knäbe et al. (2014) and Hodge (2019).

It is recommended to take spatial sub-samples in triplicate and to analyse the triplicate samples separately. The analysis of the measured residue data to derive the Tier 2 PEQ should follow the recommendations in Appendix A of this Annex.

4.2.1. Residues in pollen and nectar collected from flowers

Sampling frequency and timing for collection of pollen and nectar directly from plants depend upon the purpose of the study. For applications before flowering of the target plant, sampling can only occur when flowering has started. A single sampling from flowers immediately after application will be sufficient to obtain initial RUD values for spray applications during the flowering stage of the treated crop. For multiple applications, a single sampling directly after the final application must be done, as it is likely that concentrations in pollen and nectar are then highest at that time. For seed treatment and granules application and spray application before flowering, sampling should take place at least three times throughout the exposure period: at the beginning, middle, and towards the end of flowering. If the study is designed to provide information on the dissipation rate(s) in pollen and nectar or foliar dissipation, a minimum of 5 sampling points following application should be included (see chapter 6). Sampling points should primarily cover the first five days after application(s). Upon analysis the residues should show a clear decline over the sampling period. If this doesn't occur, then the study should be repeated, as it is key that the peak residue with respect to time is determined. For measurements in

permanent crops one year after application or in succeeding annual crops or for measurements in the treated crop after seed treatments, sampling has to be equally distributed over the flowering period because it is a priori unknown when the highest concentrations will occur. Particular attention should be paid when the crop/plants present extended flowering times or multiple flowering events. For applications with more than one application in the growing season of the crop, it is possible that the substance accumulates in the crop tissues or in the soil and it is likely that measured concentrations in pollen and nectar are then highest after the last application. It is recommended to measure the concentrations in nectar and pollen after the last application across a time frame sufficient to capture the peak residue occurrence and determine the decline over time.

Taking into consideration that nectar might be collected by bees during the day and that the nectar flow might dry in the afternoon, it is recommended to collect flowers from plants early in the morning. Additionally, when sampling both pollen and nectar produced by the same crop, it is preferable to collect nectar first and then pollen.

Sampling at the edges and ends of plots should be avoided. When the experimental design includes also a control plot, it is also recommended to take samples from the control plot before taking samples from the treated plot to avoid any cross-contamination.

The number of samples to be collected per sampling event will depend on the minimum amount of pollen and nectar required by the specific analytical method to quantify residues at the lowest level of detection. According to Human et al. (2013), for most analytical laboratories these minimum requirements are 3 g for pollen and 1.5 mL for nectar, while in the majority of the higher tier studies evaluated for the EU regulatory risk assessment of pesticides, the minimum target sample amount per sampling event was approximately 1.5 g for both pollen and nectar. However smaller amounts could be used, for example 0.2-0.3 g pollen ((Ruddle et al., 2018), (Kiljanek et al., 2021)), based on a scaled down analytical method validated according to Guidance document SANTE/12682/2019 (Kiljanek et al., 2021). Overall, it should be taken into consideration that: i) pesticide residue levels in pollen and nectar are generally in the order of parts per billion (ppb); ii) pollen production and nectar secretion varies between plants; iii) there are several factors influencing the amount of pollen and nectar at different sampling times (e.g. the age of the flower, weather conditions), and iv) depending upon the sampling method, different amounts of pollen and nectar can be collected from the same flower. Adequate knowledge of the floral biology and morphology of the test crop/plant is important in designing a sampling methodology that ensures a sufficient amount of collected material. For example, Knäbe et al. (2015) suggested that, based on the nectar yields of oilseed rape, for 200 µL nectar about 400 flowers have to be sampled. In such cases where the size of the samples collected in the field phase is lower than the sample size for which the method is validated, appropriate correction on the method validated limit of quantification (LOQ) (for the target sample size) can be applied, i.e. the LOQ should be increased to account for smaller than ideal sample availability of individual sampling events (refer e.g. to EFSA (European Food Safety Authority) (2018b)).

4.2.2. Residues in pollen and nectar collected from bees

Exposure can be refined by measuring the concentration of active substances and/or degradation products in pollen and nectar collected by sampling foraging bees that are either visiting flowers on the plant of interest or captured as they return to the hive. The recommendation for the number of sampling events should follow the recommendations in Section 4.2.1 but are also briefly summarised here. For applications during flowering a minimum of five sampling points are required; the first sampling should be performed on day before application, then immediately after application and then on at least three additional sampling points following application. Sampling points should cover the first five days after application and should occur when at least 50% of the focal crop is flowering at the time of application. For application before flowering, the collection of nectar and pollen using foraging honey bees should be scheduled to occur at least three time points (beginning, mid flowering and towards the end of flowering) over the exposure period for pre-flowering spray and granules applications and seed treatments.

Knaebe et al. (2014) state that 200 honey bees is generally the minimum number required to be collect enough nectar for analysis. Whilst this number is in line with the information from other higher tier field

studies submitted in the dossiers for market authorisation as well as in open literature papers (e.g., Rolke et al. (2016a)), this number should be adjusted to reflect the sensitivity of specific analytical techniques used to quantify residues at the lowest level of detection and will differ if sampling only for pollen.

Applicants/study directors should consider the following:

- When pollen from foragers is collected passively using pollen traps, particular attention should be paid on the trapping efficiency to plan the sampling schedule over the exposure period of the study.
- When analysing the concentration of PPP in nectar in a single sampling event, the nectar or pollen from multiple bees, this can be pooled before analysis but three replicates should be independently analysed to assess the uncertainty of the sampling method in the field.
- If a sampling event retrieves insufficient honey bees, pollen, or nectar than required in a single day the sampling can continue on the following day and both sets pooled.
- The total number of bees per time unit of sampling, the sub-samples, and the weight of the pooled sample should be recorded.

4.2.3. Knäbe et al. (2014); Rolke et al. (2016a)Further considerations on residues assessment

Pollen source analysis (palynology) can be used to ensure that bees foraged on the target crop and to evaluate the "dilution factor" of the exposure. In addition, if present, flower samples from wild flowers or crops flowering during the exposure period in the surrounding area of the treated plots, can be collected and analysed as reference checks for the pollen analysis. Visual determination method to assess the pollen source is considered too uncertain and should not be considered.

Analysis of **residues in soil** before and after pesticide application is particularly relevant for soil applied pesticides and for the exposure assessment in the permanent/succeeding crops scenarios. In this case, residues in the topsoil/root zone should be measured before the planting of succeeding crop/plant species. The soil sampling protocol should follow the principles reported in the Guidance Document for conducting pesticides terrestrial field dissipation studies (OECD (Organisation for Economic Co-operation and Development), 2016), taking into consideration that analysis of residues should be performed for soil depth segments relevant for the rooting depth of the succeeding crop to be tested. It is also recommended to collect one soil sample pooled from approximately 15-20 randomly selected locations within the study field. Additionally, the sampled soil cores should also be used for soil characterisation (soil type, particle size distribution, pH value, cation exchange capacity, calcium carbonate content and total organic carbon).

4.3. Sugar content in floral nectar and nutritional content of pollen

The effect assessment is based on the daily uptake of nectar and pollen. The daily uptake of nectar is calculated as the quotient of the sugar demand of the species divided by the sugar content of the nectar (see Section 4.2.2). Therefore, it is strongly recommended to measure in each nectar sample not only the concentration of the test substance but also **the sugar content** in order to refine the dietary consumption. Where field measurements of the sugar content are not available, the Tier 1 default sugar content (SN) values should be used in the risk assessment. The WG considered that a refinement of the SN parameter for the treated crop scenario is recommended if suitable and sufficient field data are available. This is applicable to any crop, in any Tier 1 sugar content category. As described in EFSA (European Food Safety Authority) (2013), at least five varieties have to be involved and the sugar content of the nectar in at least five locations for each variety has to be available. The WG recommends direct sampling from the nectaries in the field. The sugar concentration of the nectar can be determined in the field with hand-held refractometers (e.g., (Corbet, 2003) or, if the nectar is secreted in small quantities, alternative methods may be required (e.g., (Kearns and Inouye, 1993); (Dafni et al., 2005)). The sample size should not be fewer than 20 randomly selected individual plants at each field. Ideally, the time of sampling should be fitted to the intensive foraging period of the bees (i.e., ideal

meteorological conditions and when considerable foraging activity is observed). The samples obtained from the same field can be averaged. This sampling regime will result in at least 25 average data, which than can directly be used for refined (Tier 2) shortcut value calculations. It is, however noted, that the data available in the data base for Tier 1 (Annex C of the supplementary document) can be reused, which might reduce the number of varieties/locations to be required for the experimental data collection for this refinement step.

As regards the adjacent crop scenario and the succeeding crop scenario, the same principle applies (i.e., the SN parameter could be refined). However, any refinement for those scenarios becomes practical only if the crops of those scenarios are well defined (for which, no detailed guidance can be given). Otherwise, as in Tier 1, crops belonging to sugar category 1 will be used by default. No guidance can be given for the definition of the crops that might belong to the adjacent crop scenario and the succeeding crop scenario.

The composition of the plants of the non-crop scenarios (weeds in the field and plants at the field margin) varies in space and time. Therefore, any experimentally established value (obtained from a certain landscape at a certain time) might not be considered as sufficiently representative for a risk assessment.

Further details are available in Appendix S of EFSA (European Food Safety Authority) (2013).

As regards information on **pollen consumption**, it is recommended to measure the nutritional content (i.e., a quali-quantitative assessment of the protein ad lipid pollen mactronutrients) of each pollen sample in addition to the pesticide residues.

5. Analytical phase

For the analytical phase, the requirements outlined in the Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes (European Commission, 2021) should be fulfilled.

Basically, for higher tier field studies aiming to measure residues to refine the exposure for honey bees, the main quality criteria for the reliability of the analytical phase are (EFSA (European Food Safety Authority), 2018b, 2019):

- the analysed matrix should be clearly identified
- the analytical sample size should be appropriate
- the sample storage (including preservation during transport) and stability should be reported and considered appropriate. Stability should particularly be checked when the residues are analysed more than 30 days after sampling
- the analytical procedure (including sampling preparation, extraction and purification) should be clearly described
- linearity, accuracy, and precision should be appropriate
- the LOQ and LOD should be clearly reported
- the LOQ and LOD should be checked in order to ensure that they are low enough to detect residue levels that exert toxic effects to honey bees
- representative chromatograms should be reported
- it should be clearly indicated whether the results are expressed in terms of fresh or dry weight of the sample material
- calculations should be clearly reported

6. Assessment of residue dissipation in pollen and nectar

The higher tier exposure studies can also be used for refining the default DT50 values of 2 days (nectar) and 3 days (pollen) which are used in the dietary model for above-soil contamination (see section 5.3.13 of the Guidance Document). However, in order to do this, residue concentrations in nectar and pollen have to be measured often enough over an appropriate time period as a way to accurately reflect the

residues decline. This may require measurements at additional sampling times compared to the recommendations above. As already indicated in paragraph 4.2.1, a minimum of five quantifiable time points should be available in order to derive robust dissipation kinetics and only in some exceptional cases, four points may be enough (e.g. fast dissipation or decline of metabolites), but there should never be fewer than four time points. The principles and practical guidance to be followed in these studies can be found in Annex A to the Guidance Document on the refinement of residue dissipation in the ERA of pesticides for terrestrial organisms.

7. Assessment of residue dissipation in plants

The recommendations and suggested quality criteria provided in Annex A to the Guidance Document on the refinement of residue dissipation in the ERA of pesticides for terrestrial organisms should be followed for field residues aimed to refine the dissipation in plants.

8. Considerations for exposure refinement for the succeeding crop scenario

To date, two different approaches have been followed to investigate the potential exposure of bees to residues in flowering succeeding crops in regulatory higher tier studies (e.g. EFSA (European Food Safety Authority) (2018b)). In one case, the succeeding crop is grown on soils treated over their complete area with the test substance to obtain a theoretical plateau concentration of this substance in soil, i.e. the concentration of the test substance in pollen and nectar is the consequence of the uptake of the substance via the roots of the succeeding crop grown under conditions of "forced" soil residues. Alternatively, the untreated succeeding crops are sown in soils with a documented history of several years of use of the test substance, and thus exposed to actual accumulated residues in the agricultural soil. During the EFSA peer review of some neonicotinoids under Regulation (EC) No 1107/2009, it was agreed (e.g. EFSA (European Food Safety Authority) (2018a)) that higher tier studies with 'forced exposure' are less representative of the exposure situation under field conditions where the substance residues in soil have already undergone natural ageing processes, making them potentially less available for plant uptake. Therefore, it is recommended to measure residues in pollen and nectar from succeeding crops sown on soils with naturally aged residues established under realistic field conditions following several years of sustained application of the compound of interest. As it is very likely to be difficult to find locations with historical usage of the target substance that will also fulfil the criteria set for "minimum alternative forage" field studies (Section 1.3), it is recommended to measure residues in nectar and pollen only directly from flowers. The selection of the 5 trial sites (both semi-field and open field) for the exposure refinement for the succeeding crop exposure scenario should consequently meet the above requirements (i.e. agricultural field with a documented history of sustained use of the active substance of interest and supported by soil residue concentrations measured prior to the planting of the succeeding crops) in addition to the general specifications described in Section 1.3. The selected field can be considered suitable for the study if the measured soil concentrations of the test substance are equal to or higher than the accumulated soil predicted environmental concentration ($PEC_{plateau}$) for the specific representative use in the GAP and calculated in line with the EU regulatory guidance available at the time of the marketing authorisation application. Field studies with "forced exposure" can be considered appropriate to refine exposure of new active substances and existing substances for which it is impractical to find agricultural fields with soil accumulated residues following repeated applications over the years. Recommendations on soil sampling and analysis are given in Section 4.2.3.

9. Considerations to refine the exposure for field margins and adjacent crop scenarios

Very few field experiments have been conducted so far to measure residues in pollen and nectar in off-field crops/plants and, substantially, they were limited to field trials with neonicotinoid-treated seeds. Therefore the following recommendations are focussed on residues assessment from dust depositions to crops/plants at the field margin or at the adjacent field. The refinement of the exposure of off-field plants/crops following spray drift depositions can be based on RUDs derived from the treated crop when attractive crops are used. It should be noted that the default E_f (i.e. the deposition factor) cannot be refined by means of dedicated experiments.

The exposure assessment schemes for field margins and adjacent crops after seed treatments and granule applications include the option to measure RUD values for relevant crops after dust deposition (see relevant part of Appendix N in EFSA (European Food Safety Authority) (2013)). In these flow charts, the RUD is not considered a major driver for the 90th percentile case. Therefore, the aim of these measurements is to assess an average or median RUD for the crop/plant considered. Very little experience has been gained so far with these experiments. Therefore, it is tentatively proposed to perform two field experiments (preferably with two different field crops for the field margins and with this adjacent crop) and use the average RUD of the two. Until the SANCO draft Guidance on seed treatment (European Commission, 2012) is formally adopted, it is proposed to follow the recommendations of the Guideline 7029/VI/95 (rev. 5) and that of the Biologische Bundesanstalt für Land- und Forstwirtschaft Bundesrepublik Deutschland (1992) for exposure characterization of dust of active substances derived from solid formulations and treated seeds. In the field experiments designed to measure dust deposition during sowing of coated seed or during granule application, it is common practice to use Petri dishes (with glycerol/water or acetonitrile/water solutions) distributed horizontally on the bare soil at certain distances to the treated area (i.e. 2D measurements). Additionally, vertical gauze nets (also wetted with glycerol/water solution to enhance dust adsorption) can be placed directly adjacent to the sowing area to represent a three-dimensional (3D) structure, i.e. to measure aerial and ground dust drift deposits (e.g. Heimbach et al. (2014)). Special requirements for this study type are wind speed ranging between 1.0 and 5.0 m/s at 1 m above the crop, but at least 2 m above ground surface and wind direction of 90° ± 30° to the drilling direction. A review on measuring techniques and protocols for field measurements of dust drift from pesticide seed dressing during sowing can be found in Nuyttens et al. (2013). It is important to note that, when conducting 3D measurements with vertical gauze, it is essential to clearly report how results, expressed in g a.s./ha, are derived and what they represent. Also, we recommend developing guidance on measurement, use and interpretation of values estimating exposure to field margin vegetation or adjacent crops when measured at individual trial sites, as soon as possible.

The RUD values have to be based on the combination of measurements of (i) the dust deposition onto bare soil, and (ii) the resulting concentrations in nectar and pollen. The deposition onto bare soil is needed because the RUDs for field margins and adjacent crops cannot be based on the dose for the treated crop because the deposition onto these types of plants is highly variable. It may also be useful to measure the dust deposition on the plants considered to obtain additional information on the relationship between deposition onto bare soil and onto the flowers of the crop because this information is yet scarce. The concentrations have to be measured during the whole sowing procedure, immediately after application and on at least three additional sampling dates. Upon analysis the residues should show a clear decline over the sampling period. If this doesn't occur, then the study should be repeated as it is key that the peak residue with respect to time is determined as a basis of the RUD values.

The RUD values should be calculated as the quotient of the concentration in nectar or pollen (in mg/kg) divided by the mass deposited per surface area of bare soil (in kg/ha). In principle the course of time of the RUD values can be used to refine the default DT_{50} values which are used for calculating the chronic and larvae intake of nectar (DT50 = 2 days) and pollen (DT50 = 3 days).

10. Considerations to refine acute contact exposure

One of the higher tier options for the contact exposure assessment is to perform field studies to assess the 90th percentile case for the cumulative frequency distribution of the mass per bee due to contact exposure under practical conditions. In each of these studies the aim is to measure the residues load from spray deposits (overspray or spray drift) or dust particles directly on individual bees foraging the crop during or immediately after application. Until now, very few field investigations are available about acute contact exposure under practical agricultural conditions. For example, Koch and Weisser (1997) suggested that at least 100 bees, flying on the usual route between the hive and food sources, should be sampled immediately after the spray application and at time intervals of no more than 10 min until approximately one hour afterwards. It is recommended to select an intensively flowering field crop that is very attractive to bees (such as oilseed rape and *Phacelia* for downward spray applications or apple trees for sideward/upward spray applications) in order to ensure that bees will most likely forage inside the field plot when starting the pesticide application. The bees should be sampled in the treated field using e.g. a modified hoover/vacuum collector and collecting dead bees from the ground in front of the apiary (Girolami et al., 2012) or from bee traps (Pistorius et al., 2015) on condition that the residue

analysis excludes the bees' honey stomach and pollen loads. The residue on each individual forager should be determined because it is the aim to assess the cumulative frequency distribution of the individual foragers. In this type of field trial, the record of meteorological conditions in relation to the placement of the hive is very important in order to correctly interpret the results. In some cases (e.g. Girolami et al. (2012)), the sprayer or drilling machine is placed on the flight path of the foraging bees between the hive and a sugar dispenser to ensure that exposure is more likely to occur during the pesticide application. Alternatively, bees are kept inside mesh cages placed at the margin of the treated area (e.g. Marzaro et al. (2011)). Since very little experience has been gained so far with this type of field exposure study, we recommend developing further guidance in future.

11. Considerations to refine exposure for bumble bees and solitary bees

The majority of the higher tier studies conducted in the last years to refine pesticide exposure to bees, are concerned with honey bees. As a matter of fact, validated methodologies for semi-field and field regulatory testing have not yet been well established for bumble bees and solitary bees. An important step forward to fill in this gap has been made by Cabrera et al. (2016), who provided some recommendations on the development of higher-tier testing guidelines focused on the assessment of pesticide effects on bumble bees.

In principle, the same protocols defined to investigate the level of residues in pollen and nectar to refine exposure to honey bees under field conditions, can be applied also to semi-field and field studies conducted with solitary bees and bumble bees (e.g. Rolke et al. (2016b) and (Hodge, 2019)). However, some important aspects regarding the morphological and behavioural characteristics of non-*Apis* pollinators should be considered when designing residue studies with these bee species. In particular, in order to get the minimum amount necessary for the residue analysis, the sampling methodology of pollen and nectar should be adapted as suggested in Knäbe et al. (2015).

Landscape characterisation for residue field studies with bumble bees and solitary bees should be provided for a 1.5 km radius around the nests/colonies at the focal field in line with the recommendations provided in paragraph 1.3. This area is considered to cover a worst-case exposure situation for non-*Apis* bees taking into consideration average foraging distances (Kendall et al., 2022). The 1.5 km distance could be reduced in the future with the support of better data.

Higher tier studies investigating exposure levels from exposure routes other than oral consumption or acute contact via air particles for adult non-*Apis* foragers (e.g. contact exposure for larvae or oral exposure via plant surfaces for adult bees) are not considered in this guidance.

11.1. Bumble bees

As for honey bee studies, nectar and pollen can be sampled directly from flowers or from bumble bees returning to the nest after foraging.

Collecting pollen from bumble bees can be more challenging than for honey bees as pollen traps work by forcing a bee through a small entrance, which effectively knocks the pollen load off the bees' hind legs into a collection tray. The large variation in bumble bee worker size relative to honey bee workers can reduce the efficacy of standard pollen traps, making the selection of entrance size problematic for an effective sampling (Knäbe et al., 2014). In order to overcome this limitation, bumble bee specific pollen traps have been developed. Judd et al. (2020) proposed a new technique to remove the corbicular pollen loads from returning bumble bees foragers using 3D printed pollen traps which could be useful in this context. Although time consuming and labour intensive, an alternative method is to manually remove the pollen loads from foragers captured on flowers or at nest entrances. Bees foraging on flowers can be captured using insect nets (Leonhardt & Bluthgen, 2011;Botias et al., 2017) or with a modified hoover/vacuum collector (Sterk et al., 2016) whilst bees returning from foraging can be captured using nets, tubes or forceps as they re-enter the colony ((Arce et al., 2017); (Hodge, 2019), (Rundlöf et al., 2022)).

When collecting nectar samples, semi-field residues studies with bumble bees may be a better option than honey bees as suggested by Knäbe et al. (2017). Other advantages for working with bumble bees for residues studies are: (i) sampling can take place in regions with colder temperatures than honey

bees(Heinrich, 2004); (ii) a broader variety of crops can be sampled (Knäbe et al., 2017); (iii) bumble bee foragers visit 2-3 times more flowers per trip (Gradish et al., 2018); (iv) additional colonies can be ordered on short notice (Knäbe et al., 2017).

As regards contact exposure assessment, ~~however,~~ it should be noted that the exposure level may be lower for individual adult bumble bees compared with honey bees due to the presence of dense hair which impedes substances from making contact with their cuticle (Gradish et al., 2018). Nevertheless, the values obtained from these field experiments will represent a worst case exposure scenario in terms of Tier 2 PEQ_{co} values.

11.2. Solitary bees

To date, very few field studies have been conducted with solitary bees (e.g. (Rundlöf et al., 2015), (Peters et al., 2016; Woodcock et al., 2017; Ruddle et al., 2018)). The following considerations might be of some relevance in designing and conducting pollen and nectar residue studies:

- the typical size of 1 ha of test field trials is much more representative of the foraging area of solitary bees than honey bees (Sgolastra et al., 2019)
- the foraging range of *Osmia bicornis*, the most commonly used solitary bee species in European ecotoxicological semi-field and field studies, and for many other solitary bee species is typically less than 600 m ((Gathmann and Tscharntke, 2002; Zurbuchen et al., 2010; Kendall et al., 2022)) and therefore, the area with no other bee attractive crops near the trial site to avoid a landscape dilution can be reduced from 4 km to 1 km radius
- the flight season of a population at a given site usually lasts 1-3 months in spring or summer
- in full field conditions, solitary bees may ignore the flowering test crop, but this can be reduced if excluding alternative forage resources
- there are methods developed for collecting larval provisions in brood cells, mainly consisting of pollen, but not for collecting pollen or nectar from adult solitary bees
- to sample pollen in the brood cells of solitary bees, nesting units/blocks should be placed in a tunnel within the crop (Knäbe et al., 2014), or at the edge of the test crop and protected against wind, rain and predation by birds (Rolke et al., 2016b)
- the pollen provisions from at least two different brood cells are considered by Knäbe et al. (2014) sufficiently large to be analysed
- in order to ensure that all sampled pollen from the same nesting unit is fresh and from roughly the same time, (Rolke et al., 2016b) recommend to sample pollen from yet incomplete cells that are unsealed and do not contain any egg. Alternatively, the last closed brood cell can be marked the day before the anticipated sampling day (Knäbe et al., 2014).

Information on non-selective and selective sampling techniques of pollen and nectar collected from wild bees can be found in Fricke et al. (2020).

12. References

Arce AN, David TI, Randall EL, Ramos Rodrigues A, Colgan TJ, Wurm Y and Gill RJ, 2017. Impact of controlled neonicotinoid exposure on bumblebees in a realistic field setting. Journal of Applied Ecology, 54:1199-1208. doi: https://doi.org/10.1111/1365-2664.12792

Barnsley SL, Lovett AA and Dicks LV, 2022. Mapping nectar-rich pollinator floral resources using airborne multispectral imagery. Journal of Environmental Management, 313:114942. doi: https://doi.org/10.1016/j.jenvman.2022.114942

Beulke S, Wilde TD, Balderacchi M, Garreyn F, van Beinum W and Trevisan M, 2015. Scenario selection and scenario parameterisation for permanent crops and row crops on ridges in support of predicting environmental concentrations of plant protection products and their transformation products in soil. EFSA Supporting Publications, 12:813E

Biologische Bundesanstalt für Land- und Forstwirtschaft Bundesrepublik Deutschland, 1992. Richtlinien für die Prüfung von Pflanzenschutzmitteln und Pflanzenschutzgeräten.

Botías C, David A, Horwood J, Abdul-Sada A, Nicholls E, Hill E and Goulson D, 2015. Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. Journal of Environmental science technology, 49:12731-12740

Byrne FJ, Visscher PK, Leimkuehler B, Fischer D, Grafton-Cardwell EE and Morse JG, 2014. Determination of exposure levels of honey bees foraging on flowers of mature citrus trees previously treated with imidacloprid. Journal of Pest management science, 70:470-482

Cabrera AR, Almanza MT, Cutler GC, Fischer DL, Hinarejos S, Lewis G, Nigro D, Olmstead A, Overmyer J and Potter DA, 2016. Initial recommendations for higher-tier risk assessment protocols for bumble bees, Bombus spp.(Hymenoptera: Apidae). Journal of Integrated Environmental Assessment Management, 12:222-229

Căuia E, Siceanu A, Vișan GO, Căuia D, Colța T and Spulber RA, 2020. Monitoring the field-realistic exposure of honeybee colonies to neonicotinoids by an integrative approach: A case study in Romania. Journal of Diversity, 12:24

Corbet SA, 2003. Nectar sugar content: estimating standing crop and secretion rate in the field. Journal of Apidologie, 34:1-10

D'Andrimont R, Verhegghen A, Meroni M, Lemoine G, Strobl P, Eiselt B, Yordanov M, Martinez-Sanchez L and van der Velde MESSD, 2021. LUCAS Copernicus 2018: Earth-observation-relevant in situ data on land cover and use throughout the European Union. Earth System Science Data, 13:1119-1133

Dafni A, Kevan PG and Husband BC, 2005. Practical pollination biology.

Dominik C and Schweiger O (Helmholtz Centre for Environmental Research - UFZ), 2019. Report on landscape context of field sites. Deliverable D1.2 EU Horizon 2020 PoshBee Project.

EFSA (European Food Safety Authority), 2013. Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). Efsa Journal, 11:3295

EFSA (European Food Safety Authority), 2014. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. Efsa Journal, 5:3662. doi: https://doi.org/10.2903/j.efsa.2014.3662

EFSA (European Food Safety Authority), 2018a. Evaluation of the data on clothianidin, imidacloprid and thiamethoxam for the updated risk assessment to bees for seed treatments and granules in the EU. Efsa Journal,

EFSA (European Food Safety Authority), 2018b. Peer review of the pesticide risk assessment for bees for the active substance clothianidin considering the uses as seed treatments and granules. Efsa Journal, 16:e05177. doi: https://doi.org/10.2903/j.efsa.2018.5177

EFSA (European Food Safety Authority), 2019. Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA Supporting Publications,

ESA STAT Dust Working Group, 2011. Physical method–assessment of free floating dust and abrasion particles of treated seeds as a parameter of the quality of treated seeds–Heubach test. Version, 1:1

European Commission, 1997. Appendix B. General recommendations for the design, preparation and realization of residue trials. Annex 2. Classification of (minor) crops not listed in the Appendix of Council Directive 90/642/ EEC.

European Commission, 2011. Guidance document on the preparation and submission of dossiers for plant protection products according to the "risk envelope approach".

European Commission, 2012. Draft Guidance document on authorisation of plant protection products for seed treatment.

European Commission, 2018. Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey.

European Commission, 2019. Technical Guidelines on data requirements for setting maximum residue levels, comparability of residue trials and extrapolation of residue data on products from plant and animal origin.

European Commission, 2021. Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes.

Fricke J, Klein O and Knäbe S, 2020. Pollinator monitoring in agroecosystems-general methods for evaluations in field studies. Julius-Kühn-Archiv:129-131

García-Feced C, Weissteiner CJ, Baraldi A, Paracchini ML, Maes J, Zulian G, Kempen M, Elbersen B and Pérez-Soba M, 2015. Semi-natural vegetation in agricultural land: European map and links to ecosystem service supply. Agronomy for Sustainable Development, 35:273-283

Gathmann A and Tscharntke T, 2002. Foraging ranges of solitary bees. Journal of animal ecology, 71:757-764

Girolami V, Marzaro M, Vivan L, Mazzon L, Greatti M, Giorio C, Marton D and Tapparo A, 2012. Fatal powdering of bees in flight with particulates of neonicotinoids seed coating and humidity implication. Journal of Applied Entomology, 136:17-26

Gradish AE, Van der Steen J, Scott-Dupree CD, Cabrera AR, Cutler GC, Goulson D, Klein O, Lehmann DM, Lückmann J, O'Neill B, Raine NE, Sharma B and Thompson H, 2018. Comparison of Pesticide Exposure in Honey Bees (Hymenoptera: Apidae) and Bumble Bees (Hymenoptera: Apidae): Implications for Risk Assessments. Environmental Entomology, 48:12-21. doi: 10.1093/ee/nvy168 %J Environmental Entomology

Heimbach U, Stähler M, Schwabe K, Schenke D, Pistorius J and Georgiadis P-T, 2014. Emission of pesticides during drilling and deposition in adjacent areas. Julius-Kühn-Archiv:68

Heinrich B, 2004. Bumblebee economics, Harvard University Press.

Hodge S, Stout, J., 2019. Protocols for methods of field sampling. Deliverable D1.1 PoshBee Project

Holzschuh A, Dainese M, González-Varo JP, Mudri-Stojnić S, Riedinger V, Rundlöf M, Scheper J, Wickens JB, Wickens VJ and Bommarco R, 2016. Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. Ecology letters, 19:1228-1236

Human H, Brodschneider R, Dietemann V, Dively G, Ellis JD, Forsgren E, Fries I, Hatjina F, Hu FL, Jaffe R, Jensen AB, Kohler A, Magyar JP, Ozkyrym A, Pirk CWW, Rose R, Strauss U, Tanner G, Tarpy DR, van der Steen JJM, Vaudo A, Vejsnaes F, Wilde J, Williams GR and Zheng HQ, 2013. Miscellaneous standard methods for Apis mellifera research. Journal of Apicultural Research, 52:55. doi: 10.3896/ibra.1.52.4.10

Judd HJ, Huntzinger C, Ramirez R and Strange JPJJ, 2020. A 3D printed pollen trap for bumble bee (Bombus) hive entrances. Journal of Visualized Experiments:e61500

Kearns CA and Inouye DW, 1993. Techniques for pollination biologists, University press of Colorado.

Kendall LK, Mola JM, Portman ZM, Cariveau DP, Smith HG and Bartomeus I, 2022. The potential and realized foraging movements of bees are differentially determined by body size and sociality. Ecology, 103:e3809. doi: https://doi.org/10.1002/ecy.3809

Kiljanek T, Niewiadowska A, Małysiak M and Posyniak A, 2021. Miniaturized multiresidue method for determination of 267 pesticides, their metabolites and polychlorinated biphenyls in low mass beebread samples by liquid and gas chromatography coupled with tandem mass spectrometry. Talanta, 235:122721. doi: 10.1016/j.talanta.2021.122721

Knäbe S, Mack P, Appeltauer A and Kling A, 2017. How do Regulatory Requirements and Assumptions Correlate to Practical Experience in Residue Studies with Nectar and Pollen? Proceedings of the SETAC Europe 25th Annual Meeting

Knäbe S, Mack P and Bocksch S, 2015. Sampling Methods of Nectar and Pollen of Brassica napus (oilseed rape) and Phacelia tanacetifolia (Lacy Phacelia). Proceedings of the SETAC Annual European Meeting

Knäbe S, Mack P, Chen A and Bocksch S, 2014. Available methods for the sampling of nectar, pollen, and flowers of different plant species. Julius-Kühn-Archiv:131

Koch H and Weisser P, 1997. Exposure of honey bees during pesticide application under field conditions. Journal of Apidologie, 28:439-447

Leip A, Marchi G, Koeble R, Kempen M, Britz W and Li C, 2008. Linking an economic model for European agriculture with a mechanistic model to estimate nitrogen and carbon losses from arable soils in Europe. Journal of Biogeosciences, 5:73-94

Lückmann J and Schmitzer S, 2019. The Oomen bee brood feeding test–revision of the method to current needs and developments. EPPO Bulletin, 49:137-146

Marzaro M, Vivan L, Targa A, Mazzon L, Mori N, Greatti M, Toffolo EP, Di Bernardo A, Giorio C and Marton D, 2011. Lethal aerial powdering of honey bees with neonicotinoids from fragments of maize seed coat. Bulletin of Insectology, 64:119-126

McKenna MA and Thomson JD, 1988. A technique for sampling and measuring small amounts of floral nectar. Journal of Ecology, 69:1306-1307

Morrant DS, Schumann R and Petit S, 2009. Field methods for sampling and storing nectar from flowers with low nectar volumes. Annals of Botany, 103:533-542

Nuyttens D, Devarrewaere W, Verboven P and Foqué D, 2013. Pesticide-laden dust emission and drift from treated seeds during seed drilling: a review. Journal of Pest management science, 69:564-575

OECD (Organisation for Economic Co-operation and Development), 2007. Test No. 501: Metabolism in Crops.

OECD (Organisation for Economic Co-operation and Development), online. ENASGIPS, 2015. Europe – North America Soil Geographic Information for Pesticide Studies (ENASGIPS), Version 3.0. Available online: http://www.enasgips3.org/home.html [Accessed: Access Date].

OECD (Organisation for Economic Co-operation and Development), 2016. Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies. Available online: https://www.oecd-ilibrary.org/content/publication/82afb9d0-en

OEPP/EPPO (European and Mediterranean Plant Protection Organization/Organisation européenne et méditerranéenne pour la protection des plantes), 2010. Efficacy evaluation of plant protection products: side-effects on honey bees. PP 1/170 (4). OEPP/EPPO Bull., 40:313-319

OEPP/EPPO (European and Mediterranean Plant Protection Organization/Organisation européenne et méditerranéenne pour la protection des plantes), 2021. PP 1/239 (3) Dose expression for plant protection products. OEPP/EPPO Bull., 51:10-33. doi: https://doi.org/10.1111/epp.12704

Peters B, Gao Z and 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. doi: 10.1007/s10646-016-1729-4

Pistorius J, Wehner A, Kriszan M, Bargen H, Knäbe S, Klein O, Frommberger M, Staehler M and Heimbach U, 2015. Application of predefined doses of neonicotinoid containing dusts in field trials and acute effects on honey bees. Bulletin of Insectology, 68:161-172

Rolke D, Fuchs S, Grünewald B, Gao Z and Blenau W, 2016a. Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on honey bees (Apis mellifera). Journal of Ecotoxicology, 25:1648-1665

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

Ruddle N, Elston C, Klein O, Hamberger A and Thompson H, 2018. Effects of exposure to winter oilseed rape grown from thiamethoxam-treated seed on the red mason bee Osmia bicornis. Environ Toxicol Chem, 37:1071-1083. doi: https://doi.org/10.1002/etc.4034

Rundlöf M, Andersson GK, Bommarco R, Fries I, Hederström V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR and Yourstone J, 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature, 521:77-80

Rundlöf M, Stuligross C, Lindh A, Malfi RL, Burns K, Mola JM, Cibotti S and Williams NM, 2022. Flower plantings support wild bee reproduction and may also mitigate pesticide exposure effects. Journal of Applied Ecology, 59. doi: https://doi.org/10.1111/1365-2664.14223

Sgolastra F, Hinarejos S, Pitts-Singer TL, Boyle NK, Joseph T, Lūckmann J, Raine NE, Singh R, Williams NM and Bosch J, 2019. Pesticide Exposure Assessment Paradigm for Solitary Bees. Environmental Entomology, 48:22-35. doi: 10.1093/ee/nvy105 %J Environmental Entomology

Sterk G, Peters B, Gao Z and 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 (Bombus terrestris). Ecotoxicology, 25:1666-1678. doi: 10.1007/s10646-016-1730-y

Van der Steen J, In preparation. Assessment of the welfare of the honey bee colony in the field and laboratory, Garrido, Nanetti (Ed.).

Westphal C, Bommarco R, Carré G, Lamborn E, Morison N, Petanidou T, Potts SG, Roberts SP, Szentgyörgyi H and Tscheulin T, 2008. Measuring bee diversity in different European habitats and biogeographical regions. Journal of Ecological monographs, 78:653-671

Williams GR, Alaux C, Costa C, Csáki T, Doublet V, Eisenhardt D, Fries I, Kuhn R, McMahon DP, Medrzycki P, Murray TE, Natsopoulou ME, Neumann P, Oliver R, Paxton RJ, Pernal SF, Shutler D, Tanner G, van der Steen JJM and Brodschneider R, 2013. Standard methods for maintaining adult Apis mellifera in cages under in vitro laboratory conditions. Journal of Apicultural Research, 52:1-36. doi: 10.3896/IBRA.1.52.1.04

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 and Pywell RF, 2017. Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science, 356:1393-1395. doi: doi:10.1126/science.aaa1190

Zurbuchen A, Landert L, Klaiber J, Müller A, Hein S and Dorn S, 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. Journal of Biological conservation, 143:669-676

Appendix A – PEQ_{di} calculations from field residue data

General guidance on the generation of RUDs from field exposure studies are provided in Section 5.5.8 of the Guidance Document. This Appendix aims to present the specific steps to follow in order to calculate Tier 2 PEQ_{di} values based on the residue data measured in field exposure studies (Figure A1).

The sampling frequency and timing schedule in a field study depends on the type and time of application and whether pollen and nectar are collected directly from flowers or from bees (Sections 4.2.1 and 4.2.2). If pollen and/or nectar are collected from flowers with spray application during flowering, one sampling is considered sufficient to derive field RUD. In all the other situations, at least three sampling times are required. At each sampling event, samples are taken in triplicate, analysed separately and the geometric mean of the three residue values is calculated. When the residue detected in a trial is lower than the LOQ but greater than the limit of detection, as worst-case assumption, the residue should be considered to be equal to the LOQ for the RUD calculation. If no residue are detected, the residue should be considered to be equal to the LOD for the RUD calculation.

For each field study, the highest geometric mean residue value of the timepoints should be retained as representative of the exposure of that field.

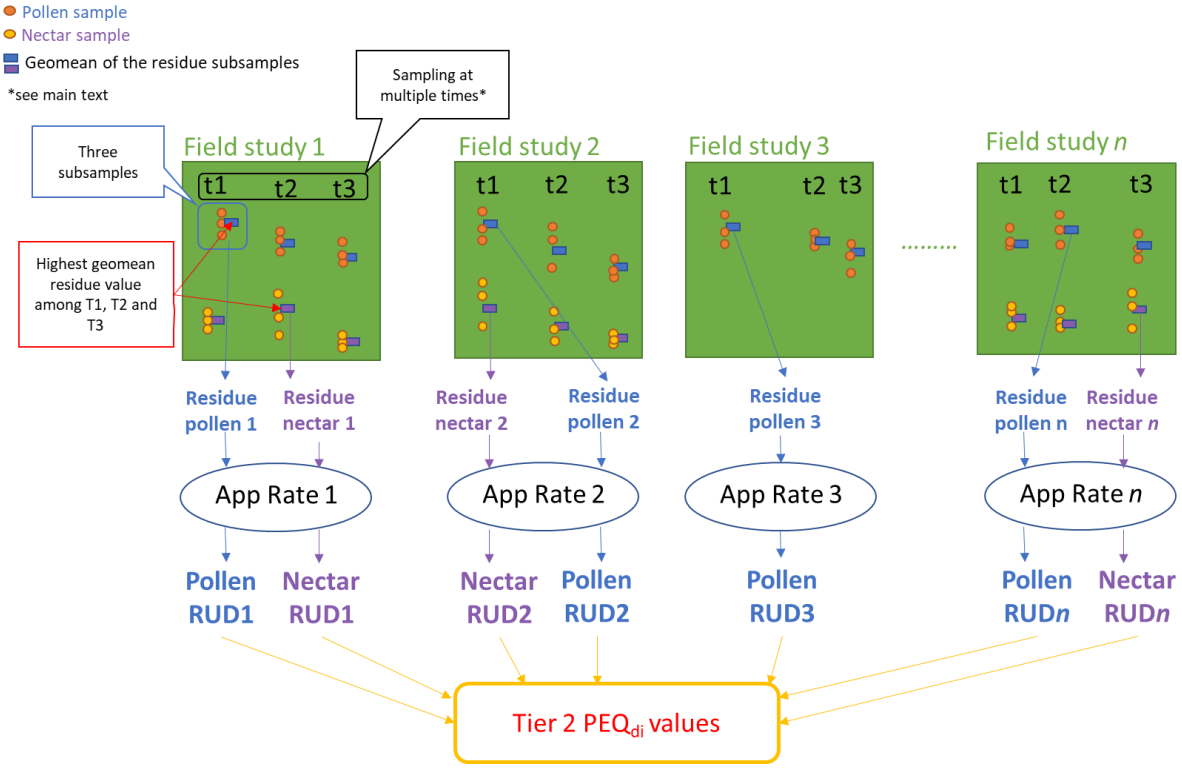

Figure A1. General overview of Tier 2 PEQ_{di} calculations with field residue data (see text for details)

The residue values selected from each field study are then normalised for the specific application rate used in the study to calculate Residue per Unit Dose (RUD) to make the residue independent from the application rate of the PPP that was applied in the specific field. A minimum of five (for "minimum alternative forage" studies) or fifteen (for "randomly selected landscape" studies) RUD values for pollen and nectar are required to perform a refined exposure assessment for each exposure scenario for each use under consideration. Finally, the overall Tier 2 PEQ_{di} value is calculated based on the exposure assessment models described in Section 5.1 of the Guidance Document, by substituting the default Tier 1 RUD_{po} and RUD_{ne} values with the RUD values measured in the field studies. It is important to note that all the selected RUDs available for the specific exposure scenario and for the use under evaluation are incorporated into the overall 90th percentile PEQ_{di} calculation.

Guidance on how to calculate EED (Estimated Exposure Dose) in higher tier effects studies can be found in Section 4.2.3 of Annex C to the Guidance Document.