DRAFT GUIDANCE OF EFSA

EFSA Draft Guidance Document on the Risk Assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)[1]

European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Guidance Document is intended to provide guidance for notifiers and authorities in the context of the review of Plant Protection Products (PPPs) and their active substances under Regulation (EC) 1107/2009. The scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA, 2012a) provided the scientific basis for the development of the Guidance Document. Specific Protection Goals were agreed in consultation with the Standing Committee on the Food Chain and Animal Health. The Guidance Document suggests a tiered risk assessment scheme with a simple and cost effective First Tier to more complex Higher Tier studies under semi-field and field conditions. Each of the tiers will have to ensure that the appropriate level of protection is achieved.

KEY WORDS

Honey bees, risk assessment, Guidance Document, Pesticides, *Apis mellifera*, *Bombus*, Solitary bees

[1] On request from [requesting party] [OR] EFSA], Question No EFSA-Q-YYYY-NNNNN, approved on DD Month YYYY.
[2] Correspondence: ppr@efsa.europa.eu
[3] Acknowledgement: EFSA wishes to thank the members of the Working Group:

Suggested citation: European Food Safety Authority; EFSA Guidance Document on the Risk Assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 20YY;volume(issue):NNNN. [202 pp.] doi:10.2903/j.efsa.20YY.NNNN. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

EFSA was asked by the European Commission to develop a Guidance Document on the risk assessment of Plant Protection Products on bees. The Guidance Document is intended to provide guidance for notifiers and authorities in the context of the review of Plant Protection Products (PPPs) and their active substances under Regulation (EC) 1107/2009. The scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA, 2012a) provided the scientific basis for the development of the Guidance Document.

The process of the development of the Guidance Document follows the methodology of definition of Specific Protection Goals (SPG) as outlined in the Scientific Opinion of EFSA's PPR Panel (EFSA, 2010). The Standing Committee on the Food Chain and Animal Health was consulted for the appropriate levels of protection (e.g. to make choices on the magnitude of effects, duration of effects and exposure percentiles).

The Guidance Document suggests proposed the implementation of a tiered risk assessment scheme with a simple and cost effective First Tier to more complex Higher Tier studies under semi-field and field conditions. Each of the tiers will have to ensure that the appropriate level of protection is achieved.

More detailed guidance on specific aspects of laboratory studies and Higher Tier risk assessments are given in the Appendices. A need was identified for test protocols for bumble bees and solitary bees. Potential protocols are available in the published literature and first proposals are made in the Appendices. It is important that fully validated test protocols are developed in future.

Note: If there is no abstract then the summary will begin on the first page and the key words section will appear after the summary.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is currently revising the European Guidance Document on terrestrial ecotoxicology elaborated by the Commission and experts from Member States. In the context of this revision, the bees risk assessment will also be addressed.

Members of the European Parliament and beekeepers' associations have expressed their concerns to the Commission as to the appropriateness of the current risk assessment scheme, and in particular on the EPPO[4] "Environmental risk assessment scheme for Plant Protection Products – Chapter 10: honeybees" revised in September 2010 with ICPBR[5] recommendations.

Considering the importance and the sensitiveness of this issue, and in line with the aim of the Commission Communication on Honeybee Health (COM (2010) 714 final)[6] adopted on 6 December 2010, the Commission considers that the revised EPPO assessment scheme would need further consideration by EFSA in an Opinion on the science behind the risk assessment for bees and that a Guidance Document on the risk assessment of Plant Protection Products on bees should be developed.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

A scientific Opinion of the PPR Panel on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus spp*. and solitary bees) will be prepared.

In particular the following issues will be addressed:

- The assessment of the acute and chronic effects of Plant Protection Products on bees, including the colony survival and development.
- The estimation of the long-term effects due to exposure to low concentrations
- The development of a methodology to take into account cumulative and synergistic effects.
- The evaluation of the existing validated test protocols and the possible need to develop new protocols, especially to take into account the exposure of bees to pesticides through nectar and pollen.

In order to have the possibility for stakeholders and the interested public to comment on the draft Guidance Document, we propose to include a round of public consultations on the draft Guidance Document. An Opinion on the science behind the Guidance Document could be delivered by April 2012 and a final Guidance Document in December 2012.

CONTEXT OF THE SCIENTIFIC OUTPUT

The Guidance Document is intended to provide guidance for notifiers and authorities in the context of the review of Plant Protection Products (PPPs) and their active substances under Regulation (EC) 1107/2009.

[4] European and Mediterranean Plant Protection Organization
[5] International Commission for Plant-Bee Relationships Statutes
[6] Communication from the Commission to the European Parliament and the Council on Honeybee Health, COM(2010) 714 final, adopted on 06/12/2010

The scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA, 2012a) provided the scientific basis for the development of the Guidance Document.

A public consultation is foreseen in order to give stakeholders and the interested public the opportunity to comment on the draft Guidance Document.

1. Introduction

A decline of some pollinator species was reported in several different regions of the world (Biesmeijer et al., 2006; Committee on the status of Pollinators in North America, 2007). Bee poisoning incidents were reported in Europe (e.g. exposure to dust from seed treatments). Pollination is a very important ecosystem service for food production and maintainance of biodiversity (Gallai et al., 2009). The question on the causes of the observed declines received a lot of attention from regulatory authorities. Research activities and monitoring of honey bee colony losses and bee poisoning incidents were initiated.

Pesticides were often considered as one of the factors contributing to the decline of some insect pollinator species. Concerns were raised by Members of the European Parliament and beekeepers' associations on the appropriateness of the current risk assessment schemes for Plant Protection Products. The European Commission tasked EFSA to issue an Opinion on the science behind the risk assessment for bees and to develop a Guidance Document on the risk assessment of Plant Protection Products on bees (*Apis mellifera*, *Bombus* spp., and solitary bees).

The process of the development of the Guidance Document follows the methodology of definition of Specific Protection Goals (SPG) as outlined in the Scientific Opinion of EFSA's PPR Panel (EFSA, 2010). Risk management choices need to be made to define the Specific Protection Goals. The Standing Committee on the Food Chain and Animal Health was consulted for the appropriate levels of protection (e.g. to make choices on the magnitude of effects, duration of effects and exposure percentiles).

The Guidance Document proposes the use of a tiered risk assessment scheme with a simple and cost effective First Tier to more complex Higher Tier studies under semi-field and field conditions. Each of the tiers will have to ensure that the appropriate level of protection is achieved.

The objective of this Guidance Document (GD) is to outline a process by which Plant Protection Products (PPPs) can be evaluated for their potential risk in causing unacceptable harm to a group of non-target organisms (bees). The maximum acceptable level of harm is defined by Specific Protection Goals (SPGs), which are set out in the GD.

In practice, the process for risk assessment has two main components: a preliminary Exposure Assessment (EA) that yields the Predicted Environmental Concentration (PEC) of the PPP that the bees are exposed to in a severe case; an effect assessment that compares the degree of harm that can result from exposure of bees to the PEC against the maximum level given by the SPGs. For example, a PPP that was unlikely to come into any contact with bees during agricultural use would have a PEC of zero and the effect assessment component of the risk assessment process would be unnecessary.

The risk assessment has several levels, or tiers. The First Tier is intended to sift out PPPs that are of negligible risk to bees and so prevent unnecessary further testing. This First Tier involves various triggers that are typically calculations based on the PEC and the known toxicity of the PPP. If the First Tier triggers indicate that the PPP potentially presents an unacceptable risk, either the assessment must be refined by including improved information and/or mitigation measures or the Higher Tier tests are invoked, which involve semi-field and field tests.

The First Tier triggers are based on comparing a Hazard Quotient (HQ) or Exposure Toxicity Ratio (ETR) against a threshold Trigger Value. The HQ or ETR is the ratio of the PEC to a standard index of the PPP's toxicity to bees (e.g. the LD_{50}). A new contribution of this GD is to produce bespoke Trigger Values that reflect the SPGs.

The Higher Tier tests were also formulated to reflect the SPGs. Thus, while there are many kinds of observations that would indicate harm to bees at some level, the semi-field and field tests presented here are designed to identify only unacceptable harm of the kind defined in the SPGs.

2. Protection goals as agreed with risk managers from Member States

Specific Protection Goals based on ecosystem services were defined according to the methodology outlined in the Scientific Opinion of EFSA (2010). In consultation with risk managers in the SCoFCAH (Standing Committee on the Food Chain and Animal Health) the Specific Protection Goals for honey bees were set as follows.

The attributes to protect were defined as survival and development of colonies and effects on larvae and bee behaviour as listed in regulation (EC) No 1107/2009. In addition, abundance/biomass and reproduction were also included because of their importance for the development and long-term survival of colonies.

The viability of each colony, the pollination services it provides, and its yield of hive products all depend on the colony's strength and, in particular, on the number of individuals it contains. It is therefore proposed to relate protection goals specifically to colony strength, which is defined operationally as the number of bees it contains (= colony size).

The magnitude of effects on colonies should not exceed 7% reduction in colony size. Forager mortality should not be increased compared to controls by a factor of 1.5 for 6 days or a factor of 2 for 3 days or a factor of 3 for 2 days.

Honey production is important for beekeepers and should therefore be included in the Specific Protection Goals. It is proposed to include honey production as an endpoint measurement in field studies.

The overall level of protection also includes the exposure assessment goals. It was decided that the exposure assessment should be done for each of the regulatory zones. By defining a certain percentile exposure assessment goal (e.g. 90%) it means that 90% of all colonies at the edge of a treated field in one regulatory zone should be exposed to a lower quantity than what is assessed in the risk assessment.

No final decision was taken by the SCoFCAH on the exposure percentiles. The current version of the Guidance Document is based on the 90^{th} percentile. If risk managers decide to choose a higher percentile after the public consultation period then the corresponding exposure values need to be changed in the final version of the GD.

For further details on setting of protection goals see Appendices A and B.

3. Exposure Assessment for bees

3.1. Introduction

3.1.1. Relationship between the exposure assessments of honey bees, bumble bees and solitary bees

This chapter deals with the exposure assessment of the bees. Except for this first section, the chapter considers only the exposure assessment of the honey bees. As will be described below, this exposure assessment focuses on the concentration in nectar and pollen in the bee hive (which is an average of the concentrations in all types of attractive plants in the foraging area). We consider the approach described for the honey bees also valid for bumble bees because they form a nest which can be considered the equivalent of a hive with respect to exposure. However, this is of course not the case

for the solitary bees. As will be described below, the approach for the honey bees is based on approaches for the different types of attractive plants in the foraging area. So for the solitary bees we propose to base the exposure assessment on the approaches described below for the different types of attractive plants.

3.1.2. Specification of the Exposure Assessment Goal

As described in Chapter 2, the proposed goal of the exposure assessment is to provide concentrations corresponding to a 90th percentile worst-case for the hives at the edges of treated fields in the area of use in the context of registration at EU level. The exposure assessment described in the following sections is based on this 90th percentile but can be changed if risk managers would decide to another percentile.

The total area to be considered for assessing this 90th percentile depends on the type of registration. Options include (i) the whole EU (e.g. for seed treatments), (ii) one of the regulatory zones, (iii) a certain climatic zone, (iv) a Member State. Usually the selected option is linked to the concept of a safe use of significant size. Let us consider for example an application of an insecticide in strawberries: the issue is then whether the SCoFCAH considers a safe use in strawberries in e.g. Greece sufficient for EU registration or would like to have a safe use in the whole southern zone. This may be different for different types of application of the substance and will need to be clarified at a later stage. This guidance will further refer to the total area to be considered as 'the area of use of the substance'.

As described in Chapter 2, the exposure assessment goal is defined as the colonies at the edges of treated field in the area of use of the substance. As will be described below, the exposure of such colonies may not only be caused by residues in nectar and pollen from plants in the treated field but also by residues in nectar and pollen from other plants: e.g. attractive adjacent crops or attractive succeeding crops. For such other plants it becomes a point of debate whether the spatial statistical distribution should be defined as (A) the hives at the edge of the treated fields or (B) the hives at the edge of the adjacent or succeeding crops. The populations A and B will be different. For example not all fields with a certain attractive succeeding crop in an area of use will have had the treated crop as its precursor crop. In order not to complicate the exposure assessment by such shifts in the definition of the spatial population of the hives, we propose to stick to the same definition of the spatial population of the hives for all types of plants: i.e. those at the edge of fields treated with the substance considered (option A). This is justified because in principle this population exists: e.g. even if the treated crop is followed by an unattractive crop, there may be a hive at the edge of this field next year because of other attractive crops in the landscape.

The exposure assessment goal used here does not prevent incidents because it assesses only the 90th percentile worst-case hive at the edge of the treated field. Incident prevention would lead to another exposure assessment goal and thus to another exposure assessment procedure. If the SCoFCAH wishes to include incident prevention in addition to the exposure assessment goal as defined above, this needs to be added at a later stage. An exposure assessment goal based on incident prevention will have to include the definition of an incident and the maximum number of incidents that is considered acceptable in the area of use of the substance.

3.1.3. Selection of the Ecotoxicologically Relevant types of Concentration

As described by EFSA (2010), any assessment of the risk to organisms has to be based on those types of concentration that are most relevant for the effect (called the ecotoxicologically relevant types of concentration). The schemes for the effect assessment for honey bees require a number of different

types of concentrations and this chapter describes how these are to be assessed. Given time limitations, we focus on the assessment of the concentrations in nectar and pollen entering the hive and ignore the other types of concentration that may be relevant for spray and seed-treatment applications (see section 3.5.1 of EFSA, 2012a). The reason for this is that the concentrations in nectar and pollen entering the hive are considered to be the most important drivers for the effects on the colony. Other types of concentration may be added at a later stage.

We consider that the most important exposure concentrations to be added are the concentration in honeydew and the concentration in the guttation water (both after spray and seed-treatment applications). High concentrations of systemic pesticides can be found in guttation droplets. However it is unclear to which extend bees use these guttation droplets and hence pose a risk to bees. At the moment it is not possible to provide a complete risk assessment method for exposure via honeydew, since concentrations in honeydew after pesticide application are not known. However, incidents with honey bees have been reported following overspray of honeydew. Therefore, the flow chart for the concentrations in the nectar and pollen following spray applications contains as a first step the option to prevent the contamination of honeydew via overspray by risk mitigation. A start has been made on Appendix E by listing plants for which honeydew formation occurs regularly and significantly. Comments and additions to this list are highly appreciated. Also for guttation water, a start has been made in Appendix F by listing crops for which guttation occurs regularly and significantly and some recommendations on how the risk to guttation water may be addressed. Comments and additions to this list are highly appreciated.

The view of stakeholders on the importance of the exposure to honeydew and guttation would be welcome. Stakeholders are kindly asked to submit information/data on these exposure routes.

The risk via systemic uptake in plants and subsequent transfer to honeydew (after spray or after solid/seed treatment) is currently not covered by the risk assessment scheme. This exposure route may be developed in the future but is considered to be less relevant than the routes via nectar and pollen. This is because the concentration of a systemic compound that could circulate in the phloem and reach honeydew without harming aphids should, in principle, not be capable of harming bees foraging on the honeydew, unless the compound is highly selective towards non-aphid insects. Selectivity information should be available in the registration dossier. If such a selectivity is highlighted, a dedicated risk assessment may be performed (e.g. risk mitigation).

The risk via direct exposure of honeydew from application of solid formulations, i.e. from ' overdust' of honeydew in adjacent crops and field margins, is also not covered by the current risk assessment scheme. This risk is considered to be less relevant than the risk from 'overdust' of nectar and pollen because the latter is expected to occur much more often.

3.1.4. Linking of Exposure and Effect Assessment based on parallel tiered approaches

The risk to bees is assessed using parallel tiered approaches for the effect and exposure assessments (EFSA, 2010, p. 46). So the guidance in this chapter delivers tiered approaches for assessing the concentrations in pollen and nectar that are needed for the tiered effect assessment scheme in Chapter 7. The tiered exposure approaches will be described in the form of flow charts (see e.g. Figure 1). So let us explain here the general legend of these charts. If a box contains a question, then it is always followed by a ‘yes’ and a ‘no’ option. If a box does not contain a question, then it is a possible next step in the tiered approach or it is a conclusion (e.g. if a box says ‘acceptable risk’). If an activity in a box leads to the conclusion that the risk is acceptable, there is no need to continue in the flow chart.

3.1.5. The concept of the Residue Unit Dose (RUD) as used in the exposure assessment

The aim of the exposure assessment is to generate concentrations in nectar and pollen. These are based on the concept of the Residue Unit Dose (RUD):

$$PEC = \delta \ RUD \qquad \text{(Eqn 1)}$$

where δ is the dose (kg/ha), RUD is the concentration in nectar or pollen (mg/kg) at a dose of 1 kg/ha and the PEC is the 'predicted environmental concentration' (mg/kg). We use the acronym 'PEC' for this endpoint of the exposure because this is commonly used for the other exposure assessments in the EU dossiers; it should be noted that the PEC for the bee exposure assessment may also be derived from measurements.

As described before, also concentrations in adjacent crops, for example, have to be assessed. In such cases, Eqn 1 does not apply because only a fraction of the dose will be deposited on this adjacent crop. Therefore we need to generalise Eqn 1 into:

$$PEC = m_{dep} \ RUD = f_{dep} \ \delta \ RUD \qquad \text{(Eqn 2)}$$

where m_{dep} is the mass deposited per area (kg/ha) and f_{dep} is the fraction of the dose deposited (-).

3.1.6. The need for an Exposure Assessment at landscape level

Bees from a hive at the edge of a treated field sample nectar and pollen not only from the treated field but also from other fields. Effects on colonies are likely to not be related to concentrations in nectar and pollen collected by an individual bee but to the average concentration in the nectar and pollen entering the hive (which is the target of the proposed exposure assessment). This average concentration depends on the concentrations in nectar and pollen in the whole foraging area of the foragers of a hive and on the sampling strategy of these foragers.

Appendix H describes a first simple model for assessing the average concentration entering a hive considering a foraging area that consists of different types of crops, i.e. a landscape-level approach. At this stage, there is not yet a consensus on a model for obtaining the average concentration in the hive based on the spatial distribution of concentrations in nectar and pollen in the foraging area of the hive. There is also no consensus on the size of the foraging area of a hive although this will be at least in the order of the radius of 1 km around a hive. Therefore we propose a conservative approach assuming that the foraging area of a hive consists exclusively of the type of plants considered (treated crop or other plants in treated field or adjacent crop etc). This conservativeness is likely to have a large effect on the resulting concentrations and may thus also have a large effect on the acceptability of a risk resulting from a certain use. This is especially the case because the conservativeness of the exposure in higher-tier effect experiments is to a large extent based on restricting the foraging area as much as possible to the treated field (e.g. by using *Phacelia* or application in tunnels). Therefore we recommend developing guidance for a landscape-level exposure assessment in the near future.

We encourage you to submit (during the public consultation period) data demonstrating that the maximum in time of the concentration in nectar or pollen in a hive at the edge of a treated field is lower than the maximum in time of this concentration in nectar or pollen in the flowers of the treated crop.

3.1.7. The hierarchy of the Exposure Assessment

We propose to structure the exposure assessment firstly on the basis of the application method of the substance and secondly on the type of plants that may generate the nectar and pollen. The justification for the application method is that this may have a very large effect on the exposure (e.g. dusts only being generated by seed treatments) and that this is linked to a certain use, and thus to the regulatory decision making (see EFSA, 2012b, for similar considerations with respect to the exposure assessment for soil organisms).

For the justification of the type of plants, let us consider for example the concentration in nectar. Bees may sample nectar from (i) the treated crop, (ii) weeds in the treated field, (iii) adjacent crops, (iv) plants in field margins, and (v) plants growing during the next growing season in the treated field. The nectar concentrations of these type of plants may differ strongly. For example, if a spray application occurs only after the flowering period of the treated crop, this is likely to lead to low or negligible exposure in the treated crop but not necessarily to low concentrations in e.g. weeds in the treated field because the weeds in the treated field may flower during application. Spray drift from orchards outside the treated field may be about 20% in the first metres (FOCUS, 2001) which may be deposited on plants that are flowering during the time of application. These examples indicate that different types of plants require different exposure assessments and thus different exposure flow charts.

Thus this chapter will consider the spray applications in Section 2 and the solid applications in Section 3 and at the start of each of these sections the different types of plants are described for which exposure assessments will be provided.

Risk mitigation through mitigation of exposure has played an important role in the regulatory risk assessment for honey bees for decades. It is therefore an essential part of the exposure assessment procedures. Thus we have integrated it in the exposure flow charts described in Sections 2 and 3.

3.2. Exposure Assessment for spray applications

3.2.1. The exposure Assessments for the different types of plants sampled by the bees

As described in Section 1 the PEC in nectar and pollen has to be assessed for all the different types of plants that are sampled by the bees. Figure 1 shows how this assessment works. The first step (box 1) is to assess the PEC in weeds in treated fields based on the full dose and conservative default RUD values. This can be seen as a screening step: in the First Tier, flowering weeds are assumed to be present at the time of application, irrespective of the crop. This will generate the highest lower-tier PEC of all types of plants and may be sufficient for non-toxic substances. If this screening step does not solve the problem, the PECs of all the types of plants in the boxes 2 to 6 have to be considered. Each of these boxes refers to an exposure assessment for which flow charts are given in the following sections. All these flow charts have to be followed in parallel and the risks resulting from these exposures have to be evaluated. As a next step (box 7) the exposure as measured in semi-field studies in tunnels may be used to account for metabolism either in the foragers during transport from the flowers to the hive or after entry of the nectar or pollen in the hive. For that purpose the courses of time of these concentrations in the flowers and in the hive have to be compared and the concentrations from the boxes 2 to 6 may be multiplied with the ratio of the maximum in the hive in the tunnel divided by the maximum in the flowers in the tunnel (nectar and pollen to be treated separately). This ratio is called the 'metabolism adjustment factor' in box 7.

There is still one complication: the flow charts for the exposure for the different types of plants contain many risk mitigation options (e.g. 'restrict application to post-flowering'). If such an option is needed to conclude on acceptable risk, the use of the substance changes and this may have also an effect of the exposure assessment for other types of plants. Therefore box 8 indicates that in such a case the flow charts in the other boxes have to be checked iteratively and this process has to continue until the assessments in the different boxes are consistent with each other.

Risk managers may wish to have some form of post-authorisation monitoring to ensure that the risk is acceptable or to confirm the underlying risk assessment. Article 66 of the EC Regulation 1107/2009 offers this possibility ('Producers of Plant Protection Products shall undertake post-authorisation monitoring on the request of the competent authorities.'). Therefore box 9 in Figure 1 offers the possibility to assess the exposure based on monitoring data in hives at the edge of treated fields. Such monitoring data have of course to be targeted to the exposure assessment goal (i.e. 90th percentile of hives at edges of treated fields in the area of use of the substance). They also have to be targeted to the most critical part of the exposure assessments in the lower tiers (e.g. if the most critical part was the concentrations in a succeeding crop then the monitoring should target hives at edges of fields of this succeeding crop). This leads to the following provisionary and non-exhaustive list of monitoring requirements:

--- all farmers in the whole foraging area (provisionally set as a circle around the hive with a radius of 3 km) should have the intention to use the substance as specified on the product label (so also following the risk mitigation measures on this label) because the concentration in the hive is influenced by the use in the whole foraging area

--- the use of the product in the foraging area during the monitoring period should be recorded

--- in view of possible effects of weather conditions, monitoring data should be available for more than one year

--- for assessment of problems in adjacent crops, monitoring should include measurements of wind direction on the day(s) when the substance is applied to the treated field

--- for assessment of problems in field margins, monitoring should include information on occurrence of field margins around the treated field in relation to the wind direction on the day(s) when the substance is applied to the treated field

--- for assessments of problems with guttation in the treated field, monitoring should include daily records of occurrence of guttation in the treated field in the period after application of the substance
--- the time course of the concentrations in nectar and pollen in the hive should be followed, starting before application(s) of the substance and continuing until the concentration has clearly passed its maximum value
--- it is advisable to perform the monitoring mainly in areas with high intensity of use of the substance because this intensity is likely to influence the 90th percentile case.
From the results of such monitoring studies the 90th percentile has to be derived using appropriate statistical analyses based on the spatial population as defined in Section 1.2 using all relevant information.

The scheme in Figure 1 does not consider the PEC in adjacent crops and field margins in the year(s) following the year of application because these PECs will be smaller than those in the treated field in the year(s) following the year of application for spray applications. The scheme chart does also not consider weeds in the year after application in permanent crops and in succeeding annual crops (either in year of application or in year after application) because the concentrations in the nectar and pollen in these weeds are also expected to be smaller than those in the weeds in the application period.

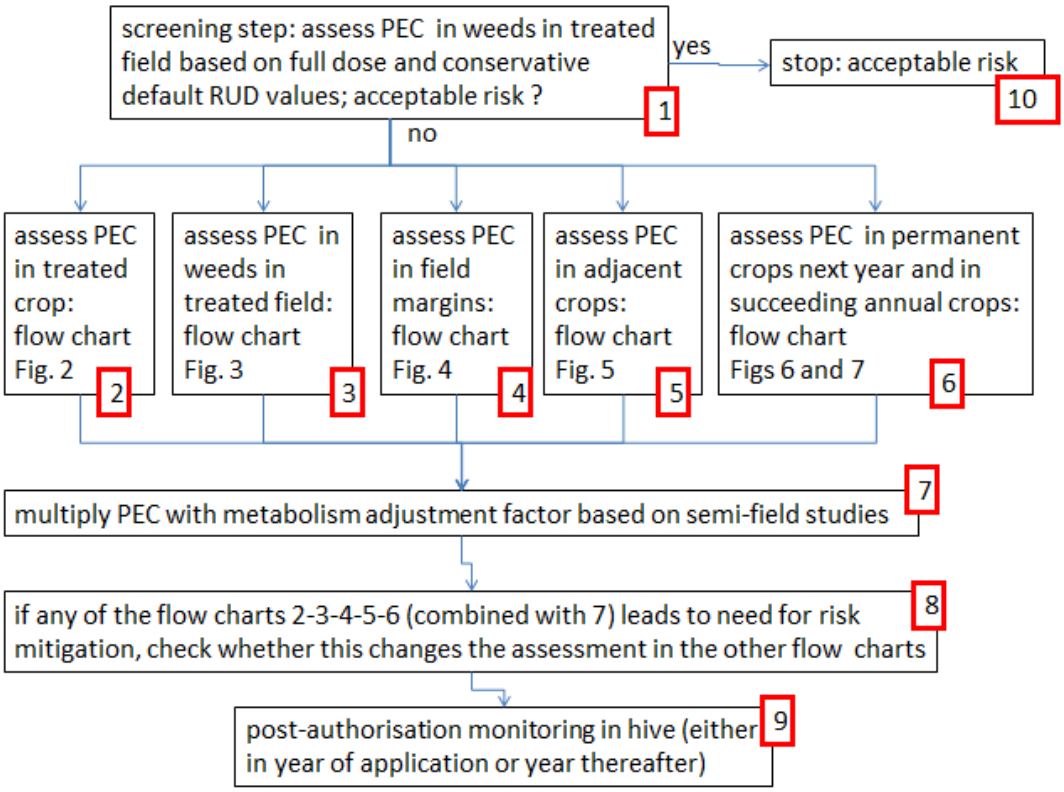


Figure 1: Scheme for the exposure assessments for the PECs in nectar and pollen collected by the bees after spray applications.

3.2.2. Conservative default values for RUDs of pollen and nectar after spray applications

The next sections describe the exposure assessments for the five different types of plants as indicated in Figure 1. Four out of these five require conservative default values for the RUD in nectar and pollen to avoid expensive residue measurements for substances that are not toxic to honey bees. These RUD values are based on the data presented in Appendix I 'Pesticide residue levels in nectar and pollen and the residue unit doses (RUDs)'. The default RUD for nectar is 21 mg/kg and that for pollen is 150 mg/kg. These are the highest values of 28 measurements for nectar and 37 measurements of pollen. The underlying assumption is that such conservative default values should be based on 99th percentiles because it is highly undesirable from a risk management point of view that a lower exposure tier would lead to acceptable risk whereas the risk would not be acceptable in reality. The highest of 28 values is the 98.2th percentile of the frequency distribution and the highest of 37 values is the 98.6th percentile of the frequency distribution (so close to the 99th percentile).

3.2.3. Concentrations in pollen and nectar in the treated crop

The exposure assessment for the PECs for nectar and pollen in the treated crop is described in the flow chart of Figure 2. At the start (box 1) it is checked whether honeydew may occur and if so, it is recommended (in box 2) to put on the label that the substance should not be applied if there is honey dew present if the HQ exceeds the trigger value for oral exposure to avoid this complication for non-toxic substances. The next step (box 3) is to check whether this crop has flowers or extrafloral nectaries during the growing season (if not, there is no nectar and pollen) and if it is attractive to bees (if not no nectar and pollen is transported to the hive). Then it is checked to see whether the substance is sprayed before or during flowering (box 4). If the substance is sprayed before flowering and not systemic (box 5) then no exposure can be expected. Otherwise the concentrations in nectar and pollen have to be assessed and as a first step this can be based on the default values described in Section 2.2 (box 6). If the risk is still not acceptable, the 90th percentile PEC in the area of use has to be assessed (box 7) by field measurements under normal agricultural conditions (see Appendix J for guidance for performing such measurements). Such measurements will also include automatically the uptake of substance via the crop roots and its transport to pollen and nectar. If this box 7 does not lead to acceptable risk, the exposure may be mitigated by restricting the application to the post-flowering period (box 8).

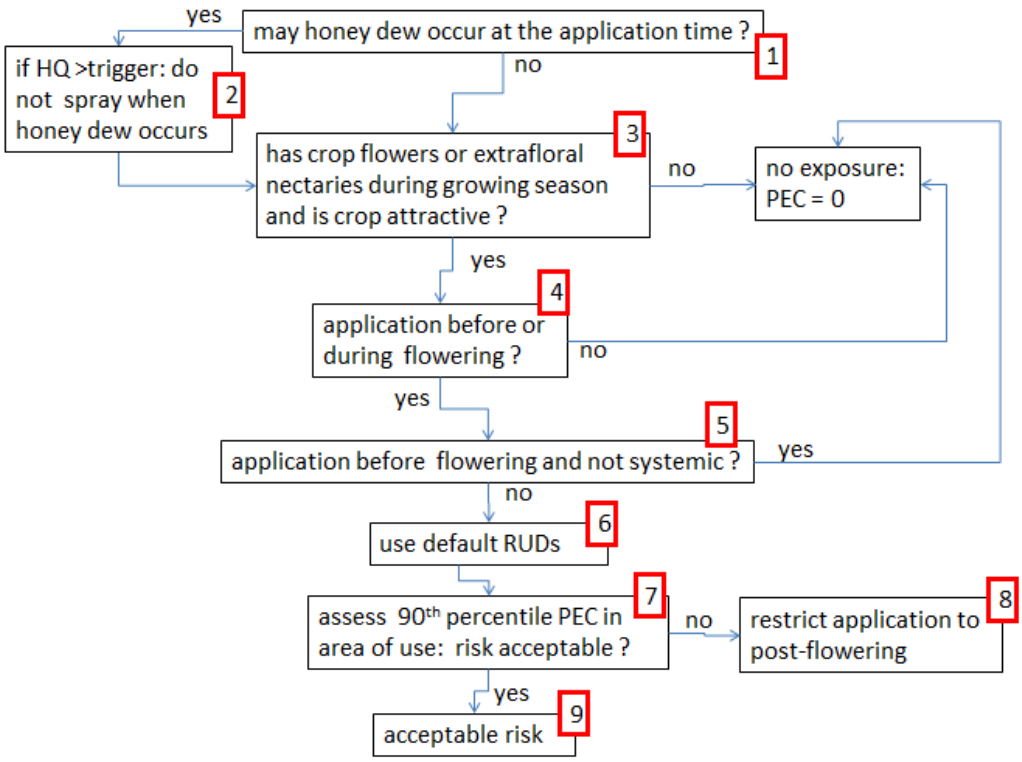


Figure 2: Flow chart for the exposure assessments of the PECs for nectar and pollen in the treated crop after spray applications. The box numbers refer to the general text above.

3.2.4. Concentrations in pollen and nectar in weeds in the treated field

The first step for the PECs for weeds in the treated field is to estimate the PEC using default RUD values (Section 3.2.2) in combination with the full dose (box 1 in Figure 3).

These plants may flower at any time, so the application time does not have an influence on these RUDs. If this gives an unacceptable risk, it may be checked whether it is likely that a significant fraction of the surface area of treated fields is covered by weeds at the application time. If this will happen at less than 10% of the area of use of the substance, no weeds will occur in a 90th percentile case and thus their exposure can be ignored (box 2). For example, weeds are usually abundant in annual crops: abundant weed growth is more likely to occur in e.g. orchards. However, at this moment no guidance for this assessment of the abundance of weeds is available for the most relevant crops. We recommend therefore to develop guidance for this at EU level in the near future. As long as this guidance is not available, the box can be ignored and the risk assessor can go immediately to box 3 or 4 (conservative approach).

Next there are two parallel steps in the flow chart: (i) mitigate the risk by not applying when flowering weeds are present (box 3) or (ii) refine the exposure by taking into account the fraction of the dose deposited on the weeds (box 4). Guidance for this fraction of the dose deposited can be found in Appendix E of EFSA (2009). In case box 4 does not lead to acceptable risk, we propose to refine the RUDs for the weeds by using RUDs measured for this substance in a number of different types of field

crops (box 5). An alternative is to measure RUDs in *Phacelia* as a proxy for the weeds. This approach of using other plants than the weeds is based on the assumption that the RUD of a substance is more driven by substance properties than by plant properties. This is likely to be the case but it is uncertain whether this assumption is defensible for the full range of plants and substances. Therefore we recommend to underpin this approach by analysing available data and further research. The alternative would be to measure RUD for the most relevant weed species; we do not advise this because the composition of attractive weed species in treated fields is likely to be very variable and we are not aware of data on their distribution in treated fields across the EU.

The flow chart in Figure 3 considers only exposure via spray application and thus ignores the exposure of the weeds via root uptake in the soil and subsequent accumulation in nectar and pollen of the weeds. This possibility was ignored because it is likely to lead to lower concentrations in nectar and pollen than overspray.

Because flowering weeds will often be present in the field at the time of application, the assessment of the PEC in the weeds in the treated field will often trigger the biggest exposure assessment problems of all the assessments in the flow chart of Figure 1 if the risk mitigation option (box 3) is for some reason impossible. In such case the landscape-level exposure assessment (yet to be developed) could be a useful higher-tier solution because weeds are unlikely to be present on a large fraction of the surface area of the treated field.

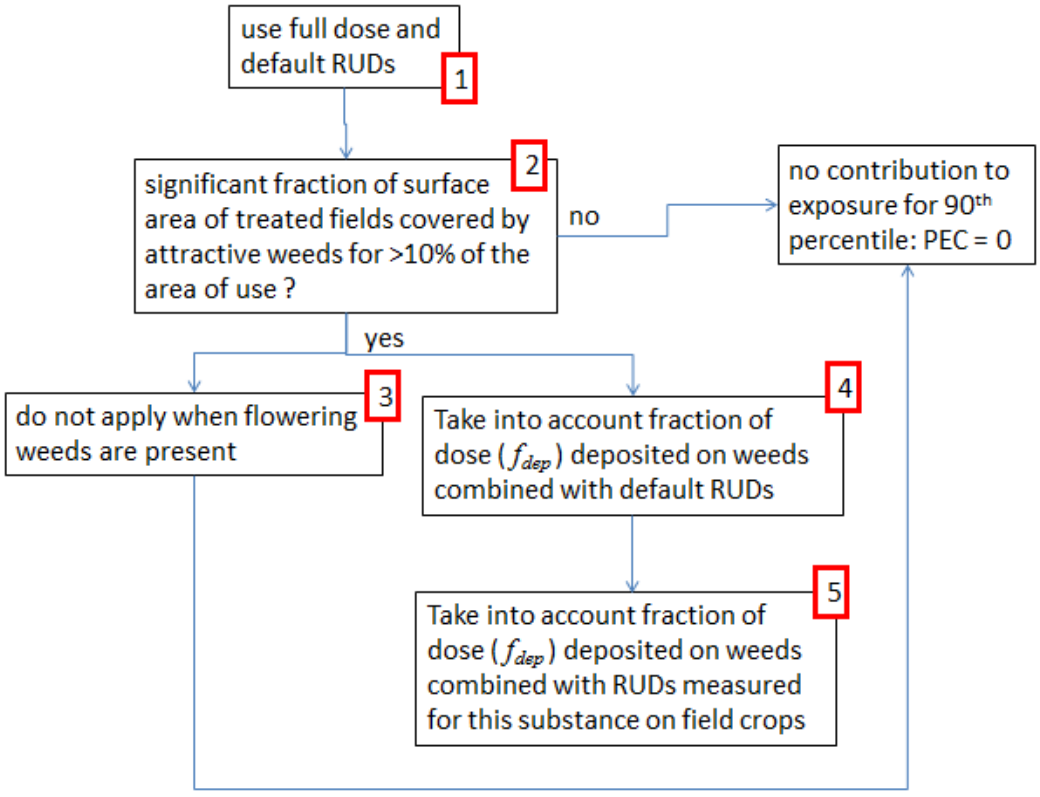


Figure 3: Flow chart for the exposure assessments of the PECs for nectar and pollen in weeds in the treated field after spray applications. The box numbers refer to the general text above.

3.2.5. Concentrations in pollen and nectar in plants in field margins

Flowering field margins can always be present at the application time, so their exposure has to be assessed. The target is the 90th percentile of the average concentration in nectar or pollen that enters a hive at the edge of the treated field. So it therefore seems justifiable to consider the average concentration of all attractive plants in the whole field margin of a treated field as the basis of the assessment: there are a priori no reasons to assume that the bees would preferably forage more on contaminated parts of the field margin than on parts that are not contaminated (e.g. because they were upwind during application).

The first step to assess pollen and nectar concentration in field margins is to calculate PECs with Eqn 2 using default RUDs and default conservative spray drift deposition (box 1 of Figure 4). See Appendix K for interim guidance for the spray drift deposition. If the risk is not acceptable then spray drift can be reduced with risk mitigation measures (box 2). The alternative is to refine the RUDs for the weeds by using RUDs measured for this substance in field crops (box 3). This is the same approach as proposed for the weeds in the treated field in the previous section and has thus the same uncertainties. If the risk is not yet acceptable, drift reduction measures can be applied (box 4). If the risk is still not acceptable, the spray drift can be refined by calculating a 90th percentile deposition using a stochastic model (box 5); see Appendix K for the proposed approach based on this stochastic model.

As described before, the exposure assessment is based on the conservative assumption that the foraging area of a hive consists exclusively of the type of the plant considered (here the flowering plants in the field margin). This is likely to overestimate exposure especially for plants in field margins because the surface area of field margins is relatively small at the landscape level.

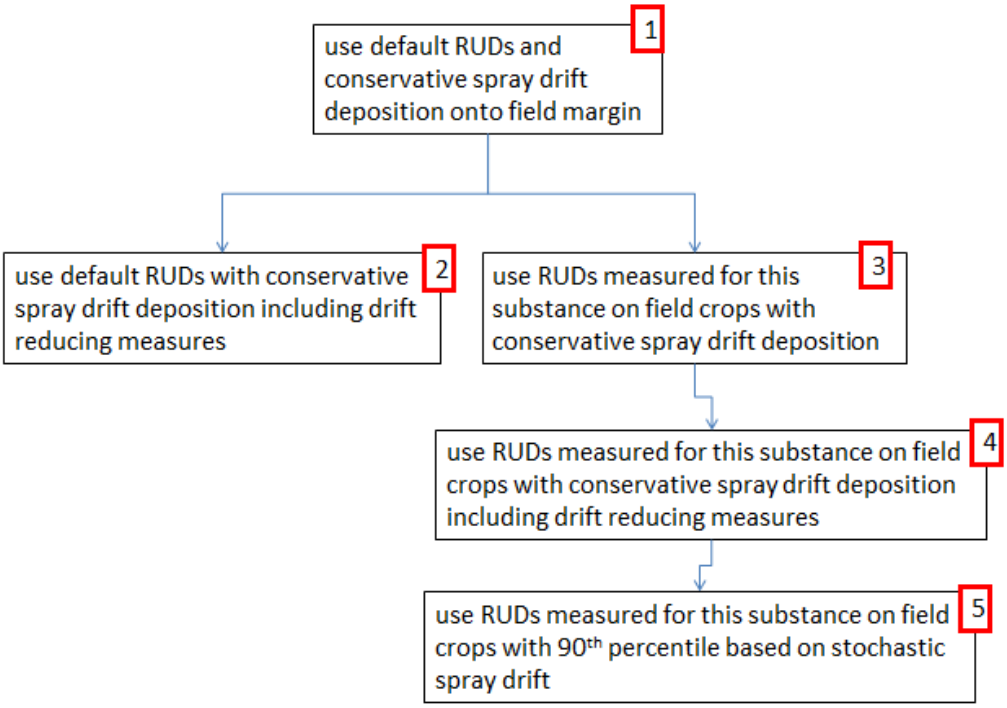


Figure 4: Flow chart for the exposure assessments of the PECs for nectar and pollen in the field margin of treated crops after spray application(s). The box numbers refer to the general text above

3.2.6. Concentrations in pollen and nectar in adjacent crops

As described before, a substance that is sprayed onto a treated crop that is not flowering at the time of application, may lead to effects on an adjacent crop that is flowering at the time of application. Consider for example two adjacent apple orchards of which the treated orchard is not flowering whereas the adjacent orchard is flowering or a potato crop that is sprayed whereas adjacent to the potato crop there is a flowering oil seed rape field.

Following the same reasoning as that for the field margins, we propose to consider the average spray drift deposition in the whole adjacent-crop field: there is a priori no reason to assume that the bees would preferably forage more on the contaminated strip of adjacent crop that is closest to the treated field.

The first step in the exposure assessment of adjacent crops (box 1 in Figure 5), is to calculate the PEC with Eqn 2 based on the default RUDs and conservative default spray drift deposition (f_{dep} in Eqn 2). See Appendix K for interim guidance for the spray drift deposition. If the risk is not yet acceptable, the exposure can be mitigated by applying drift reduction measures (box 2). If the risk is acceptable and the notifier considers the drift reduction measures no problem (box 3), then the problem is solved. Otherwise it can be checked whether there is an attractive adjacent crops area bigger than 10% of the surface area of the treated fields (box 4). If this is not the case, the 90th percentile hive is unlikely to be influenced by an attractive adjacent crop and the exposure resulting from these plants can be ignored. At this moment the assessment in box 4 cannot be performed easily because no geostatistical analyses of the desired frequencies of occurrence of attractive crops are available. We recommend to perform such analyses at EU level using crop maps that are currently available at a resolution of 1 km^2 for all EU countries (e.g. http://eusoils.jrc.ec. europa.eu/library/Data/EFSA/).

As long as the results of these analyses are not available, this box can be ignored and the exposure assessment can continue assuming that this percentage is indeed above 10% (conservative approach because the exposure has to be assessed then anyhow). The next step is to check whether application is after flowering of the attractive adjacent crops (box 5). If yes, the PEC can be assumed to be zero. Next step (box 6) is to check whether application is before flowering of all attractive adjacent crops and if the substance is not systemic. If yes, the PEC can be assumed to be zero again. If no, the substance is applied during flowering or it is both systemic and applied before flowering. Then there are two options. The first is to measure RUDs for the relevant adjacent crops (box 7). Relevant means only those attractive adjacent crops that would in isolation lead to 'no'-answers in the boxes 5 and 6. The second is to refine the 90th percentile spray drift deposition based on a modelling study based on a stochastic wind angle and wind speed (box 8; see Appendix K for details of the modelling study). The 90th percentile PEC has to be based on the spatial population of hives as defined in the exposure assessment goal, i.e. all hives at the edge of treated fields. So if the relevant attractive adjacent crops only occur for e.g. 20% of the treated fields, then the 90th percentile PEC can be assessed by taking the 50th percentile PEC of the spray drift deposition probability density function (because the 90th percentile is the 50th percentile of the top 20% of the statistical population). See Appendix L for the general approach for assessing such percentiles.

As described before, geostatistical analyses of the frequencies of occurrence of attractive adjacent crops are currently not available. As a consequence, it can be assumed that the relevant attractive crops are adjacent to all treated fields (conservative assumption).

If the risk is still not acceptable, box 9 provides the risk mitigation option of spray drift reducing measures.

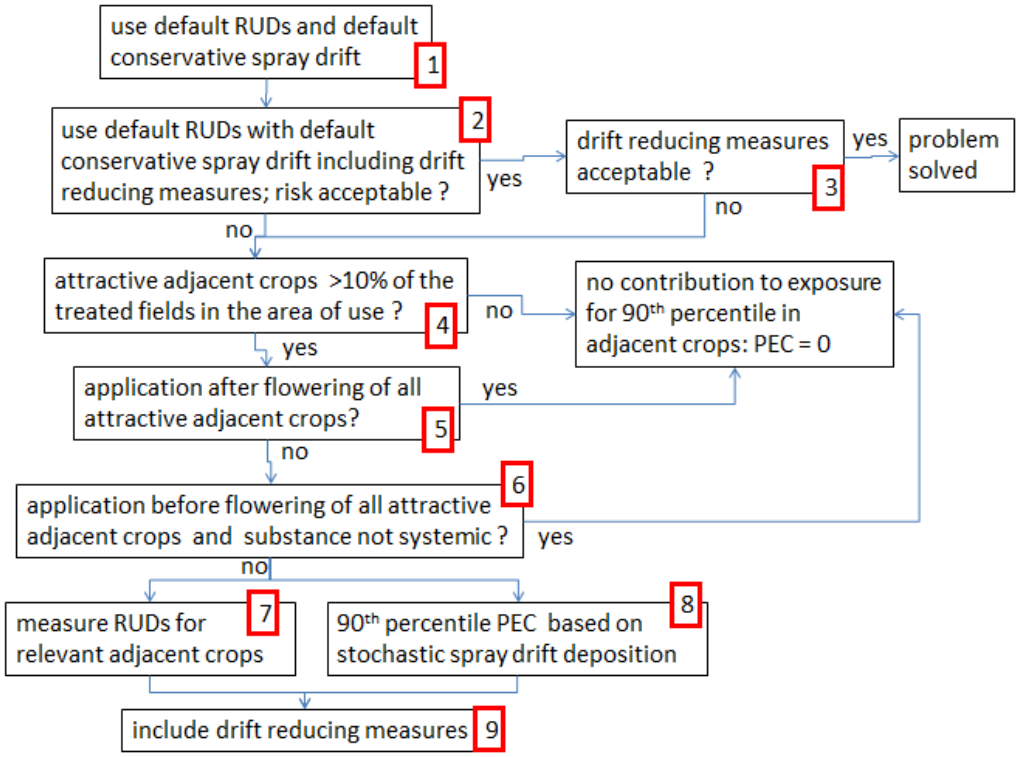


Figure 5: Flow chart for the exposure assessments of the PECs for nectar and pollen in adjacent crops after spray applications. The box numbers refer to the general text above

3.2.7. Concentrations in pollen and nectar in plants in permanent crops in the next year and in succeeding annual crops

For permanent crops it is possible that soil residues of substances lead to root uptake in the following year and are subsequently transported via the plants to nectar and pollen (especially for systemic substances). This may also happen for annual crops that are grown one year after the treated annual crop. Vegetables such as cabbage, carrots and beans may be grown two times in a growing season (e.g. six of the nine FOCUS groundwater scenarios have been parameterised for such double crops; FOCUS, 2009). So a spray application to the first crop may lead to uptake of substances via the roots in the second crop and accumulation in nectar and pollen of this second crop. This may be relevant for attractive double crops such as beans. This section provides guidance for the exposure assessment of the concentrations in nectar and pollen in these three types of crops.

Root uptake of substances seems to occur for all organic micropollutants and seems to be mainly a function of the octanol-water partition coefficient and the molar mass (Sur et al., 2012). So it is impossible to exclude a priori that non-systemic substances are transported to nectar and pollen. Therefore this exposure assessment applies to both non-systemic and systemic substances. We recommend analysing available data on residues in nectar and pollen resulting from root uptake to underpin that non-systemic substances will not be transported to nectar and pollen in amounts that could become relevant for the risk assessment of bees. If this indeed can be underpinned, this exposure assessment could be limited to systemic substances.

There is a consensus in literature that the plant uptake of Plant Protection Products and their metabolites at a certain depth in soil is proportional to their concentration in the pore water in the soil

at that depth. This concept has already been used for decades in the simulation models that have been used for the regulatory assessment of leaching to groundwater and surface water at national and EU levels (e.g. Leistra & Dekkers, 1976). We therefore propose using the average pore water concentration in the root zone of the plant as a criterion to assess the likelihood of significant plant uptake (as a lower tier approach).

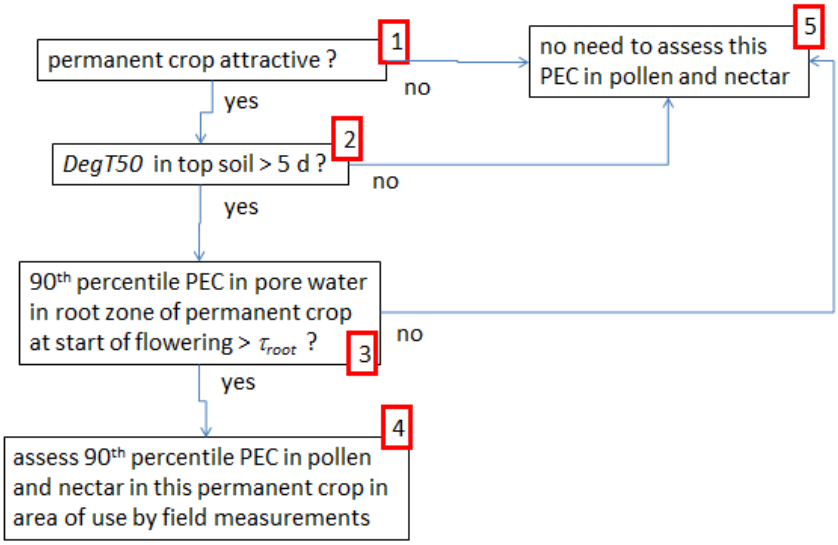
The next question is then what value of this pore water concentration should be used for triggering further work. The first consideration is that the concentration in the nectar and pollen can be considerably larger than the concentration in the water that is taken up by the roots (especially for systemic substances). The second consideration is that the density of pollen and nectar is in the order of 1 kg/L, so a concentration of 1 µg/L in nectar or pollen corresponds to about 1 µg/kg. Combining these two, we propose that the trigger concentration in pore water (in µg/L) should be ten times smaller than a 'safe' concentration in nectar and pollen (in µg/kg). It seems appropriate to use, as the safe concentration, the regulatory acceptable concentration in nectar or pollen due to oral exposure (RAC_{oral}) that will be assessed in Chapter 7. So we propose:

$$\tau_{root} = 100 \; RAC_{oral} \qquad \text{(Eqn 3)}$$

with τ_{root} in µg/L and RAC_{oral} in mg/kg (the factor 100 is needed because of the unit mg/kg for the RAC_{oral}; the basis of the logic is that if RAC_{oral} is e.g. 1 µg/kg τ_{root} has to be 0.1 µg/L).

We consider first the exposure assessment for permanent crops in the year after the application (Figure 6). Box 1 tests whether the permanent crop is attractive. The next step is a simple trigger for the $DegT_{50}$ in top soil at 20°C and at moisture content at field capacity. The DegT50 is the half-life in the soil matrix in soil (so excluding dissipation processes at the soil surface). This is part of the endpoint list and thus available. The concept behind this trigger is that if this $DegT_{50}$ is short enough, the pore water concentration in the root zone will be low enough a year after application. We propose tentatively $DegT_{50} > 5$ d. The trigger value has to be chosen so that the later steps in the flow chart are unnecessary even for the most toxic substance, the most critical scenario and the highest application rate. The proposed value of 5 d is tentative and will have to be underpinned by scenario calculations for the full range of substance properties. If this trigger is exceeded, the 90th percentile of the average pore water concentration in the root zone at the time of the start of the flowering next year has to be assessed and compared to τ_{root} (box 3). This 90th percentile refers to the area of use of the substance (considering of course the variability in meteorological conditions from year to year). No scenarios have yet been developed for this 90th percentile. As long as these scenarios are not available, we propose to use the FOCUS groundwater scenario that is most relevant for the area of use of the substance (these scenarios have been parameterised for apples for all nine scenario locations; FOCUS, 2009). These FOCUS scenarios intend to assess the 90th percentile of the pore water concentration leaching at 1 m depth. A scenario selection procedure depends on the target quantity: so it can be expected that a 90th percentile scenario for the leaching concentration at 1 m depth will differ significantly from a 90th percentile scenario for the average pore water concentration in the root zone. However, development of a scenario targeted to the concentration in the root zone will take time. When such scenarios are developed, they can be best targeted to the total mass taken up from the start of the growing season to the moment of flowering because this is likely to be a better indicator of the concentration in nectar and pollen than the average concentration in the root zone.

If the assessment in box 3 of Figure 6 does not solve the problem, the 90th percentile PEC in nectar and pollen has to be assessed via field measurements (box 4); see Appendix J for guidance on how this should be done.



788
789
790

791 **Figure 6:** Flow chart for the exposure assessments of the PECs for nectar and pollen in permanent
792 crops in the year after one or more spray application(s). The box numbers refer to the general text
793 above

794
795 So we can now move on to the exposure assessment for nectar and pollen of succeeding annual crops
796 (Figure 7). As described before, both succeeding crops in the application year are considered as well
797 as succeeding crops in the next year. The first step (box 1) is to check whether the DegT50 in top soil
798 at 20°C and at a moisture content at field capacity are low enough to prevent exposure. We propose a
799 trigger of 2 days for succeeding crops in the application year and 5 days for crops grown the year after.
800 Also these triggers need to be underpinned by scenario calculations for the full range of substance
801 properties. The next step (box 2) is to check whether attractive succeeding crops occur for more than
802 10% of the area of use of the substance. If not, less than 10% of statistical population of the hives will
803 be exposed via these types of plants and these types of plants can thus be ignored when assessing the
804 90th percentile exposure of the hives. If they do occur above 10%, then box 4 indicates that the 90th
805 percentile of the average concentration in the pore water in the root zone at the start of flowering
806 should be assessed and compared to τ_{root} (box 3).
807
808 For the annual crops grown in the next year, we propose to follow the same approach as for the
809 permanent crops: use the FOCUS groundwater scenario that is most relevant to the area of use of the
810 substance. FOCUS (2009) parameterised scenarios for some twenty annual crops including e.g. oil
811 seed rape. This should be considered as an interim approach just like for the permanent crops (see
812 previous paragraph for explanation). For the succeeding crops grown in the year of application of the
813 substance, the FOCUS leaching scenarios seem less appropriate because leaching is a process of years
814 whereas the exposure of these crops has to be assessed e.g. three months after application of the
815 substance (FOCUS, 2009). For these crops we recommend to use the guidance developed by EFSA
816 (2012b) for assessment of the 90th percentile of the average pore water concentration in the top 20 cm
817 of soil in the context of the risk assessment for soil organisms.
818
819 In view of the above, we recommend developing targeted scenarios for assessing the plant uptake of
820 substances in attractive permanent and in attractive annual succeeding crops and that these are also
821 used to support the selection of the combinations of soil and meteorological conditions that are likely
822 to lead to the highest risk of carryover of residues to plants growing next year.
823

If box 3 of Figure 7 does not solve the problem, field measurements of concentrations in nectar and pollen are needed to assess the 90th percentile PEC. The spatial statistical population of the hives consists of the hives at the edge of the treated fields (Section 1.2). So the 90th percentile PEC in pollen and nectar should be assessed considering the frequency of all succeeding crops. Let us assume for example that there is only one attractive succeeding crop that occupies 30% of the area of use of the substance in the year after application. These 30% are now considered to be the upper 30 percent of the distribution of the PEC values. In such a case the 90th percentile can be calculated as the 67th percentile of the frequency distribution of the measured PECs in nectar and pollen (because 90 is at 2/3 between 70 and 100; see Appendix L for the general approach to calculate such a percentile). So we recommend selecting the succeeding crop that will deliver the 90th percentile based on a ranking of the attractiveness of the succeeding crops in combination with their surface area in the area of use of the substance (box 4). Next the target percentile X for this attractive succeeding crop corresponding to the overall 90th percentile can be assessed (box 5; see Appendix L for details) by measuring the concentrations of nectar and pollen in field experiments (box 6).

Should it be difficult to assess the spatial distribution of succeeding crops, the exposure assessment can of course always be simplified by using conservative assumptions (e.g. assessing the 90th percentile of the most attractive succeeding crop).

As indicated in Figure 7, there is also the risk mitigation option to not grow the succeeding crop that causes the problem or to delay sowing or planting of this crop until the soil residues have declined to an acceptable level (box 7).

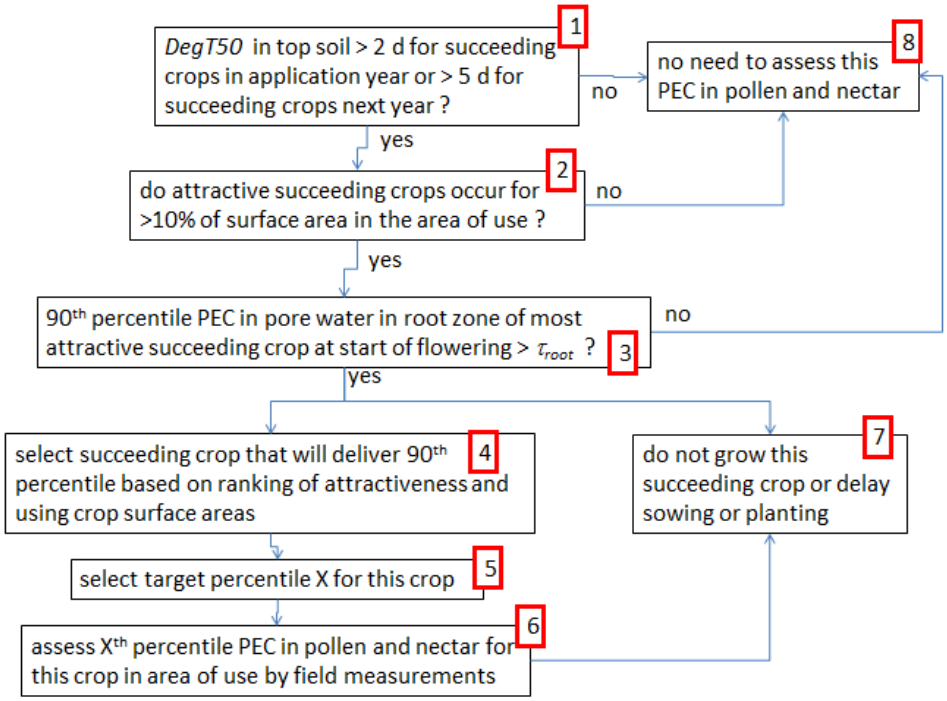


Figure 7: Flow chart for the exposure assessments of the PECs for nectar and pollen in succeeding annual crops following one or more spray application(s) in the treated crop. The box numbers refer to the general text above

For non-toxic substances τ_{root} may be larger than 100 µg/L. In such cases, it may be overkill to assess the 90th percentile PEC in pore water in the root zone by simulations with numerical models and it may suffice to use a worst-case upper limit of this PEC. At this stage, it is still impossible to give this upper limit because no experience with such scenario calculations has yet been gained.

3.2.8. The likely hierarchy of the Exposure Assessments for the different types of plants in regulatory practice

Currently, the risk assessor has to first apply the conservative screening (box 1 of Figure 1) and thereafter go through all flow charts in parallel (Figure 1). It would be easier if we could define a hierarchy between these flow charts. However, the flow charts of Figures 2 to 6 are in general complex and most of them contain options to reduce the exposure via risk mitigation. As described in Figure 1, risk mitigation measures may lead to the need for going iteratively through part of the flow charts because applying a risk mitigation measure may lead to another use of the substance. Nevertheless we attempt here to shed some light on this hierarchy.

The assessment for the treated crop (Figure 2) and for crops grown after the treated crop (Figures 6 and 7) have no link to any of the other assessments and also have no link to each other. The assessments for (i) the weeds in the treated field (Figure 3), (ii) the plants in the field margins (Figure 4), and (iii) adjacent crops (Figure 5) have in common that their exposure is based on the possibility that these plants flower at the time of application of the substance. So an option for a hierarchy could be to start with weeds in the treated field because they may receive the full dose (but not always: see box 4 of Figure 3), then to continue with the plants in field margins where the deposition is usually less and then to end with the adjacent crops.

The 90th percentile exposure PEC for the adjacent crops is likely to be lower than that for the field margins for two reasons. The first is that flowering attractive adjacent crops are only present at a fraction of the border of treated fields at the application time whereas flowering plants in field margins may always be present at the application time (it can only be different in the highly exceptional case that the adjacent crop would have much higher crop-specific RUD values than other field crops). The second reason is that the average concentration in the nectar and pollen in an attractive adjacent crops is lower than in flowering plants in field margins because spray drift deposition decreases strongly with distance to the treated field. So probably the exposure assessment for the adjacent crops is superfluous now because it will lead to lower exposure than for the field margins. However, the whole exposure assessment is based on the conservative assumption that the foraging area of a hive consists exclusively of the type of plant considered (see Section 3.1.6). In the longer term this conservative approach is likely to be replaced with a more realistic landscape-level exposure approach (see Appendix H). Then it may occur that flowering of certain plant species in the field margin of a field may lead to less exposure of the hive than e.g. an adjacent flowering oil seed rape crop because the number of these plants in the field margin is much less than the number of crop plants in the first few metres of the adjacent field. So the flow chart for the adjacent crops is likely to have little added value now but will probably have its come back after landscape-level approaches have been developed.

3.3. Exposure Assessment for solids

3.3.1. Introduction

Solids are defined as seed treatments, pellets, granules etc. Solid formulations (e.g. wettable powders) that are mixed with water and then sprayed are part of the spray exposure assessment. The EU regulation (article 3, item 17) prescribes that Plant Protection Products that are used as seed treatments are registered at the EU level, so not at zonal or Member State level. This is based on the concepts (i) that the use of the Plant Protection Product is linked to the coating of the seed, so not to the sowing of the seed, and (ii) that there should be free trade of treated seeds across the EU. So the area of use of the substance for seed treatments is the whole surface area in the EU where the crop of treated seed is grown.

The EU regulation does not prescribe registration of granules at the EU level. So the exposure assessments of seed treatments are different in this respect. Therefore we describe here first the guidance for seed treatments and thereafter that for granules.

3.3.2. Exposure Assessment for seed treatments

3.3.2.1. The exposure assessments for the different types of nectar and pollen collected by the bees

Following the same reasoning as for the spray applications, the PEC in nectar and pollen after seed treatments has to be assessed for all the different types of plants sampled by the bees. The scheme in Figure 8 shows the same types of plants as for the spray applications (in Figure 1) except the weeds in the treated field. The weeds in the treated field are unlikely to be an issue in view of the application via the seed treatment: no weeds will be present in the field when the crop is sown and uptake of weeds via the roots is unlikely because the substance is concentrated around the treated seed. Therefore uptake via the roots of weeds is likely to be negligibly small in the application year. Admittedly weeds may lead to higher exposure in the treated field than the treated crop if this does not flower. However, there is currently no up-to-date guidance for soil exposure resulting from seed treatments: EFSA (2012b) developed such guidance for spray applications but not for other types of application such as seed treatments. Therefore we recommend to develop such guidance for seed treatments and to use this to assess the uptake by the weeds in the treated field. As long as this has not yet happened, we suggest ignoring these plants in the bee exposure assessment.

The flow chart in Figure 8 (in box 5) also contains the option to use the metabolism adjustment factor as described in Section 2.1. If such an adjustment factor has already have been derived from studies with spray applications, then this factor may be used here as well because there are a priori no reasons to assume that the metabolism in the bee or in the hive is influenced by the route of exposure of the nectar or pollen in the flower. The flow chart in Figure 8 (in box 7) also contains the option of post-authorisation monitoring as in Figure 1. See Section 2.1 for guidance on the monitoring procedure.

The mechanism of the exposure in the treated crop (box 1) and in succeeding annual crops (box 2) differs completely from that in the field margin and in an attractive adjacent crop (boxes 3 and 4). The treated crop is exposed because its seed is coated with the substance which leads to uptake by the roots of the crop. This substance is then taken up and transported to the nectar and pollen of the treated crop. Similarly the roots of succeeding crops may take up soil residues from seed treatments. However,

plants in field margins and of an attractive adjacent crop are exposed through the dust that is generated by sowing the treated crop and that is deposited onto them. Therefore we describe first the exposure assessments driven by root uptake and then those driven by dust deposition.

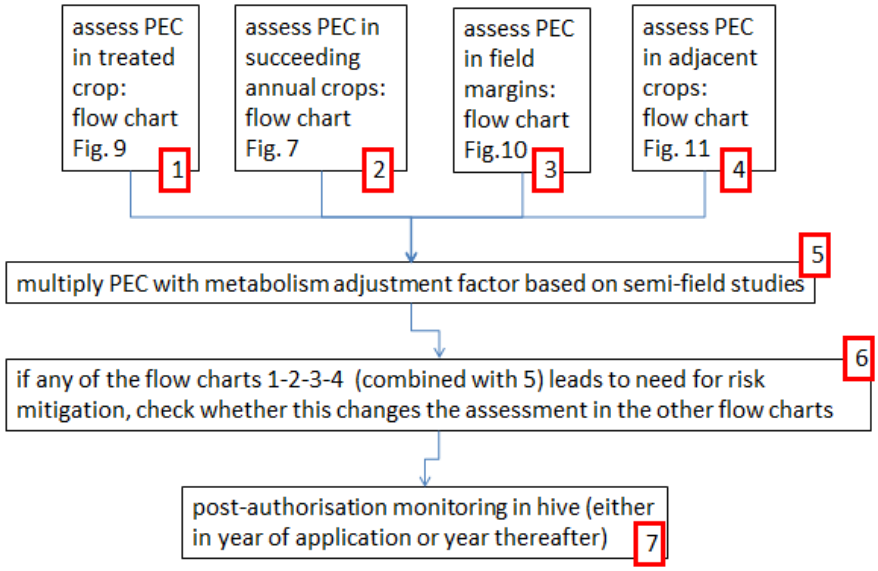


Figure 8: Scheme chart for the exposure assessments for the different types of plants sampled by the bees after seed treatments.

In principle it is possible that dust deposition will occur on bees that are foraging on honeydew in field margins or in adjacent crops or that such dust deposition will contaminate such honeydew which is then taken up by foraging bees. For the spray applications we included a risk mitigation option to avoid this (box 2 in Figure 2). We propose not assessing this exposure of honeydew due to dust deposition because we expect that it will lead to less exposure of the bees than the flowering plants in the field margin.

3.3.2.2. Concentrations in pollen and nectar in the treated crop

The first two steps (Figure 9, boxes 1 and 2) in the exposure assessment for the treated crop are the same as for the spray applications: there is only exposure in the hive if the crop has attractive sources for nectar or pollen. See Appendix G. The next step is to use a conservative default value for the PEC from seed treatments (box 3). We propose to use for this purpose a PEC of 1 mg/kg irrespective of the dosage and the type of seed. The Appendix I 'Pesticide residue levels in nectar and pollen and the residue unit doses (RUDs)' contains data which would lead to less conservative default values. However, there are only data for three insecticides that belong to the same chemical class. We feel that this is too weak a basis for setting conservative RUD values for the whole population of Plant Protection Products and therefore propose the conservative PEC of 1 mg/kg.

If this would still not lead to acceptable risks, the 90th percentile PEC could be derived by residue analysis in five field studies in the area of use of the substance (i.e. in this case the whole cropped area in the EU) as described in Section 3.2.4.

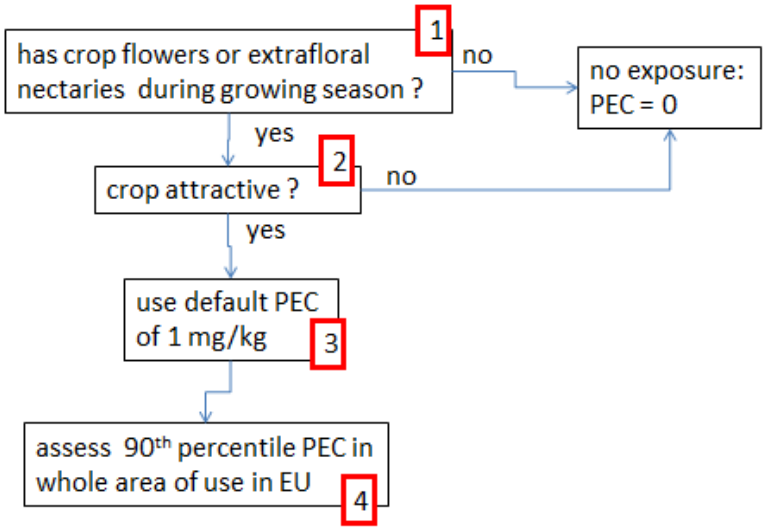


Figure 9: Flow chart for the exposure assessments of the PECs for nectar and pollen in the treated crop after seed treatments. The box numbers refer to the general text above

3.3.2.3. Concentrations in pollen and nectar in succeeding annual crops

After the growing cycle of the seed-treated crop, another attractive crop may be grown in the same year or in the next year. So it is possible that part of the substance brought into the soil with the seed is taken up by succeeding annual crops which may lead to concentrations in pollen and nectar that may cause problems. We expect that this exposure will usually be small because it can be expected that a large part of the substance brought into the soil with the treated seed will be taken up by the crop plant that grows from this seed and because the remaining soil residue probably will behave as a slow-release formulation. In view of time constraints, we are unable to analyse the available relevant information in the literature and the dossiers in detail. Therefore we propose to assess this exposure with the same flow chart as for the spray applications (Figure 7), but of course using the whole surface area grown with this seed-treated crop in the EU as a basis for the assessment of the 90th spatial percentile (see Section 3.2.1). The flow chart for the spray applications uses the groundwater scenarios developed by FOCUS (2009) and the soil exposure scenarios developed by EFSA (2012b). However, these scenarios have been developed for spray applications and do not consider the processes resulting from application with the seed. As described above, these scenarios probably overestimate the soil exposure resulting from seed treatments. As a consequence, the flow chart in Figure 7 may trigger field studies (in box 6) while this is not strictly necessary. Therefore we recommend developing soil exposure scenarios for seed treatments in analogy to the scenarios developed for spray applications by EFSA (2012b).

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1023

1024 3.3.2.4. Concentrations in pollen and nectar in field margins

1025

1026 Introduction

1027
1028 Also for the seed treatments we are interested in the average concentration in nectar and pollen in the
1029 whole field margin of the treated field, so also considering the parts of the field margin that are not
1030 exposed because they were upwing during application.
1031
1032 As described before, field margins are exposed because of dust drift deposition. As described in
1033 Appendix K, the emission of the substance via the dust is almost completely determined by
1034 technological factors (quality of the seed coating and the sowing equipment). Severe bee-killing
1035 incidents have been reported as the result of dust emission after sowing seeds pneumatically and
1036 considerable improvements have been achieved in recent years to reduce these emissions by using
1037 better equipment in a number of Member States (e.g. Germany); see EFSA (2012a). As described in
1038 Section 3.1, the area of use of substances applied as seed treatments is the whole surface area in the
1039 EU where the crop, whose seed is treated, is grown. If we base the exposure assessment of a seed
1040 treatment on this total area, the 90th percentile case is likely to be a case with a sowing equipment with
1041 a comparatively high level of emission. This would have the consequence that one part of the EU
1042 cannot use a substance applied as a seed treatment because technological developments in another part
1043 of the EU are lagging behind. It is uncertain whether this is the intention of the SCoFCAH. An
1044 alternative approach would be to link an authorisation at EU level to a certain class of sowing
1045 machines (similar to the classes for emission reduction of spray drift; see Huijsmans & van de Zande,
1046 2011). These two approaches are fundamentally different: the first approach assesses the exposure
1047 based on the current reality of sowing equipments used across the EU whereas the second approach
1048 prescribes the class of sowing equipment needed for a certain seed treatment (which would have the
1049 consequence that the use is considered not acceptable for classes of sowing equipment that generate
1050 more dust emission). We describe below exposure assessment methodologies for both approaches so
1051 that the SCoFCAH can make an informed choice.
1052
1053

1054 Approach based on sowing equipment as used in reality in the EU

1055
1056 The first step of the exposure assessment (box 1 of Figure 10) is whether the combination of treated
1057 seed and sowing equipment will lead to dust emission (see Appendix G for detailed guidance). The
1058 next step (box 2) is a simple conservative step in which it is assumed that the dose in the treated field
1059 (kg/ha) is sprayed over the field margin. This has the consequence that the acute risk assessment can
1060 be based on HQ < trigger for contact exposure. If this criterion is not fulfilled (box 3), the exposure is
1061 assessed using conservative default dust deposition figures combined with default RUD values for
1062 pollen and nectar derived from spray applications multiplied with a factor 5. Use of RUDs from sprays
1063 may seem strange at first, however the background is as follows: Spray applications usually consist of
1064 spraying a liquid volume of 500 L/ha; this is a water layer of 0.05 mm. Evaporation rates of water
1065 during daytime are in the order of 10 mm/d in Europe in spring and summer, so in the order of 0.5
1066 mm/h. This means that the water of the spray application usually evaporates within an hour. So a spray
1067 liquid will usually become a solid in the field within less than an hour. Therefore it seems justified to
1068 assess the concentration in nectar and pollen based on RUDs from spray applications. However there
1069 are important differences between spray applications and dust deposition: the dust particles may stick
1070 to the hairs of the foragers and the foragers may collect them (assuming that they are pollen) whereas
1071 this is unlikely to occur with dried remnants of a spray solution. Therefore we tentatively introduce a

safety factor of 5. We recommend underpinning this in near future by analysing existing data on dust deposition and resulting concentrations in pollen and nectar reaching the hive.
We propose the following conservative default dust deposition (mass of substance per surface area of the adjacent field expressed as percentage of the mass of substance applied per surface area of treated field) to be used z1% for oil seed rape, 1.3% for cereals, 0.003% for sugar beets and 2.3% for any other crop (see Appendix K for justification).

The next step (box 4) is to assess the distribution of the different sowing equipments (mechanical sowing, pneumatic sowing with and without deflectors) across the EU. These have to be ranked in order of increasing dust emission and the percentage of the surface area of this crop that is sown with this equipment, needs to be estimated (e.g. based on an EU wide questionnaire). Then the sowing equipment has to be selected that will deliver the 90th percentile assuming that only the sowing equipment determines the emission. For example, if the equipment with the highest deposition is used on 15% of the surface area, this equipment will deliver the 90th percentile. If the equipment with the highest deposition is used on 7% of the surface area, then this equipment will not deliver the 90th percentile and the equipment with the one but highest deposition has to be considered. Furthermore the target percentile X for this equipment needs to be assessed in box 4. Let us assume for example that 50% of the cereals is sown mechanically and 50% pneumatically. The pneumatic equipment will lead to more deposition so this is the upper 50% of the frequency distribution. So taking the 80th percentile of the pneumatic exposure should then give the overall 90th percentile. This 80th percentile is the 'target percentile *X* for this equipment' as described in box 4. See Appendix L for the general calculation procedure of this target percentile.

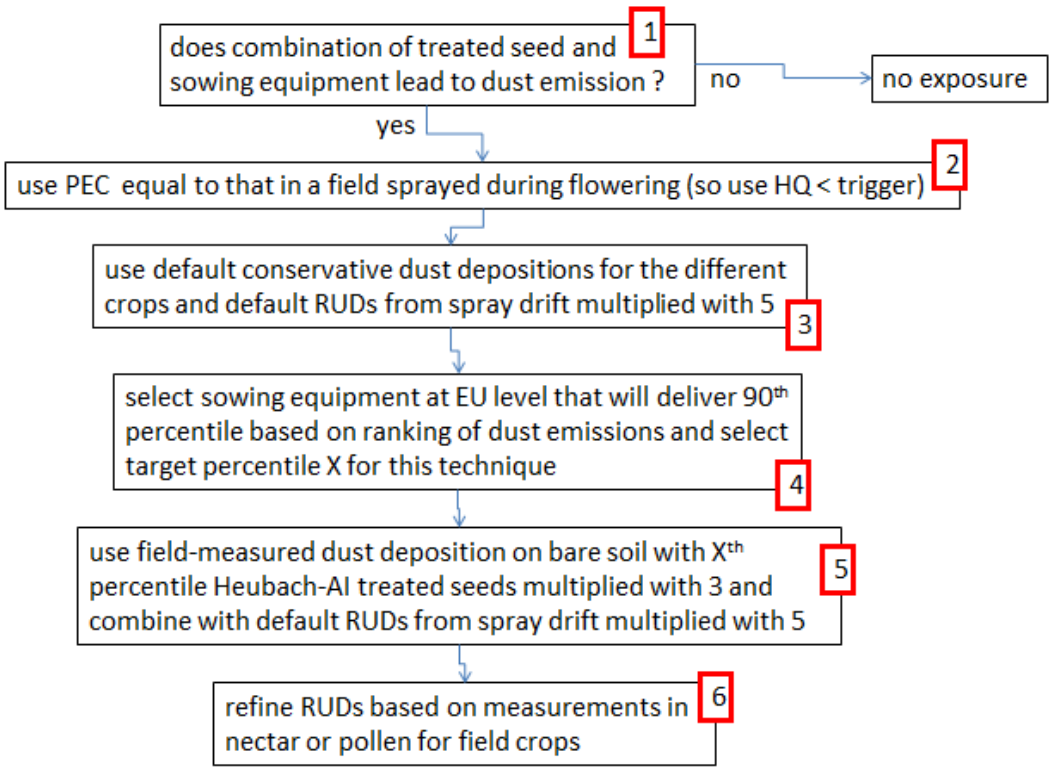


Figure 10: Flow chart for the exposure assessments of the PECs for nectar and pollen in field margins after seed treatments based on the sowing equipments as used in reality across the EU. The box numbers refer to the general text above

So now we know now which percentile to assess considering all field margins adjacent to treated fields where this application equipment is used and move to box 5. As described before, the dust emission is strongly driven by the mass of dust released in the Heubach test and the concentration of active ingredient in this dust. We propose to combine these two factors by defining the 'Heubach-AI' value as the mass of active ingredient per 100 kg seeds or 100 000 seeds in the Heubach test. We propose to base the assessment of this X^{th} percentile on measurements of the Heubach-AI on portions of seed sampled from all seed treatment facilities for this crop-substance combination in the EU. The population of seed treatment facilities differs strongly for the different crops: e.g. in Germany there are about 15 such facilities for oil seed rape and about 1000 such facilities for cereals. Also the variation in the Heubach-AI values is likely to differ strongly for the different crops. Taking again the example of Germany: the variation of Heubach-AI values for oil seed rape is likely to be much smaller than that for maize because the 15 facilities for oil seed rape have agreed to work on the basis of the same protocol whereas the about 1000 facilities for cereals have not yet done so. For the assessment of the 90^{th} percentile exposure case this is not a problem: the sampling of the seed treatment facilities across the EU will take care of the current reality.

The above approach assumes that the sowing equipment has a much larger effect on the emission than the Heubach-AI value and that these are not correlated. They may be correlated if e.g. the sowing equipment with the highest emission is used in a certain region of the EU and the farmers in this region have a preference for seed treatment facilities in this region and if these facilities produce treated seeds with Heubach-AI values that differ systematically from the other facilities in the EU. Then this approach will lead to a systematic error in the estimated 90^{th} percentile. Therefore we recommend to underpin or refine the proposed approach by analysing relevant information in the literature and the dossiers.

So based on Heubach-AI tests using seeds sampled from the relevant population of seed treatment facilities, a portion of treated seed can be identified that corresponds to the X^{th} percentile of the Heubach-AI value. We recommend as a next step (box 5) performing a field experiment in which the deposition of the substance on bare soil is measured as a function of the distance of the treated field (at least over 20 m) using this portion of treated seed. In such experiments the wind angle and wind speed has to be measured continuously (e.g. every minute) at different heights above the soil surface up to at least 5 m. The wind angle during application should be within 30^{o} of the line along which the collecting vessels for the dust deposition have been placed. If the angle appears to be larger at the end, the measured deposition should be corrected (no guidance yet available, so for the time being this correction can be ignored). Wind speed should be between 2 and 3 m/s. The background of this recommendation is that little is yet known about the effect of wind speed on dust deposition in which case experiments can be best carried out at an intermediate wind speed. The deposition in the first hour after application should be measured but also the deposition in the next 23 hours. Also the mass of active ingredient applied to the treated field should be carefully assessed.

The resulting deposition should be multiplied by 10 to account for the filtering capacity of the plants in the field margin and be divided by 3 to account for the overestimation of the average dust deposition because the wind angle in the measurements is limited to 60^{o} of the possible 360^{o} (see Appendix K). These factors 10 and 3 are preliminary figures that should be underpinned by further research. The factor 10 is based on the draft SANCO Guidance Document in which a worst-case study is reported in which 12.4 times more substance was recovered in a vertical gauze net than in Petri dishes on the soil surface. So in combination this shows that the resulting deposition should be multiplied by 10/3 which is rounded to 3.

The deposition of substance resulting from the above exercise has to be combined with the default RUDs from spray drift multiplied by 5 (box 5); this is the same approach as was used in box 3.

In case box 5 does not lead to an acceptable risk, we propose to refine the RUDs of the plants by using RUDs measured for this substance on field crops in dust deposition experiments. This is based on the assumption that the RUD of a substance is more driven by substance properties than by plant

properties. This is likely to be the case but it uncertain whether this assumption can be extended for the full range of plants and substances. Therefore we recommend underpinning this approach by analysing available data and further research. The alternative would be to measure RUD for the most relevant weed species; we do not advise this because the composition of attractive weed species in treated fields is likely to be very variable and we are not aware of data on their distribution in treated fields across the EU.

It is of course possible to add a risk mitigation box at the bottom of Figure 10 that says 'exclude sowing equipment with highest dust emission' with an arrow that goes back to box 4. This would be a compromise between the approach in this section and that in the next section.

Approach based on certain classes of sowing equipments

We now need to consider the alternative approach: i.e.to link an authorisation at EU level to a certain class of sowing machines. This is just a simplification of the approach in the previous section because only one class of sowing equipment needs to be considered.

The first three boxes in the flow chart in Figure 11 are identical to those in Figure 10. In box 4 the same approach is used as in box 5 of Figure 10 with the simplification that a portion of treated seeds should be used that represents a 90th percentile Heubach-AI value. Box 5 is again identical to box 6 of Figure 10. If this class of sowing equipment results in unacceptable risks then there is a risk mitigation option to select a less problematic class of sowing equipment (box 6 in Figure 11) and to go back to box 4.

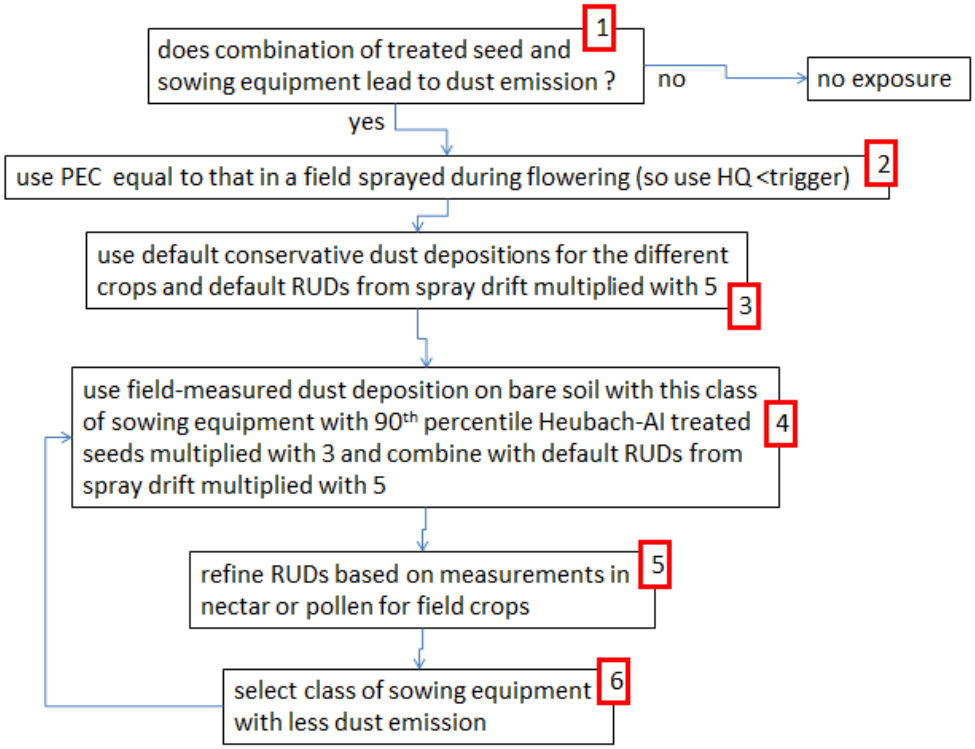


Figure 11: Flow chart for the exposure assessments of the PECs for nectar and pollen in field margins after seed treatments considering a certain class of sowing equipments. The box numbers refer to the general text above

The approach in Figure 11 is stricter than the compromise discussed at the end of the previous section because Figure 11 checks each class of sowing equipment separately whereas the compromise considers all classes of sowing equipments as one pool (e.g. not considering the worst class of equipment if this class was used for less than 10% of the treated fields).

Concentrations in pollen and nectar in adjacent crops

Also for the seed treatments we are interested in the average concentration in nectar and pollen over the full width of the field of the adjacent crops.

For the assessment of the concentrations in pollen and nectar in adjacent crops there is the same choice as that for the field margins: either base the assessment on the sowing equipments that are used in reality across the EU or base it on a certain class of sowing equipments.

We limit the assessment for the adjacent crops to the option of the equipments that are used in reality across the EU. The option of assessing a certain class of sowing equipments can be developed quickly in analogy to Figure 11 after the SCoFCAH has decided between the two options.

The first three steps (boxes 1-2-3 in Figure 12) are the same as in Figures 10 and 11 but note that the values for the conservative dust deposition in box 3 of Figure 12 are higher than those in Figures 10 and 11: as described in Appendix K these should here be 3.4% for maize, 1.4% for oil seed rape, 1.9% for cereals, 0.005% for sugar beets and 3.4% for other crops. The values for the adjacent crop are higher than those for the field margin because the 'dilution factor' for the decline with distance to the treated field for the adjacent crop is 0.48 whereas the 'dilution factor' for the wind angle for the field margin is 0.33 (see Appendix K for details).

The next step (box 4) is to assess whether attractive adjacent crops exist that flower at the time of application or thereafter (otherwise the dust emission will not lead to exposure of hives). If more than one such crop exists, then it should be checked whether they will occur at the border of more than 20% of the treated fields (box 5). The background of this 20% is as follows: only 50% of adjacent crops will be exposed because 50% will be upwind during application so will receive no dust deposition. So if less than 20% of the treated fields have attractive adjacent crops, less than 10% of the hives at the edges of treated fields will be exposed via foraging of the adjacent crop. If this is the case, the exposure resulting from the adjacent crops can be ignored because this exposure will probably not influence the concentration for the 90^{th} percentile of all hives at the edge of treated fields. Please note that this 20% is only justified because seed treatments are by definition applied only once per growing season. In case of spray applications which may be repeated many times in a growing season (especially in fruit crops), the statistics of the drift deposition are more complicated than here. Therefore the limit in box 4 of Figure 5 (spray applications) was set to 10% whereas in box 4 of Figure 12 this 20% is used.

In principle it is possible (using crop maps available at EU level) to analyse the statistics of occurrence of attractive adjacent crops at zonal and member state level and to use the results for all future risk assessments for seed treatments (thus making the use of this flow chart considerably easier and increasing harmonisation of these risk assessments at zonal and member state level). We recommend therefore that this exercise is carried out.

So after having passed box 5, we have one or more attractive crops that in total occur at the border of more than 20% of the treated fields and we have to assess the frequency distribution of the average concentration in nectar and pollen of the population of all these adjacent crop fields. The question is now which factors drive mainly the variability of this frequency distribution. The main factors are likely to be (i) the sowing equipment and the Heubach-AI values of the treated seed (influencing emission), (ii) wind direction and wind speed (influencing deposition), (iii) RUDs of the adjacent crop (influencing the relationship between deposition and concentration in nectar and pollen). The attractiveness of an adjacent crop does not of course influence the concentrations in nectar and pollen in this crop, so this is not considered here. However, this may become important at a later stage when the average concentration in the hive is assessed (using a landscape-level exposure assessment). Of these main factors, the sowing equipment, the Heubach-AI values and the RUDs do not depend on the weather at the moment of application. However, the wind speed and wind direction are of course influenced by this weather. Therefore we propose assessing the effect of wind speed and wind direction differently from the other factors, i.e. by stochastic modelling (Monte-Carlo simulations) based on the natural variability of wind speed and wind direction; see Appendix K for details.

We consider the sowing equipment the most important driver of the concentrations, so we start in box 6 by selecting the sowing equipment that will give the 90th percentile case. Furthermore the target percentile X of this subpopulation of crop and sowing equipment is selected that will give the overall 90th percentile. The procedure is somewhat complicated so it is best explained via an example. Let us assume that there are attractive adjacent crops for 30% of all treated fields and that there are two classes of sowing equipment: i.e. mechanic and pneumatic. Pneumatic gives the highest dust deposition and is used in 80% of the cases whereas mechanical is used in 20% of the cases. First step is to divide the total percentage of adjacent crops by 2 because half of the fields are upwind during application. So we have 15% treated fields left of which 12% is pneumatic and 3% is mechanic. So of these 30% of the treated fields, 12% have the combination of a downwind attractive crop and pneumatic sowing. The target percentile X of this subpopulation is then 100 × (2/12) = 17 because 2 of the 12% are below the 90th percentile. See Appendix L for the general calculation procedure of such percentiles.

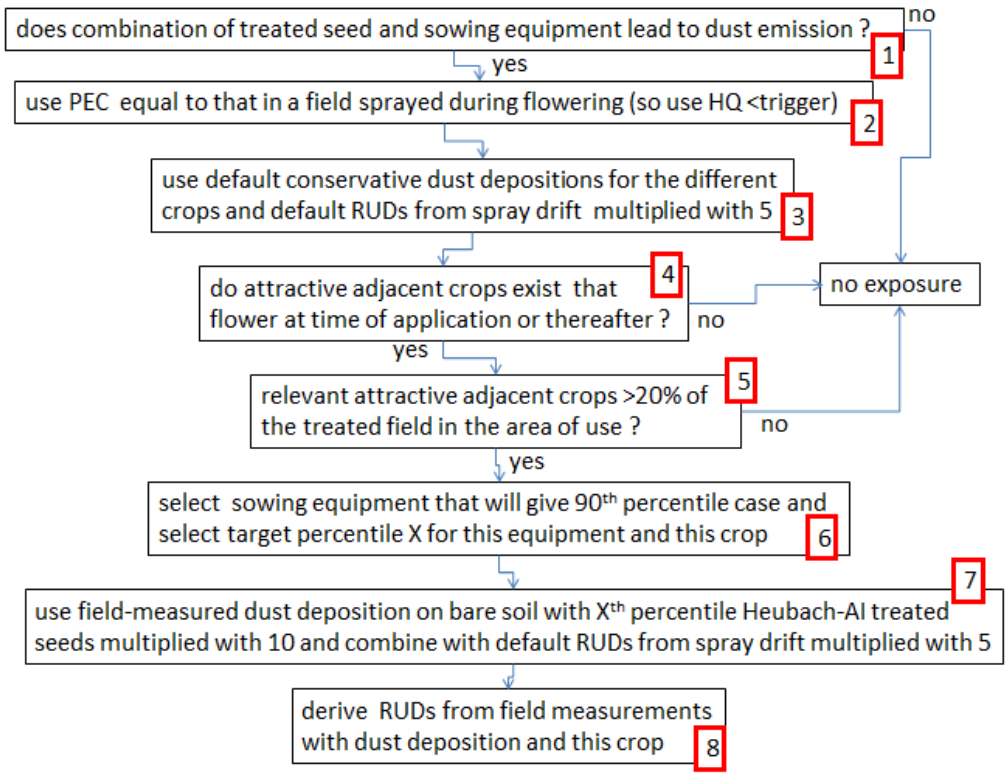


Figure 12: Flow chart for the exposure assessments of the PECs for nectar and pollen in adjacent crops after seed treatments based on the sowing equipments as used in reality across the EU. The box numbers refer to the general text above

The next step (box 7) is the same step as in Figures 10 and 11 but the field measurements here are multiplied by 10 and not by 3 as in Figures 10 and 11 because of the difference in statistics of the drift depositions between the field margin and the adjacent crop as explained in Appendix K. The last step (box 8) is to measure RUDs for this crop and dust deposition which can then replace the default RUDs.

If certain steps in the flow chart are impossible due to lack of available information, it is always an option to use a more conservative and more simple approach. For example, in case of the above example of 30% adjacent crop and 60% pneumatic, it could have been assumed that 100% of this adjacent crop occurs in combination with 100% pneumatic, which would give X = 80 (because 50% of adjacent crops are upwind and have zero deposition) instead of X = 17 in the above case.

The relationship between the assessments for the field margin and the adjacent crop is for seed treatments different from that for the spray applications. As described in Section 2.8, the PEC for the adjacent crop is expected to be lower than that for the plants in the field margin for the spray applications. However, for the seed treatments the situation is not clear because there are two opposite effects: as described before, the average dust deposition onto a downwind adjacent attractive crop is higher than the average dust deposition onto a field margin (Appendix K) but downwind adjacent crops will occur only for a fraction of the treated fields which will lower the target percentile X in box 6 of Figure 12.

3.3.3. Exposure Assessment for granules

3.3.3.1. Introduction

The assessment of the concentrations in pollen and nectar resulting from granule applications has similarities with both that resulting from spray applications and that resulting from seed treatments. The similarities with the spray applications are that the substance is usually applied to the whole soil surface (so not only to the seeds) and that registration decisions are made at national level. The similarity with the seed treatments is that granule application also leads to dust emission. Therefore the exposure assessment for the granules contains both elements of the assessment for the spray applications and elements of the assessment for the seed treatments.

Granules can be applied in different ways: (i) simply broadcasted, (ii) incorporated into the soil, and (iii) buried with the seed. They can be applied both in permanent and in annual crops. When buried with the seed, the similarity in behaviour of the substance with the seed treatments is of course larger than for the other application methods. Our guidance intends to cover all granule application methods. During application, dust is formed from the granules which can be deposited onto the crop (if present) or onto plants in field margins or onto adjacent crops. In view of all these possibilities we propose to use the scheme of Figure 1 to assess the concentrations in nectar and pollen from the different types of plants. The first screening step (box 1) is very conservative for granules because it is unlikely that a granule grain will end up in the flower of a weed and because the dust deposition onto the treated field is probably only a small fraction of the dose.

3.3.3.2. Concentrations in pollen and nectar in the treated crop

For the assessment of the concentrations in pollen and nectar in the treated crop we propose using the flow chart of Figure 2 (designed for the spray applications). The only complication is the estimation of the default RUDs in box 6. As indicated in Figure 2, the exposure via the treated crop is only considered relevant for systemic substances that are applied before flowering.

We propose the following procedure for box 6: (i) if granules are applied before emergence, the default RUDs for granules is based on the information available for the seed treatments (in which case a PEC of 1 mg/kg would be recommended; see box 3 of Figure 9), so RUDs based on uptake via the roots instead of based on overspray; (ii) if granules are applied after emergence, the default RUDs from spray applications are used (which will often lead to much higher PECs than this 1 mg/kg).

In both cases the PECs in pollen and nectar as estimated in box 6 of Figure 2 for the granules will usually be overly conservative. In the first case because the substance applied as treated seed is in much closer contact with the plant roots than when applied as a granule (except in the case of granules that are buried with the seed). In the second case because the treated crop is likely to catch much less substance from a granule application than from a spray application (same argumentation as in previous section for weeds in the treated field).

3.3.3.3. Concentrations in pollen and nectar in weeds in the treated field

For the assessment of the concentrations in pollen and nectar in weeds in the treated field we propose to use the flow chart of Figure 3 (designed for the spray applications). Also here the default RUDs are likely to overestimate exposure.

3.3.3.4. Concentrations in pollen and nectar in plants in field margins

Also for the granules we are interested in the average concentration in nectar and pollen in the whole field margin of the treated field, so considering also the parts of the field margin that are not exposed because they were upwind during application.

For the assessment of the concentrations in pollen and nectar in plants in field margins, we propose using a new flow chart (Figure 13) because the flow charts for the seed treatments (Figures 10 and 11) cannot be used without modifications. Unlike seed treatments, granule applications are not registered at EU level. At Member State level it does not seem to make sense to assess 90th percentiles for different types of application equipments so we follow here the same approach as in Section 3.2.4.2 and in Figure 10, i.e. assessing the 90th percentile that will occur in agricultural reality.

The first step (box 1) is to assume that the field margin has a PEC that is equal to that in a field sprayed during flowering, based on the HQ for contact exposure (so very conservative). The second step is to use default conservative dust depositions in combination with default RUDs from spray drift multiplied with 5. The background of the RUDs from spray drift multiplied by 5 is described at the start of Section 3.2.4.2. We propose to set the conservative default dust deposition to 11% (see Appendix K).

The next step (box 3) is to select the application technique that will deliver the 90th percentile dust deposition (similar to the approach in Figure 10 and Figure 12). EFSA (2004) showed that a spinning disc will generate considerably less dust deposition than a boom spreader. So this implies that it is necessary to estimate which percentage of the granule applications is with a spinning disc and with a boom spreader. From this information the target percentile X for this technique has to be derived (see Appendix K for details).

It is a point of debate which factor should be used to determine the select the case for the percentile X. In principle there are two candidates: the dustiness of the formulation or the meteorological conditions (wind speed). For the seed treatments we proposed to use the Heubach-AI value considering the different seed treatment facilities in the area of use. EFSA (2004) sent a questionnaire to all Member States asking for the information on granule dust measurements that they require from notifiers. Twelve Member States responded; the conclusion was that there are no generally accepted criteria for this in granular formulations. So it is likely that there is considerable variation between the dustiness of different portions of granule formulation. Based on this we propose to assess this target percentile X on the basis of the Heubach-AI value of the granule. There is of course the problem that Heubach-AI values are not part of the current regulatory dossier. However, it may be possible to estimate the Heubach-AI values with the existing CIPAC methods to measure the dustiness of granules (see EFSA, 2004, for a description of these methods). To bridge the gap between the Heubach test and the CIPAC methods, data are needed on Heubach-AI values for a range of granules for which the CIPAC information is already available. We recommend generating this data as it may facilitate the introduction of this new approach in regulatory practice.

So the next step (box 4) is to perform a field experiment on dust deposition on bare soil with a portion of granule formulation that approaches the Xth percentile of the Heubach-AI values. See Section 3.2.4.2 for instructions on the experimental conditions. The measured result has to be multiplied by 10 to account for the filtering capacity of the plants. The result has to be combined with default RUDs

from spray drift multiplied by 5 as described before. The next step is to refine the RUDs based on dust deposition experiments on field crops. If the risk is still unacceptable, it may be an option to mitigate the exposure by excluding the application technique that gives the highest deposition (box 6) and to go back to box 3.

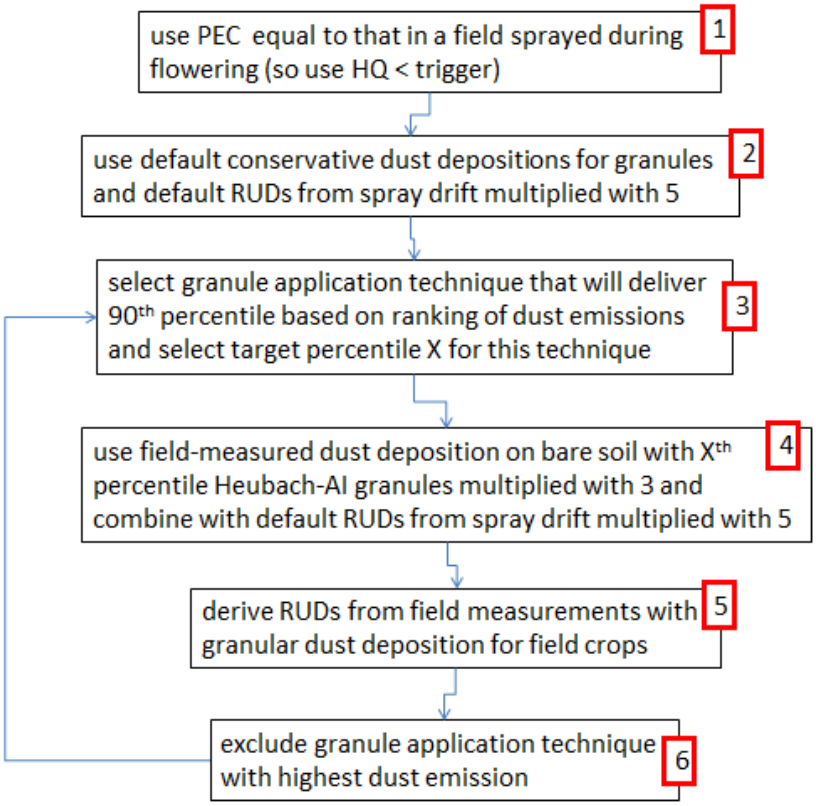


Figure 13: Flow chart for the exposure assessments of the PECs for nectar and pollen in field margins after granule applications. The box numbers refer to the general text above

3.3.3.5. Concentrations in pollen and nectar in adjacent crops

The assessment of the concentrations in pollen and nectar in adjacent crops can be assessed following the same principles as for the spray applications and seed treatments (Figures 5 and 12 in Sections 2.6 and 3.2.5). As in the case of the seed treatments, it is a priori unknown whether the 90th percentile concentrations in the adjacent crops are higher or lower than those in the plants in the field margin because there are two opposite effects: the average dust deposition on a downwind adjacent attractive crop is higher than the average dust deposition on a field margin (Appendix K) but downwind adjacent crops will occur only for a fraction of the treated fields which will lower the 90th percentile concentration.

We propose the flow chart in Figure 14. This differs only slightly from that for the seed treatments in Figure 12, so only those parts that are different from Figure 12 are discussed here. Unlike Figure 12 there is no first box that checks whether the combination of granule and application equipment leads to dust emission because dust emission can never be excluded for granules. The conservative default dust

deposition for the granules in box 2 is 15% (as explained in Appendix K) which is considerably higher than the values for the seed treatments (Table K1). The trigger percentage in box 4 is 20% for single applications and 10% for multiple applications because, unlike seed treatments, granule applications may occur several times in a growing season. Unlike Figure 12, Figure 14 contains a risk mitigation box at the bottom that allows for elimination of application techniques that lead to too high risks.

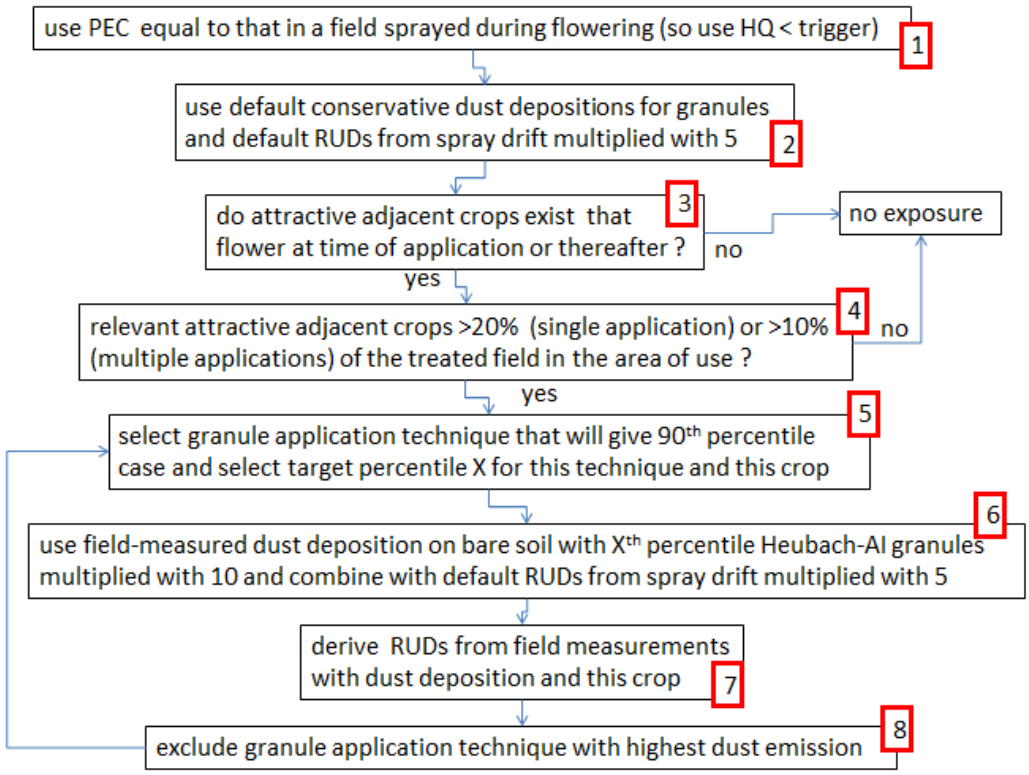


Figure 14: Flow chart for the exposure assessments of the PECs for nectar and pollen in adjacent crops after granule applications. The box numbers refer to the general text above.

3.3.3.6. Concentrations in pollen and nectar in permanent crops in the next year and in succeeding annual crops.

The assessment of the concentrations in pollen and nectar in permanent crops in the next year and in succeeding annual crops can be based on the flow charts for the spray applications (Figures 6 and 7).

3.4. Recommendations for further work to improve or underpin the proposed exposure assessment guidance

We recommend developing guidance for a landscape-level approach for the exposure of the average concentration in nectar and pollen entering the hive because without such guidance the exposure assessment of this concentration is likely to be unnecessarily conservative. Such guidance has to be based on a quantitative model for assessing these concentrations considering a variety of attractive crops within the foraging surface area. We recommend developing the quantitative model (see Appendix H for a first attempt) and underpining this by extensive field calibrations. Special attention should be paid to the effect of differences in attractiveness of different crops on the average concentration entering the hive because this may influence the assessment of the 90th percentile in case of different attractive adjacent crops.

We recommend developing guidance at EU level for assessing whether a significant fraction of the surface area of treated fields is likely to be covered by attractive weeds for more than 10% of the area of use of substances. This guidance is likely to become a useful element of the exposure assessment of concentrations in nectar and pollen in weeds in treated fields.

We recommend analysing available data on RUDs in attractive weeds and crops resulting from spray applications to underpin the hypothesis that the RUD of a substance in attractive weeds can be predicted from the RUD of this substance in treated crops. If the available data are insufficient, we recommend performing research to test this hypothesis. This hypothesis offers a higher-tier option for the exposure assessments of concentrations in nectar and pollen in weeds in (1) treated fields and (2) field margins.

We recommend performing geostatistical analyses (using currently available crop maps; e.g. http://eusoils.jrc.ec.europa.eu/library/Data/EFSA/) to assess the likelihood of occurrence of attractive crops (1) grown adjacent to the treated crop and (2) grown in the treated field after the treated crop. We recommend summarising the results of these analyses in the form of user-friendly software that produces the frequency distributions of these attractive crops for all major crops at Member State and zonal level. We also recommend analysing the width of these adjacent fields and their geometry in relation to the treated field because these have a large effect on the average deposition of spray drift on these adjacent fields.

We recommend performing spatial analyses to identify the most relevant crops adjacent to seed treatment applications at zonal and member state level to streamline the exposure assessment resulting from dust deposition in adjacent crops.

We recommend performing (1) a geostatistical study to underpin or revise the proposed field margin width of 2 m and to check to what extent all edges of the field are surrounded by field margins, (2) a modelling study in which the spray drift deposition onto field margins and onto adjacent fields with attractive crops is simulated as a function of a stochastic wind angle and a stochastic wind speed from which the 90th percentile spray deposition cases can be derived (see van der Zande et al., 2012, for an example of such a study for spray deposition on surface water). This modelling study should also consider the effect of repeated applications. Furthermore we recommend analysing all spray drift data available in the EU to underpin the assumptions on which this modelling study should be based. We also recommend considering in this analysis the fact that the plants in field margins and of the adjacent crop may perhaps 'catch' more drift than bare soil.

We recommend developing targeted scenarios for assessing the plant uptake of substances in attractive permanent and attractive annual succeeding crops because such scenarios may be useful for assessing the need of residue analyses in nectar and pollen in such crops. It is advisable to check whether the

proposed trigger DegT50 < 5 d (box 1 of Figure 6) is appropriate after these scenarios have been developed.

In view of the large uncertainty in the average concentration in nectar and pollen entering the hive in higher-tier experiments, we recommend measuring this concentration in such future higher-tier experiments.

We recommend analysing available data on residues in nectar and pollen resulting from root uptake to underpin that non-systemic substances are not transported to nectar and pollen in amounts that could become relevant for the risk assessment of bees.

We recommend performing research to underpin or revise the assumption that differences in RUD values between different adjacent crops play only a minor role in the assessment of the 90th percentile exposure concentration in nectar and pollen in adjacent crops (both for spray applications and solid applications).

We recommend analysing existing data on concentrations in nectar and pollen in the hive that result from exposure to dust deposition (originating both from seed treatments and granules) in order to assess to what extent these can be predicted on the basis of RUDs resulting from spray drift deposition multiplied by a factor of 5.

We recommend performing research to underpin or refine the factor 10 used to extrapolate dust deposition on bare soil to dust deposition on attractive plants in field margins and on attractive adjacent crops.

We recommend performing stochastic simulation studies using calibrated physical models in which the dust deposition on attractive adjacent crops is simulated as a function of wind speed and wind angle to obtain a less conservative and thus more realistic assessment of the 90th percentile deposition.

We recommend developing soil exposure scenarios for seed treatments in analogy to the scenarios developed for spray applications by EFSA (2012b) in order to improve the exposure assessment of weeds in the treated field and of attractive crops grown after the treated-seed crop.

We recommend analysing relevant information in the literature and the dossiers on the effect of sowing equipment and Heubach-AI values (as defined in Section 3.4.2.1) on emission of dust during sowing of treated seeds to underpin the assumption that the sowing equipment (mechanical versus pneumatic, with and without deflectors) has a much larger effect than the Heubach-AI value.

We recommend measuring Heubach-AI values for a range of granules and to try to correlate these to information in the dossier on the dustiness of these granules (CIPAC methods).

We recommend collecting and analysing all available data on dust deposition of granules on plants in adjacent crops in order to reduce the 15% conservative default deposition.

4. Laboratory, semi-field and field studies

Several points for improvement and future research related to the available test protocols were identified in EFSA, 2012a. Weaknesses were identified in particular in relation to field studies. In order to rely on the studies in the risk assessment it is recommended that the points listed in the relevant appendices (Appendices M, N, O) are systematically checked for each study that is submitted and included in the risk assessment. Studies which do not address the points should not be relied on in the regulatory risk assessment.

4.1. Acute laboratory (oral+ contact LD 50), 10-d laboratory adult (LC50), Aupinel larvae test

The endpoints from these studies should be collated as follows:

Toxicity study	Endpoint
LD50 contact (Appendix M)	µg/bee
LD50 oral (Appendix M)	µg/bee
LC50 adult (Appendix M)	mg/kg
NOEC larvae (Appendix M)	mg/kg

Please see the relevant Appendix M for further details.

Proposals are made for test protocols for studies with *Bombus terrestris* and *Osmia* spp. (see Appendices P and Q).

4.1.1. Test for bioaccumulative toxicity in oral dose administered to honey bees

Testing protocol

1. Using at least 3 cages of 10 newly enclosed workers per dose with ad libitum access to feeder syrup (using a minimum of 4 doses plus a control and measure intake at 24 at 48 hrs (replace with fresh feed)), determine the concentration of the PPP compound (µg L^{-1}) in dietary syrup necessary to cause 50% mortality after 48 hours of exposure. Denote this concentration (units of µg L^{-1}) by $LC_{50,48h}$.

2. Using the same experimental units and conditions, administer feeder syrup at two concentrations of the compound: $LC_{50,48h}$ and $0.25LC_{50,48h}$ (which has a molarity of one quarter that of $LC_{50,48h}$) and measure syrup consumption rates (replace with fresh feed each day) and mortality daily until each cage has accumulated 50% mortality. Cages receiving syrup of the lower concentration ($0.25LC_{50,48h}$) are expected to reach this mortality in approximately eight days or less (see below). The suggested level of replication is 10 cages of each concentration (but see power requirements in step 4 below).

3. For each cage, determine the total (cumulative) quantity of compound (µg) consumed in each cage when 50% mortality occurred. For a cage exposed to the high concentration ($LC_{50,48h}$) treatment, denote this amount by Q_H and by Q_L for a cage at the low concentration ($0.25LC_{50,48h}$).

4. For each separate concentration ($LC_{50,48h}$ and $0.25LC_{50,48h}$), determine the mean quantity of compound consumed (total) in each treatment group of cages, denoted as $E(Q_H)$ and $E(Q_L)$ respectively. If E (Q_L) is lower than E (Q_H), there is potential for bioaccumulation, so test the difference between these two means with an appropriate statistical analysis (e.g. one-tailed t-test if the assumptions are met). The experiment is valid if the power of the test to detect a 35% difference in E

(Q) between the two concentration treatments is at least 80%. This difference is calculated relative to the high concentration treatment: % difference = 100* [$E(Q_H)$ - $E(Q_L)$]/ $E(Q_H)$. A procedure for power analysis is given in Fig 14.

5. Designate the PPP as showing a potential for bioaccumulation if $E(Q_L)$ is lower than $E(Q_H)$ and the statistical test shows a significant difference between the two sets of Q_H and Q_L and the estimated half life of the toxicant is ≥ 1 day (calculate $E(Q_L)/E(Q_H)$ and estimate the half-life by using this value on the x-axis of Fig 15; the estimated half-life is the corresponding value on the y-axis). This threshold is chosen for the following reason: once an animal is no longer exposed to a toxicant, the expected time for the toxicant to be virtually eliminated from the animal's body is five time the toxicant's half-life because $0.5^5 < 5\%$. Five days is a significant proportion of an adult bee's lifespan.

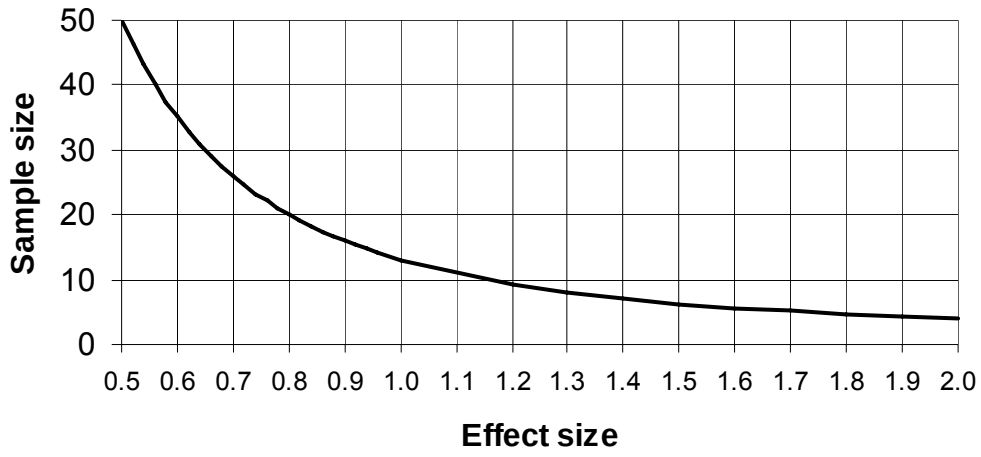


Figure 15: Sample size required to detect the size of a given effect using a one-tailed t-test with 80% confidence, which is the conventional requirement for adequate statistical power. 'Effect size' is calculated as: E = (0.35×mean measurement of control group)/standard deviation of control group. Relationship obtained using power.t.test (d = ∗, sd = 1, sig.level = 0.05, power = 0.8, type = "two.sample", alternative="one.sided") in **R** statistical software, where ∗ denotes the effect size (E).

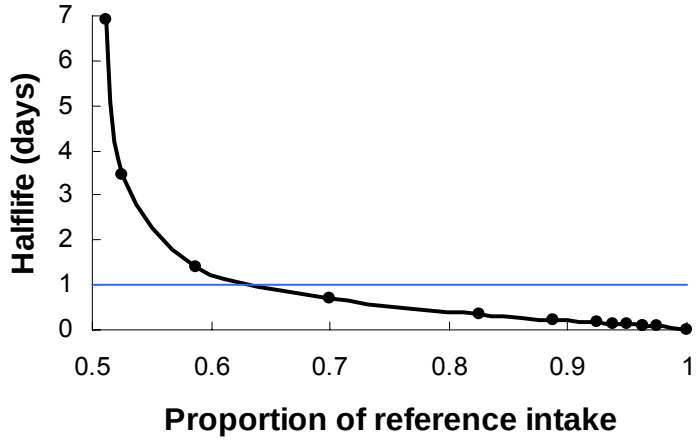


Figure 16: Idealized relationship between estimated half-life and the observed total dietary intake of the compound in the low concentration exposure that precedes 50% mortality as a proportion of the total intake in the high concentration exposure (i.e. *proportion of reference intake* = $E(Q_L)/E(Q_H)$).

Principles of the test

Haber's law predicts the same level of response under two exposures that produce an equivalent constant toxic load, where toxic load is defined as the product of the environmental concentration and time. If L denotes the toxic load necessary to cause a given effect among exposed subjects, C is the exposure concentration and t is the exposure duration, then Haber's Law is given by

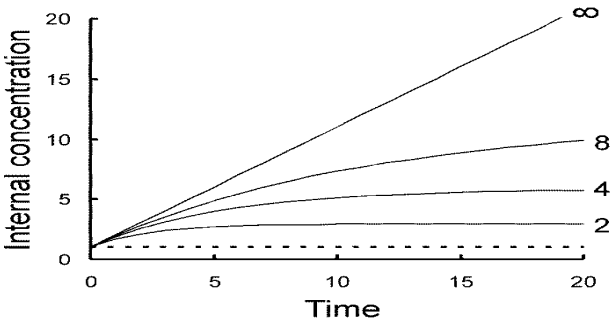
$$C \times t = L \qquad \text{(Eqn 4)}$$

When Eqn 4 applies, the effect shows 'first order time-dependence'.

Assuming that daily consumption of syrup is constant and independent of the concentration of toxicant, an equivalent toxic load is produced by $C_1 = LC_{50,48h}$ for $t_1 = 2$ days and $C_2 = 0.25(LC_{50,48h})$ for $t_2 = 8$ days because the fourfold reduction in dietary concentration is compensated by the fourfold increase in the duration of the exposure. If the daily consumption rates of syrup are approximately equivalent, regardless of the concentration of the toxicant, then the total amount of toxicant consumed will be directly proportional to the duration of exposure and the use of Q_H and Q_L to test Haber's Law simply involves a transformation of measurement units.

If, on the other hand, the toxicant is an antifeedant, the consumption rates of syrup depend on the concentration of the toxicant and so the daily consumption rates may be higher in the cages exposed to the low concentration syrup ($0.25(LC_{50,48h})$. In this case, the cages exposed to low concentration syrup manifest 50% mortality faster than expected simply through rapid consumption of the toxicant and $t_2 <$ 8 days is not due to bioaccumulation. However, the test protocol has taken this into account. Toxic load has units of 'molar hours' because it is the product of concentration and exposure time and, in principle, it is proportional to the number of molecular contacts between the toxicant and its target site. For a perfectly non-accumulating toxicant, each molecule is eliminated instantly after contacting the target site and so the toxic load is also equivalent to the total amount of toxicant ingested. Since an equivalent effect is expected from an equivalent toxic load, it is appropriate to test Haber's Law by comparing the total amount of toxicant consumed to bring about a fixed endpoint, such as 50% mortality, between the two exposures.

For a persistent toxicant that bioaccumulates during continuous ingestion, Haber's Law, as stated in Eqn 4, will fail to describe the exposure-concentration relationship because the concentration of the toxicant at its site of action increases with time even when the dietary concentration is constant (Figure 16).



1648

1649 **Figure 17:** Relationships between the internal concentration of a toxicant and time for five
1650 compounds with various degrees of persistence and bioaccumulation. Each curve relates to a
1651 hypothetical individual that ingests one unit of dietary toxicant per unit time but the five compounds
1652 vary in their biological half-life; one is eliminated completely by the end of each time unit (dashed
1653 horizontal line) and

1654 If the toxicant's effects become disproportionately large as the duration of the exposure increases
1655 despite constant dietary concentration, the effect shows 'second order time-dependence', i.e. the toxic
1656 load necessary for a given level of fatalities is:

1657 $C \times t^b = L$ (Eqn 5)

1658 where $b > 1$.

1659

1660 If we consider $C_1 = LC_{50,48h}$ and $t_1 = 2$ days, the exposure duration required to produce an equivalent
1661 toxic load $C_2 = 0.25(LC_{50,48h})$ when $b > 1$ is found by solving

1662

1663 $C_1 \times 2^b = 0.25C_1 \times t_2^b$ (Eqn 6)

1664 which yields

1665

1666 $t_2 = \sqrt[b]{4 \times 2^b} = 2\sqrt[b]{4}\sqrt[b]{(4 \times 2^b} = 2\sqrt[b]{4}$ (Eqn 7)

1667

1668 Note that this relationship does not depend on the concentration of a.i. in the syrup used for the short
1669 exposure. For a non-bioaccumulative toxicant ($b = 1$), the required exposure duration is 8 days, as
1670 required. For a bioaccumulative toxicant (e.g. $b = 2$), the required exposure duration is less than 4
1671 days.

1672

1673

1674

4.2. Semi-field and field studies

Semi-field and field studies are required when concerns have not been adequately addressed at lower tiers. This could mean that the First Tier assessment and/or the refined assessment using more appropriate exposure data has not been satisfactorily addressed to enable a decision to be made as regards whether the use can be permitted without risk mitigation measures. The choice and design of any Higher Tier study should be such that it addresses concerns highlighted at lower tiers. Guidance is provided below on when it is appropriate to either carry out a semi-field or field study. Detailed protocols are presented in Appendices N and O, outlining how a semi-field or field study for regulatory purposes should be carried out.

When to do a semi-field or field study

The table below is a guide on when to carry out either a semi-field or field study. This guidance is aimed at addressing the risks/concerns highlighted at the First Tiers of the risk assessment scheme.

Risk quotient breached	**Discussion**	**Proposed study**
Risk quotient for a spray application is breached for adult acute oral LD50 only	When ***only*** the risk quotient for adult acute oral LD50 is breached concern is only related to acute oral effects, i.e. all other risk quotients pass, hence in order to determine the 'real' risk under more realistic conditions it is proposed that a semi-field study is conducted.	As the effects are short-term a study according to EPPO170 with a focus on mortality.
Risk quotient for a spray application is breached for adult acute contact LD50 only	When ***only*** the risk quotient for adult acute contact LD50 is breached concern is only related to acute contact effects, i.e. all other risk quotients pass, hence in order to determine the 'real' risk under more realistic conditions it is proposed that a semi-field study is conducted.	As the effects are short-term a study according to EPPO170 with a focus on mortality.
Risk quotient for a spray application is breached for adult chronic oral LC50 only	When ***only*** the risk quotient for adult chronic oral LD50 is breached concern is only related to chronic oral effects, i.e. all other risk quotients pass, hence in order to determine the 'real' risk under more realistic conditions it is proposed that a semi-field study is conducted that is appropriately extended to ensure that long-term effects on adult bees and the colony can be determined.	As the effects are short-term a study according to OECD 75 – the Opinion and the Defra R and D (PS2367) highlighted some potential changes.
Assessment for bioaccumulative risk highlights a concern	When ***only*** the assessment for bioaccumulative risk raises a concern, then for the 'real' risk under more realistic conditions it is proposed that a semi-field study is conducted that is appropriately extended to ensure that long-term effects on adult bees and the colony can be determined.	As the effects are short-term a study according to OECD 75 – the Opinion and the Defra R and D (PS2367) highlighted some potential changes.
Risk quotient for a spray application is breached for larvae assessment only	When ***only*** the risk quotient for the larvae assessment is breached concern is only related to effects on larvae, i.e. all other risk quotients pass, hence in order to	As the effects are short-term a study according to OECD 75 – the Opinion and the Defra

Risk quotient breached	Discussion	Proposed study
	determine the 'real' risk under more realistic conditions it is proposed that a semi-field study is conducted that is appropriately extended to ensure that effects on larvae and the colony can be determined.	R and D (PS2367) highlighted some potential changes.

If a risk assessment breaches more than one risk quotient, e.g. the risk quotient for acute oral adults and larvae are breached, then it is proposed that the applicant should carry out the most comprehensive study, i.e. the study should be designed to address all the concerns raised at lower tiers.

If as a result of conducting a semi-field study, concern is highlighted, then there is either a need to ensure that appropriate risk mitigation is used to ensure that exposure and hence risk to honey bees is kept to a minimum, or a field study should be conducted to address the concerns raised at all the tiers.

Design of a semi-field and field study

If as a result of the initial risk assessment concern is raised, i.e. one or more risk quotients are breached, then further work is required. To avoid further studies, it may be possible to refine the risk assessment by refining either the exposure assessment or the effects assessment; in addition, it may also be possible to refine the risk assessment using risk mitigation measures (this is in effect refining the exposure estimate to an 'acceptable' level). If an unacceptable risk remains, it must be further investigated by studies, which are described below.

Details as to how to carry out and interpret semi-field and field studies and as to how to use them in risk assessment are provided in Appendices N and O. In carrying out field studies it is important to ensure that adequate exposure has been achieved and it is therefore necessary to carry out residue studies (see Appendix J) to determine the likely residues in pollen and nectar of flowers in treated fields. It is also necessary to carry out semi-field studies so that the residue in pollen and nectar in flowers on treated plants can be compared to that likely to be present in pollen and nectar in the hive. Briefly, the rationale is as follows: semi-field studies typically force bees to forage exclusively on treated flowers, which means that the in-hive residues will be at their highest levels. In-hive residues may have lower concentrations than the residues in nectar and pollen from flowers for various reasons (compound degradation, metabolism by bees). Once determined in a semi-field study, the differential between floral and in-hive residues can be used to evaluate whether in-hive residues have reached adequate levels in field studies, i.e. information about the flower-hive differential is used, along with the residue data set collected according to Appendix J to determine if exposure in a field study has been sufficient. This is illustrated by the following:

- As part of the exposure assessment, it is necessary to determine the residues in pollen and nectar in flowers from treated plants from residue studies (see Appendix J). These data indicate that the residues in pollen and nectar are P_{flower} and N_{flower} mg/kg respectively.
- Semi-field studies are conducted and the residues in pollen and nectar in the treated plants are P^*_{flower} and N^*_{flower} mg/kg, whilst the residues in the pollen and nectar in the hive are P_{hive} and N_{hive} mg/kg respectively.
- This information is used to calculate two adjustment factors, i.e. $P^*_{flower} / P_{hive} = A_{pollen}$, for the pollen adjustment factor; and $N^*_{flower} / N_{hive} = A_{nectar}$, for the nectar adjustment factor. These factors are used to determine the expected level of residues in the hive under field conditions. Specifically, if the residues in the treated flowers at the field study are P'_{flower} and N'_{flower} then the in-hive residue levels are expected to be $A_{pollen} \times P'_{flower}$ and $A_{nectar} \times N'_{flower}$. The effect of this calculation is illustrated by considering a hypothetical pesticide that degrades before it

reaches the hive. In this case, the in-hive residue in the semi-field study is zero, $A_{pollen} = A_{nectar} = 0$, and the expected in-hive residues in a field study are $A_{pollen} \times P'_{flower} = 0$ and $A_{nectar} \times N'_{flower} = 0$.

- These factors are used to adjust the exposure estimates and the risk assessment re-run (see Figure 1 of Chapter 3).
- If a field study is conducted, then the in-hive concentration of pollen and nectar should be greater than that measured under semi-field conditions.

In addition to the conventional semi-field and field studies and in order to address concerns raised in EFSA (2012a) regarding the ability of field studies to adequately assess potential adverse effects on behaviour of bees, and in particular effects on orientation and a subsequent effect on the ability of bees to return to the colony, it is proposed that a homing study should be carried out. Details are provided in Section 3 of Appendix O.

5. Trigger values

The risk assessment scheme and associated trigger values need to ensure that the protection goal (negligible effects on colonies, see chapter 2) is achieved at all levels of the tiered risk assessment.

In defining the Specific Protection Goal (SPG) reference has been made to the level of mortality that colonies next to a treated crop can sustain over a certain time period without undue harm. (i.e. the colony will not be lost).

In order to determine if a Plant Protection Product and its associated use pose an acceptable risk, and hence the SPG can be met, it is necessary to develop appropriate trigger values.

Currently, in risk assessments carried out under 1107/2009 a Hazard Quotient, or HQ, approach is used to determine whether the acute risk from a pesticide applied as a spray poses an 'acceptable'[7] risk. A HQ is the ratio between the application rate in g/ha and the LD50oral or LD50contact in µg/bee, i.e. g/ha ÷ LD50. If the resulting ratio is 50 or less, then the risk is deemed to be acceptable. A key issue to consider is whether a HQ of 50 or less is comparable to the protection goal.

The HQ trigger has been reviewed by Mineau et al., (2008) and Thompson and Thorbahn (2009). There are several limitations (see Appendix R in EFSA, 2012a) which make it difficult to link the HQ of 50 to the suggested protection goal of negligible effects on colonies. Therefore an alternative method to derive trigger values is suggested in the current Guidance Document and described in Appendix U.

It was considered appropriate to use the same trigger values for solid formations as for spray formulations (see Appendix U).

The risk assessment scheme and associated trigger values enable an assessment that, if met, would protect x % of sites (i.e. treated fields) where honey bee colonies are situated on the edge of treated fields. The trigger values are set so that an individual colony can tolerate an impact on foragers of a certain magnitude for a certain period of time (for negligible effects this is for example an increase of average daily mortality compared to controls by a factor of 1.5 for 6 days).

In order to calculate trigger values which should ensure that the protection goals are met, it was necessary to find information on background mortality of foragers under natural conditions. In the published literature only 7 studies were found where natural background levels of forager mortality could be derived. In 5 studies information was given on the forager mortality or on life span of foragers and in 2 studies only the total life span of adult bees was given. In order to increase the dataset also these two studies were included in the analysis and the forager life span was calculated assuming that the in-hive life span is 20 days. The average daily forager mortality rate ranged from 5.3% to 20.8%. The 10^{th} percentile was 7.9% and the median value was 13% (see Appendix T). The conservativeness of the trigger value depends on the choice of the background mortality. The lower the number of natural background mortality that is chosen for derivation of the trigger value the more conservative will be the resulting trigger value. Given the limited dataset it is proposed to use the lowest background mortality rate found in literature to derive the trigger values. This may be refined further as soon as more studies become available. For the calculation of the trigger values and further details see Appendix U.

The following trigger values are proposed for honey bees:

[7] The term 'acceptable' is not defined, i.e. it is not related to a level of mortality or sub-lethal effects.

The trigger value for acute oral and acute contact toxicity are for hazard quotients. HQ = application rate (in g a.s./ha) / toxicity (µg a.s./bee).

The trigger values for chronic oral toxicity and the larvae (NOEC) are for ETRs (ratio of Exposure and Toxicity, ETR = Exposure/Toxicity).

In order to conclude that the protection goal is met, the calculated HQ or ETR value needs to be lower than the suggested trigger value.

Acute oral toxicity (LD50): HQ < 33

Acute contact toxicity (LD50): HQ < 11

Chronic oral toxicity (LC50): ETR < 0.03

Larval toxicity (NOEC): < 0.1

The endpoint for larval toxicity is based on a concentration that does not cause any effects in the laboratory study compared to controls (NOEC). Therefore the protection goal of negligible effects is achieved if the 90th percentile exposure estimate does not exceed the NOEC. No additional assessment factor is needed to ensure that the protection goal is achieved. However, there are uncertainties related to potential differences in sensitivity in honey bee subspecies and lab to field extrapolation. An assessment factor of 10 is proposed in order to account for these uncertainties.

The following trigger values are proposed for bumble bees:

Bumble bee workers have a longer flight span than honey bee workers and thus lower daily mortality rates. The trigger value calculation was based on a daily background mortality of 4.4% (see Annex X on mortality rates). Bumble bee colonies are particularly susceptible to reduction of worker bee numbers because only large colonies produce queens (see Whitehorn et al., 2012). In order to account for the higher susceptibility to worker losses it is suggested to add an additional assessment factor of 5 to the trigger value established for honey bees.
The endpoint from the honey bee larvae test is used in the risk assessment for bumble bees. In order to account for uncertainties related to potential differences in sensitivity between honey bee larvae and bumble bee larvae it is suggested to add an additional assessment factor of 10.

Acute oral toxicity (LD50): HQ < 5.5

Acute contact toxicity (LD50): HQ < 1.76

Chronic oral toxicity (LC50): ETR < 0.024 or <0.0024*

Larval toxicity (NOEC): < 0.01

*an additional assessment factor of 10 should be added to the ETR trigger if the assessment relies on the endpoint from honey bees in order to account for potential differences in species sensitivity.

The following trigger values are proposed for solitary bees:

The trigger values for acute effects were calculated based on a daily background mortality of 5% (based on a flight span of 20 days for Osmia taken from Bosch et al. 2008). An assessment factor of 5 is suggested in order to account for uncertainties related to potential differences in sensitivity among solitary bees.
The endpoint from the honey bee larvae test is used in the risk assessment for solitary bees. In order to account for differences in sensitivity between honey bee larvae and solitary bee larvae it is suggested to add an additional assessment factor of 10.

Acute oral toxicity (LD50): < 6.3

Acute contact toxicity (LD50): < 2

Chronic oral toxicity (LC50): ETR < 0.027 or <0.0027*

Larval toxicity (NOEC): < 0.01

*an additional assessment factor of 10 should be added to the ETR trigger if the assessment relies on the endpoint from honey bees in order to account for potential differences in species sensitivity.

Please note that the natural background mortality has a strong influence on the proposed trigger values for acute toxicity (contact and oral) and chronic oral toxicity. The proposed trigger values are based on the lowest values of background mortality found in literature as a precautionary approach because of the low number of studies available. If more data becomes available this value may be refined.

The trigger values include assessment factors to account for uncertainties related to lab to field extrapolation and potential differences in species sensitivity. These uncertainties could be reduced if more data becomes available.

Therefore it would be welcome if stakeholders could provide data to address these uncertainties in order to refine the trigger values.

6. Introduction to the risk assessment scheme for honey bees

6.1. Acute and chronic risk assessment

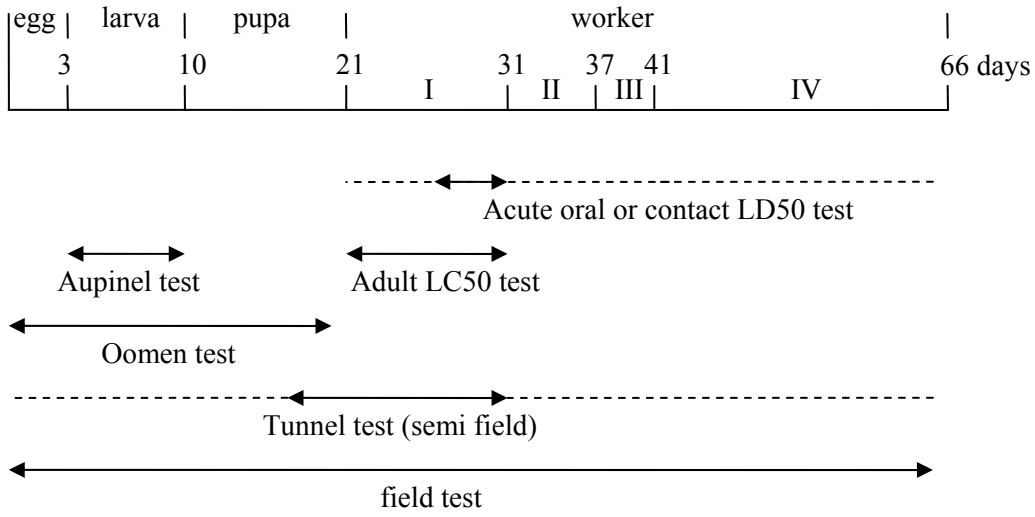
For risk assessment of adult honey bees following a spray application, the contact and oral acute (single dose) LD50 should be generated (using OECD guidelines 213 and 214) as these reflect the hazard associated with single acute exposures. Both routes of exposure should be evaluated as there is currently insufficient data to predict the contact LD50 from the oral LD50 and vice versa. It is important that the OECD guidelines are complied with in detail, e.g. that the study is extended if increasing mortality is observed and all sub-lethal effects are reported. Data on the toxicity of the active ingredient and the formulation should be reported (LD50, ECx and slope) as effects may differ, e.g. co-formulants may alter the rate of uptake and products may contain more than one active ingredient. These data are used to generate the Hazard Quotient (HQ) using the lowest of the LD50 estimates and the application rate (µg a.i. or µg product as appropriate) at the First Tier. Although the HQ is not based on a detailed assessment of exposure to sprayed products it is a measure of risk which has been validated using field trial and incident data (Thompson and Thorbahn, 2009).

For systemic pesticides applied as seed and soil treatments, exposure may be by intake of contaminated nectar or pollen, through guttation water or via dusts. As for the sprayed compounds, the acute oral LD50 should be evaluated but the contact exposure route is less relevant.

It is recognised that single acute exposure scenarios are not representative of the exposure of foragers or in-hive honey bees for compounds which may persist for more than a single day in the environment, or in nectar and/or pollen returned to the hive. Currently there is insufficient evidence that toxicity following extended exposures can be reliably predicted from acute oral LD50 data. Until this can be demonstrated, a more extended oral toxicity study is recommended; in practice even when the database supports prediction for existing classes of active ingredient, it is recommended that these are conducted for active ingredients for new classes of active ingredient. Oral extended exposure studies should be undertaken for both the active ingredient and the product (detailed harmonised guidelines for their conduct are required) and again any observed sub-lethal effects should be reported. The data should be used to determine both the LC50 and NOEC and ECx and to investigate whether there are any indications of cumulative effects according to Chapter 4. Currently there is no data to support an HQ approach and therefore a more standard ETR approach is recommended based on the exposure of the adult honey bees and the LC50, NOEC and ECx.

Insect growth regulators are a specific class of insecticides known to affect brood and not adult honey bees. Therefore all active ingredients and formulations with IGR properties must be assessed using the Oomen et al. (1992) brood dosing study to generate a NOEC as this covers all stages until emergence. Although Oomen et al. (1992) is not recognised as a fully validated guideline, the test methodology has been used for a number of years and there is extensive experience in its conduct and interpretation. It is recommended that it is submitted for consideration as an international guideline.
For compounds within the hive, acute exposure of larvae is unlikely to occur and a chronic exposure is a more realistic scenario. At present there are insufficient data available to predict the toxicity to larvae from that in adults. Therefore until data is available to support such predictions chronic toxicity studies (exposure for the developmental period of the larvae as a minimum) should be conducted with both the active ingredient and the product (for spray applications) to ensure the safety of co-formulants returned to the hive on pollen and in nectar after spray applications are assessed. These studies may be conducted with a laboratory study (similar to that proposed by Aupinel et al. (2009) but adapted to cover the chronic dosing scenario) or by adaptation of the Oomen et al. (1992) study to generate dose-response data. Neither of these test methods are currently recognised as validated guidelines and it is recommended that this is considered as a priority. The data should be used to both determine the NOEC and ECx and to investigate whether there are any indications of cumulative effects according to Chapter 4 (for bee-toxic compounds it is more appropriate to use a laboratory study where daily assessments are possible). Again a more standard ETR approach is appropriate based on the exposure of the larvae and the NOEC or ECx.

In Figure 17 the parts of a bee life cycle covered by the toxicity tests are depicted. The acute oral or contact test only covers a small part of the honey bee worker stage (preferably bees from the cleaning and feeding phase of the worker bee life cycle). The Aupinel test covers the larval stage and the Oomen tests the egg, larval and pupal stage through to emergence. The semi-field exposure phase within the tunnel is limited to 10-14 days as this is as long as a colony can be kept within a tunnel without adverse effects on development, but they can be moved outside and kept for as long as is required. A field test can be kept as long as required, for instance when the hive is kept for 63 days in the field it will cover 3 brood cycles.



I = cleaning and feeding phase, II = wax producing and cell construction phase, III guiding and ventilating phase, and IV forager phase

Figure 18: Part of the bee life cycle (i.e. worker bee) potentially covered by toxicity tests

6.2. Semi-field studies

Well-designed semi-field studies are considered as the worst-case exposure scenario (equivalent to at least 95% exposure scenario) as honey bees are confined to the treated crop. Due consideration should be given to the design of the semi-field studies to ensure that the crop is highly attractive (e.g. *Phacelia*) and that colonies are exposed to the treated crop, e.g. spray applications during periods of active foraging, removal of stores prior to exposure. For systemic compounds it is recognised that the exposure may be limited in semi-field studies due to the area of forage available. Therefore it is recommended that consideration be given to improvements in the OECD75 test design for systemic pesticides to extend the exposure period, e.g. by providing supplementary pollen and sucrose sources which contain the same residue levels as the treated crop and extension of the study to encompass a suitable post-exposure assessment period depending on the persistence of the chemical. The conduct of the semi-field studies should always take into account the findings in previous studies, e.g. if the study is triggered by concerns about adult acute mortality and sub-lethal effects then these aspects should be studied in detail in an EPPO 170 test design, e.g. behaviour of foragers, behaviour at the hive entrance, if the study is triggered by the larval study then a OECD 75 study design is appropriate. If concerns are raised by effects on both adults and larvae then further adaptation of OECD 75 is required to address adult effects identified in EPPO 170, e.g. behaviour of foragers, behaviour at the hive entrance and daily mortality in addition to detailed assessments of brood.

A detailed description can be found in Appendices N and O.

6.3. Field studies

Field studies are considered as realistic but not worst case when compared to semi-field studies and if well-designed may be identified as realistic worst case (i.e. the x^{th} percentile). However, to achieve this, due consideration should be given to ensuring that exposure is maximised in the study, e.g. the use of a highly attractive crop and minimisation of alternative forage sources around the treated area, removal of stores prior to exposure and extension of the assessment period to ensure effects can be detected. As for semi-field studies the endpoints should be directed primarily to the concerns raised by the previous studies but also encompass sub-lethal effects, e.g. on foraging activity.

Details regarding methodology for assessment of uncertainties have not been included in discussion of the proposed risk assessment approach as these should be established as part of the development of the Guidance Document.
Risk management has not been included in the discussion of the proposed risk assessment approach as these should also be established as part of the development of the Guidance Document.

6.4. Exposure assessment in the risk assessment scheme

The risk assessment schemes for honey bees, bumble bees and solitary bees require exposure concentrations in order to calculate the ETR quotients at a number of places. The aim of the exposure assessment is to consider a x^{th} percentile case. So all the exposure concentrations in these risk assessment schemes should be equal to or higher than a x^{th} percentile case. These risk assessment schemes contain semi-field or field studies in the Higher Tiers at a number of places. These studies usually only consider one treatment level that is compared to an untreated control. To be consistent with the exposure assessment aim, the exposure in these semi-field or field studies should be equal to or higher than a x^{th} percentile case.

6.5. Risk assessment for bumble bees and solitary bees

The primary concerns for bumble bees and solitary bees were considered to be from insecticides, insecticidal and IGR pesticides and therefore the risk assessment proposed is primarily for these modes of action. A lower trigger should be used in the First Tier of the bumble bee and solitary bee risk assessment than that used in the honey bee risk assessment to take account of the cross-species extrapolation following acute and chronic exposure. Additional exposure scenarios, highlighted in Chapter 3, may be important for bumble bees and solitary bees, e.g. soil, and further research is needed to determine their relative importance and, if required, inclusion in risk assessment.

There is a need for research to develop relevant standardised semi-field and field test designs for bumble bees and solitary bees. In some cases, e.g. bumble bees, these may be relatively straightforward, but for other species, such as univoltine solitary bees, methodology requires significant further work.

6.6. Systemic compound

The definition of a systemic working mechanism is: property of a chemical substance which causes the substance to be taken up by the plant, be transported into the plant via the sap stream, and in this way be effective in several parts of the plant.

If a substance is systemic, the risk to bees via nectar, pollen and honeydew must be assessed. An easy decision criterium to determine whether a substance will occur in nectar, pollen or honeydew is currently not available. A substance should therefore be considered systemic unless proven otherwise in a reasoned case or by providing actual residue measurements (both for spray and for SST compounds). As a refinement, the actual residue level in nectar, pollen or honeydew can be measured in supervised residue trials.

Examples of sources to consult when determining whether a substance is systemic are:

- Information on mode of action (Annex IIA 3 / Annex IIIA 1);
- Plant metabolism studies and residue trials (Annex IIIA 6.2.1, 6.3 / Annex IIIA 8)
- Input parameters of EU groundwater leaching model (FOCUS groundwater, Annex AIII 9.6; for systemic substances a Plant Uptake Factor of 0.5 is used; if no information otherwise the PUF is 0)
- Books or internet databases with pesticide properties (e.g. Pesticide Manual).

7. Risk assessment schemes

The risk assessment schemes and the associated trigger values are based on initial considerations to follow a precautionary principle when not sufficient data were available. The proposed scheme is therefore very conservative in comparison to risk assessments for other groups of non-target organisms. The reviewers are invited to express their ideas on how to address the uncertainties appropriately and in particular to help expand the scientific background with more data.

7.1. Risk assessment scheme for honey bees

7.1.1. Risk assessment scheme for honey bees for spray applications

1 Is exposure for honey bees negligible (see Note 1)?

if yes, classify risk as negligible
if no, go to 2

2 Assessment of the risk from the sprayed application

The following data are required on the toxicity of the active substance/product (Note 2) to adults (see note 3):

- acute oral toxicity to adults conducted according to OECD 213
- acute contact toxicity to adults conducted according to OECD 214
- chronic toxicity study according to Appendix M

The following data are required on the toxicity of the active substance to larvae:

If the above acute data indicate that the compound is of low toxicity to adult bees, i.e. the LD50contact and LD50oral is >100 µg/bee and the LC50 is >100 mg/kg, then a study according to Appendix M (Aupinel method) is required.
If, however, the above data indicate that the a.s. is toxic to adult bees then a study according to Appendix M (Oomen study) is required.
The logic behind this is that in the latter scenario there is the potential for the a.s. to have some adverse effects on adult honey bees and the study covers potential brood care effects. In situations where brood care is not considered to be an issue, it is considered necessary only to assess the risk to larvae. Please note that toxicity has been used as a trigger to determine which study should be conducted; this is due to the fact that application rates may not be known when carrying out the First Tier studies. If the application rates are known, then the selection of the appropriate study can be based on risk, where a low risk is defined as one where the risk quotient for HQcontact and HQoral and ETRadult are not breached.

If the active substance is an insect growth regulator (IGR) a study according to Oomen (Appendix M) is always required because of the mode of action of the compound's potential to affect the growth/development of insects, which may also cause effects on adult bees.

The endpoints from these studies should be collated as follows:

Toxicity study	Endpoint
LD50 contact	µg/bee
LD50 oral	µg/bee
LC50 adult	mg/kg*
NOEC larvae (Appendix M)	mg/kg
NOEC bee brood (Appendix M)	mg/kg

*: This endpoint needs to be expressed also as µg/bee/day

Calculate the Hazard Quotient (HQ) between the application rate and the lower of the LD50 toxicity values (g ha^{-1} /LD50 in µg per bee).

Calculate the Exposure Toxicity Ratio (ETR_{adult}) between the amount of residues that may be ingested by an adult bee in 1 day (see note 4) and the LC50 value.

Calculate the ETR_{larvae} between the concentration of residues that may occur in the feed of a larva (see note 4) and the no observed effect level (NOEC).

Note: the above assessment should be made either for the treated crop, adjacent crop, following crops, weeds in the treated field or the field margin, which represents the highest exposure. As a conservative screening step, the scenario for weeds in the treated field might be considered. See Chapter 3 for further information.

Assess whether there is evidence of cumulative toxicity according to Haber's Law in the toxicity tests with adult and larval honey bees (see Chapter 4.1.1.).

if HQ (oral) < 33 and HQ (contact) < 11 and ETR_{adult} < 0.03 and if ETR_{larvae} < 0.1 and no evidence for cumulative toxicity go to 4
if HQ (oral) ≥ 33 or HQ (contact) ≥ 11 or ETR_{adult} ≥ 0.03 or ETR_{larvae} ≥ 0.1 or evidence of cumulative toxicity go to 3

Please see Chapter 5 for a summary regarding the derivation of these trigger values.

3 Refinement of the risk assessment

It is assumed that at least one risk quotient has been breached and therefore further work is required before the use and associated product can be authorised. This further work can involve either the refinement of the toxicity and/or the refinement of the exposure. Refinement of the toxicity can take the form of carrying out either semi-field and/or field studies. Further information on possible approaches are provided in Chapter 4 and Appendices N and O.

As regards the refinement of exposure, this can be carried out by determining exposure estimates for the product and use under appropriate conditions. Further information is provided in Chapter 3 and associated Appendices. It should be noted that the refinement of the exposure may also include the use of risk mitigation measures (see Chapter 9).

If it is proposed to refine the risk assessment via the production of revised exposure estimate then it is necessary to re-run the assessment carried out at Point 2 above to ensure that the risk is acceptable. If it is proposed to carry out refined effects studies, it is essential to ensure that the exposure scenario is appropriate and reflects the exposure estimates determined in Chapter 3.

It is essential to determine whether the refined risk assessment will ensure that the Specific Protection Goals (see Chapter 2) are met or not.

The refined risk assessment should include an estimate of the uncertainty of the assessment (see step 4)

4 Assessment of uncertainty

Analyze uncertainties in the risk assessment as well as the underlying data to determine the uncertainty in the assessment and in particular whether the SPG will be met (see Chapter 10).

Notes

Note 1 Bees can be exposed to pesticides both directly and indirectly. Direct exposure may result from spray of liquid formulations. Indirect exposure may result from systemic activity in plants. See the exposure flowchart for more detailed information. Examples of when exposure of bees is negligible: food storage in enclosed spaces, wound sealing and healing treatments and use in glasshouses without honey bees as pollinators.

Note 2 According to the data requirements for 1107/2009, formulation data are stated as required on honey bees. It may be possible to extrapolate data (toxicity endpoints) between similar formulations and also sometimes to estimate formulation toxicity from effects obtained in studies conducted with the technical active substance.

Testing of the formulation is required if:

1. the toxicity of a formulation containing one active substance cannot be reliably predicted to be either the same or lower than the active substance(s)
2. the product contains more than one active substance

As regards point (1), the toxicity to bees of an active substance may be increased by formulations containing significant quantities of organic solvents and/or surfactants. Therefore, the toxicity of formulations should be assessed using the standard laboratory toxicity studies conducted with the proposed or similar formulation. A case should be made if data on the formulation are not considered necessary; such cases should include a justification as to why the co-formulants are unlikely to increase the toxicity of the formulation compared to the active substance on its own.
As regards point (2), in principle the requirements for studies for formulations that contain more than one active substance are the same as for single active substance formulations. For a new formulation it would be expected that formulation studies should be submitted unless data from a similar product are available or can be accessed, or if a well reasoned case for non-submission can be provided.
If formulation data are submitted on the toxicity of the two separate actives and a case made that the new formulation containing both active substances will not have a higher toxicity than the single active formulations, then there should be supporting evidence that there will not be synergistic or additive toxicity.
Currently available evidence indicates that synergistic effects between two or more active substances are quite rare; however additive effects are more common and may be expected where two (or more) active substances have the same effect on honey bees. Therefore, where the toxicological action in honey bees of component active substances are similar, it would be appropriate for applicants to provide formulation toxicity studies. Alternatively, it may be possible to calculate the formulation toxicity on the assumption of additive toxicity and hence reduce the need for additional testing (see Chapter 8 for further details).

Note 3 According to the regulatory requirements for active substances and products (SANCO, 2011) reports of acute oral and contact tests and a chronic toxicity test shall be submitted.
There is a need to improve the testing protocols concerning bees, in particular to better address the chronic risk to bees and the identification and measurement of sub-lethal effects (e.g. effects on memory, learning capacity, orientation) to be used in the risk assessment. Pending the validation and adoption of new test protocols and of a new risk assessment scheme, all efforts shall be made to comprehensively address, with the existing protocols, the acute and chronic risk to bees, including those on colony survival and development.
The tests shall provide the EC10, EC20, EC50 (or an explanation if they cannot be estimated) together with the NOEC. Sub-lethal effects, if observed, shall be reported.

Note 4 Appendix S gives practical advice on how to calculate the amount of residues that may be ingested by an adult bee or the concentration to which bee larva may be exposed. For the screening step the following shortcut values can be used (based on default RUDs):

	the overall residue intake (µg/bee/day) to be used in calculation of ETR_{adult}	overall residue concentration (mg/kg) to be used in calculation of ETR_{larvae}
Honey bee	16.2	21.8
Bumble bee	**23.5**	37.2
Solitary bee	16.1	**137.1**

As the next step, PEC values (still based on default RUD values) may be calculated. The corresponding ETR values can be calculated by using the following equations:

	the overall residue intake (µg/bee/day) to be used in calculation of ETR_{adult}	overall residue concentration (mg/kg) to be used in calculation of ETR_{larvae}
Honey bee	forager: 0.773 x PECnectar nurse: 0.305 x PECnectar + 0.0115 x PECpollen	0.9935 x PECnectar + 0.0065 x PECpollen
Bumble bee	0.906 x PECnectar + 0.0299 x PECpollen	0.8741 x PECnectar + 0.1259 x PECpollen
Solitary bee	0.696 x PECnectar + 0.0102 x PECpollen	0.0996 x PECnectar + 0.9004 x PECpollen

7.1.2. Risk assessment scheme for honey bees for solid applications

In this context a solid application is defined as a Plant Protection Product that is applied as a solid or on a solid and hence honey bees are exposed to a solid rather than a spray or liquid. Examples of solid formulations are pellets, granules, baits, dusts and seed treatments (pelleted and non-pelleted). It does not include a solid formulation that is mixed with water and applied as a spray, for example water dispersible granules.

1 Is exposure for honey bees negligible (see Note 1)?

if yes, classify risk as negligible
if no, go to 2

2 Assessment of the risk from solid applications

The following data are required on the toxicity of the active substance/product (Note 2) to adults (see Note 3):

- acute oral toxicity to adults conducted according to OECD 213
- acute contact toxicity to adults conducted according to OECD 214
- chronic toxicity study according to Appendix M

The following data are required on the toxicity of the active substance to larvae:

If the above acute data indicate that the compound is of low toxicity to adult bees, i.e. the LD50contact and LD50oral is >100 µg/bee and the LC50 is >100 mg/kg then a study according to Appendix M (Aupinel method) is required.
If, however, the above data indicate that the a.s. is toxic to adult bees then a study according to Appendix M (Oomen study) is required.
The logic behind this is that in the latter scenario there is the potential for the a.s. to have some adverse effects on adult honey bees and the study covers potential brood care effects. In situations where brood care is not considered to be an issue, it is considered necessary only to assess the risk to larvae. Please note that toxicity has been used as a trigger to determine which study should be conducted; this is due to the fact that application rates may not be known when carrying out the First Tier studies. If the application rates are known, then the selection of the appropriate study can be based on risk, where a low risk is defined as one where the risk quotient for HQcontact and HQoral and ETRadult are not breached.

If the active substance is an insect growth regulator (IGR) a study according to Oomen (Appendix M) is always required because the compound's mode of action will have the potential to affect the growth/development of insects and may also cause effects on adult bees.

The endpoints from these studies should be collated as follows:

Toxicity study	Endpoint
LD50 contact	µg/bee
LD50 oral	µg/bee
LC50 adult	mg/kg*
NOEC larvae (Appendix M)	mg/kg
NOEC bee brood (Appendix M)	mg/kg

*: This endpoint needs to be expressed also as µg/bee/day

Calculate the Hazard Quotient (HQ) between the application rate and the lower of the LD50 toxicity values (g ha^{-1} ⁄LD50 in µg per bee).

Calculate the Exposure Toxicity Ratio (ETR_{adult}) between the amount of residues that may be ingested by an adult bee in 1 day (see note 4) and the LC50 value.

Calculate the ETR_{larvae} between the concentration of residues that may occur in the feed of a larva (see note 4) and the no observed effect level (NOEC).

Note: the above assessment should be made either for the treated crop, adjacent crop, following crops, weeds in the treated field or the field margin, which represents the highest exposure. As a conservative screening step, the scenario for weeds in the treated field might be considered. See Chapter 3 for further information.

Assess whether there is evidence of cumulative toxicity according to Haber's Law in the toxicity tests with adult and larval honey bees (see note 5):

if HQ (oral) < 33 and HQ (contac) < 11 and ETR_{adult} < 0.03 and if ETR_{larvae} < 0.1 and no evidence for cumulative toxicity go to 4
if HQ (oral) ≥ 33 or HQ (contact) ≥ 11 or ETR_{adult} ≥ 0.03 or ETR_{larvae} ≥ 0.1 or evidence of cumulative toxicity go to 3

Please see Note 6 for a brief summary regarding the derivation of these trigger values.

3 Refinement of the risk assessment

It is assumed that at least one risk quotient has been breached and therefore further work is required before the use and associated product can be authorised. This further work can involve either the refinement of the toxicity and/or the refinement of the exposure. Refinement of the toxicity can take the form of carrying out either semi-field and/or field studies. Further information on possible approaches are provided in Chapter 4 and Appendices N and O.

As regards the refinement of exposure, this can be carried out by determining exposure estimates for the product and use under appropriate conditions. Further information is provided in Chapter 3 and associated Appendices. It should be noted that the refinement of the exposure may also include the use of risk mitigation measures (see Chapter 9).

If it is proposed to refine the risk assessment via the production of revised exposure estimate then it is necessary to re-run the assessment carried out at Point 2 above to ensure that the risk is acceptable. If it is proposed to carry out refined effects studies, it is essential to ensure that the exposure scenario is appropriate and reflects the exposure estimates determined in Chapter 3.

It is essential to determine whether the refined risk assessment will ensure that the Specific Protection Goals (see Chapter 2) are met or not.

The refined risk assessment should include an estimate of the uncertainty of the assessment (see step 4)

4 Assessment of uncertainty

Analyze uncertainties in the risk assessment as well as the underlying data to determine the uncertainty in the assessment and in particular whether the SPG will be met (see chapter 10).

Notes

Note 1 Bees can be exposed to pesticides both directly and indirectly. Direct exposure may result from spray of liquid formulations. Indirect exposure may result from systemic activity in plants. See the exposure flowchart for more detailed information. Examples of when exposure of bees is negligible: food storage in enclosed spaces, wound sealing and healing treatments and use in glasshouses without honey bees as pollinators.

Note 2 According to the data requirements for 1107/2009, formulation data are stated as required on honey bees. It may be possible to extrapolate data (toxicity endpoints) between similar formulations and also sometimes to estimate formulation toxicity from effects obtained in studies conducted with the technical active substance.

Testing of the formulation is required if:

1. the toxicity of a formulation containing one active substance cannot be reliably predicted to be either the same or lower than the active substance(s)
2. the product contains more than one active substance

As regards point (1), the toxicity to bees of an active substance may be increased by formulations containing significant quantities of organic solvents and/or surfactants. Therefore, the toxicity of formulations should be assessed using the standard laboratory toxicity studies conducted with the proposed or similar formulation. A case should be made if data on the formulation are not considered necessary; such a case should include a justification as to why the co-formulants are unlikely to increase the toxicity of the formulation compared to the active substance on its own.
As regards point (2), in principle the requirements for studies for formulations that contain more than one active substance are the same as for single active substance formulations. For a new formulation it would be expected that formulation studies should be submitted unless data from a similar product are available or can be accessed, or if a well reasoned case for non-submission can be provided.
If formulation data are submitted on the toxicity of the two separate actives and a case made that the new formulation containing both active substances will not be any more toxic than the single active formulations, then there should be supporting evidence that there will not be synergistic or additive toxicity.
Currently available evidence indicates that synergistic effects between two or more active substances are quite rare; however additive effects are more common and may be expected where two (or more) active substances have the same effect on honey bees. Therefore, where the toxicological action of component active substances are similar in honey bees , it would be appropriate for applicants to provide formulation toxicity studies. Alternatively, it may be possible to calculate the formulation toxicity on the assumption of additive toxicity and hence reduce the need for additional testing (see Chapter 8 for further details).

Note 3 According to the regulatory requirements for active substances and products (SANCO, 2011) reports of acute oral and contact tests and a chronic toxicity test shall be submitted.
There is a need to improve the testing protocols concerning bees, in particular to better address the chronic risk to bees and the identification and measurement of sub-lethal effects (e.g. effects on memory, learning capacity, orientation) to be used in the risk assessment. Pending the validation and adoption of new test protocols and of a new risk assessment scheme, all efforts shall be made to comprehensively address, with the existing protocols, the acute and chronic risk to bees, including those on colony survival and development.
The tests shall provide the EC10, EC20, EC50 (or an explanation if they cannot be estimated) together with the NOEC. Sub-lethal effects, if observed, shall be reported.

Note 4 Appendix S gives practical advice on how to calculate the amount of residues that may be ingested by an adult bee or the concentration to which bee larvae may be exposed. For the

screening steps, shortcut values and simplified equations may be used as reported in Note 4 of the risk assessment scheme for honey bees for spray applications, but the figures need to be multiplied with the adjustment factor of 5. For PECnectar and PECpollen for seed treatment for the target crop, the default of 1 mg/kg needs to be used.

7.2. Risk assessment scheme for bumble bees

7.2.1. Risk assessment scheme for bumble bees for spray applications

The proposed risk assessment scheme for bumble bees is only in a preliminary phase. There is no reason to use a different type of scheme than that of honey bees. But before it will be possible to run this scheme additional research has to be done (see EFSA, 2012a, chapter 5).

2 Is exposure for bumble bees negligible (see Note 1)?

if yes, classify risk as negligible
if no, go to 2

2 Is the compound an insecticide, or an insect growth regulator or does the compound have insecticidal activity (see Note 2)?

if yes, go to 3
if no, go to 8

Remark: A risk assessment for bumble bees is only carried out for insecticides, insect growth regulators or compounds with insecticidal activity, in contrast to honey bees where the risk is assessed for each compound.

3 Assessment of the risk from sprayed applications

The following data are required on the toxicity of the active substance/product (Note 3) to adult bumble bees (see Note 4):

- acute oral toxicity to adult bumble bees
- acute contact toxicity to adult bumble bees
- chronic toxicity study for adult and larval honey bees as surrogate species (depending on the findings for honey bees, the study for larvae is either a study similar to the Aupinel method or a study similar to the Oomen method when the assessment for the brood care should also be taken into account (see honey bee scheme for more information)).

If the active substance is an insect growth regulator (IGR) a study according to Appendix M (Oomen study) should be carried out because the compound's mode of action will have the potential to affect the growth/development of insects and may also cause effects on adult bees.

The endpoints from these studies should be collated as follows:

Toxicity study	Endpoint
LD50 contact	µg/bumble bee
LD50 oral	µg/bumble bee
LC50 adult	mg/kg*
NOEC larvae (Appendix M)	mg/kg
NOEC bee brood (Appendix M)	mg/kg

*: This endpoint needs to be expressed also as µg/bee/day

Establish adult oral and contact LD50 for bumble bees (see Note 5).

Calculate the hazard quotient (HQ) between the application rate and the LD50 toxicity values (g ha^{-1} /LD50 in µg of active ingredient per bumble bee).

Assess possible longer term impacts on adult bumble bees (Note 6) using the endpoints of the LC50 study with *Apis* worker bees **as a surrogate** for bumble bees.

Calculate the exposure toxicity ratio (ETR_{adults}) of the amount of residues that may be ingested by bumble bees in 1 day and the LC50 value.

Assess possible impacts on bumble bee larvae (Note 6) using *Apis* larvae test endpoint **as a surrogate** for bumble bee larvae.

Calculate the exposure toxicity ratio (ETR_{larvae}) between the concentration of residues that may be occur in the feed of bumble bee larvae and the no observed effect level (NOEC).

Note: the above assessment should be made either for the treated crop, adjacent crop, following crops, weeds in the treated field or the field margin, which represents the highest exposure. As a conservative screening step, the scenario for weeds in the treated field might be considered. See Chapter 3 for further information.

Assess whether there is evidence for cumulative toxicity according to Haber's Law in the toxicity tests (see Note 7).

If HQ (oral) < 5.5 and HQ (contact) < 1.76 and ETR_{adult} < 0.024/<0.0024 and if ETR_{larvae} < 0.01 and no evidence for cumulative toxicity go to 5
If HQ (oral) ≥ 5.5 or HQ (contact) ≥ 1.76 or ETR_{adult} ≥ 0.024/≥ 0.0024 or ETR_{larvae} ≥ 0.01 or evidence of cumulative toxicity go to 4

Please see Chapter 5 for a brief summary regarding the derivation of these trigger values.

4 Refinement of the risk assessment

It is assumed that at least one risk quotient has been breached and therefore further work is required before the use and associated product can be authorised. This further work can involve either the refinement of the toxicity and/or the refinement of the exposure. Refinement of the toxicity can take the form of carrying out either semi-field and/or field studies. Further information on possible approaches are provided in Chapter 4 and Appendices N and O.

As regards the refinement of exposure, this can be carried out by determining exposure estimates for the product and use under appropriate conditions. Further information is provided in Chapter 3 and associated Appendices. It should be noted that the refinement of the exposure may also include the use of risk mitigation measures (see Chapter 9).

If it is proposed to refine the risk assessment via the production of revised exposure estimate then it is necessary to re-run the assessment carried out at Point 2 above to ensure that the risk is acceptable. If it is proposed to carry out refined effects studies, it is essential to ensure that the exposure scenario is appropriate and reflects the exposure estimates determined in Chapter 3.

It is essential to determine whether the refined risk assessment will ensure that the Specific Protection Goals (see Chapter 2) are met or not.

The refined risk assessment should include an estimate of the uncertainty of the assessment (see step 4)

4 Assessment of uncertainty

Analyze uncertainties in the risk assessment as well as the underlying data to determine the uncertainty in the assessment and in particular whether the SPG will be met (see Chapter 10).

Notes

Note 1 Bumble bees can be exposed to pesticides both directly and indirectly. Direct exposure may result from spray of liquid formulations. Indirect exposure may result from systemic activity in plants. See the exposure flowchart for more detailed information. Examples of when exposure of bees is negligible: food storage in enclosed spaces, wound sealing and healing treatments and use in glasshouses without bumble bees as pollinators.

Note 2 The outcome of the honey bee assessment scheme can be used for deciding whether risk assessment for bumble bees also has to be carried out. A compound has to be assessed for bumble bees in case it was necessary in the honey bee scheme to revise the default exposure values or when an assessment of Higher Tier studies had to be carried out. Data for the non-target arthropods could also be used for assessing the potential insecticidal activity of a compound. For most of the compounds the two standard non target arthropods are tested (*Typhodromus pyri* and *Aphidius rhopalosiphi*). When the quotient of the application rate multiplied by a MAF factor and the LR50 is greater than 2 the compound could be considered as having insecticidal activity. In addition efficacy studies with other insects or studies carried out with insects in the screening process could be a source for assessing potential insecticidal activity.

Note 3 Note when to assess product or not. According to the data requirements for 1107/2009, formulation data on honey bees are stated as required. It may be possible to extrapolate data (toxicity endpoints) between similar formulations and also sometimes to estimate formulation toxicity from effects obtained in studies conducted with the technical active substance.

Testing of the formulation is required if:

1. the toxicity of a formulation containing one active substance cannot be reliably predicted to be either the same or lower than the active substance(s)
2. the product contains more than one active substance

As regards point (1), the toxicity to bees of an active substance may be increased by formulations containing significant quantities of organic solvents and/or surfactants. Therefore, the toxicity of formulations should be assessed using the standard laboratory toxicity studies conducted with the proposed or similar formulation. A case should be made if data on the formulation are not considered necessary; such a case should include a justification as to why the co-formulants are unlikely to increase the toxicity of the formulation compared to the active substance on its own.

As regards point (2), in principle the requirements for studies for formulations that contain more than one active substance are the same as for single active substance formulations. For a new formulation it would be expected that formulation studies should be submitted unless data from a similar product are available or can be accessed, or a well reasoned case for non-submission can be provided.

If formulation data are submitted on the toxicity of the two separate actives and a case made that the new formulation containing both active substances will have a higher toxicity than the single active formulations, then there should be supporting evidence that there will not be synergistic or additive toxicity.

Currently available evidence indicates that synergistic effects between two or more active substances are quite rare; however additive effects are more common and may be expected where two (or more) active substances have the same effect on honey bees. Therefore, where

the toxicological action of component active substances are similar in honey bees it would be appropriate for applicants to provide formulation toxicity studies. Alternatively, it may be possible to calculate the formulation toxicity on the assumption of additive toxicity and hence reduce the need for additional testing (see Chapter 8 for further details).

Note 4 In the definitive version of regulatory requirements for active substances (SANCO, 2011) bumble bees as such are not mentioned.
There is a need to improve the testing protocols concerning bumble bees, in particular to better address the chronic risk to bumble bees and the identification and measurement of sub-lethal effects (e.g. effects on memory, learning capacity, orientation) to be used in the risk assessment. Pending the validation and adoption of new test protocols and of a new risk assessment scheme, all efforts shall be put in place to comprehensively address, with the existing protocols, the acute and chronic risk to bumble bees, including those on colony survival and development.
The tests shall provide the EC10, EC20, EC50 (or an explanation if they cannot be estimated) together with the NOEC. Sub-lethal effects, if observed, shall be reported

Note 5 *Bombus terrestris* is proposed as test species. Test protocols for this species are suggested in Appendix P.

Note 6 Appendix S gives practical advice on how to calculate the amount of residues that may be ingested by an adult bee or the concentration to which bee larvae may be exposed. For the screening steps shortcut values and simplified equations may be used as reported in Note 4 of the risk assessment scheme for honey bees for spray applications.

Note 7 Either assume that honeybees are an adequate surrogate for bioaccumulative toxicity or replicate design of test but using bumblebees.

Note 8 At the moment no standardized guidelines are available for Higher Tier testing but protocols for semi-field and field studies are proposed in Appendix P. Endpoints measured in these tests are: bee mortality rate, queen production rate, progeny survival.

7.2.2. Risk assessment scheme for bumble bees for solid applications

The proposed risk assessment scheme for bumble bees is only in a preliminary phase. There is no reason to use a different type of scheme than that for honey bees. But before it will be possible to run this scheme additional research has to be done (see EFSA, 2012a, chapter 5).

In this context a solid application is defined as a Plant Protection Product that is applied as a solid or on a solid and hence honey bees are exposed to a solid rather than a spray or liquid. Examples of solid formulations are pellets, granules, baits, dusts and seed treatments (pelleted and non-pelleted). It does not include a solid formulation that is mixed with water and applied as a spray, for example water dispersible granules.

3 Is exposure for bumble bees negligible (see Note 1)?

if yes, classify risk as negligible
if no, go to 2

2 Is the compound an insecticide, or an insect growth regulator or does the compound have insecticidal activity (see Note 2)?

if yes, go to 3
if no, go to 8

Remark: A risk assessment for bumble bees is only carried out for insecticides, insect growth regulators or compounds with insecticidal activity, in contrast to honey bees where the risk is assessed for each compound.

3 Assessment of the risk from solid applications

The following data are required on the toxicity of the active substance/product (Note 3) to adult bumble bees (see note 4):

- acute oral toxicity to adult bumble bees
- acute contact toxicity to adult bumble bees
- chronic toxicity study for adult and larval honey bees as surrogate species (depending on the findings for honey bees, the study for larvae is either a study similar to the Aupinel method or a study similar to the Oomen method when the assessment for the brood care should also be taken into account (see honey bee scheme for more information)).

If the active substance is an insect growth regulator (IGR) a study according to Appendix M (Oomen study) should be performed because the compound's mode of action will have the potential to affect the growth/development of insects and may also cause effects on adult bees.

The endpoints from these studies should be collated as follows:

Toxicity study	Endpoint
LD50 contact	µg/ bumble bee
LD50 oral	µg/ bumble bee
LC50 adult	mg/kg*
NOEC larvae (Appendix M)	mg/kg
NOEC bee brood (Appendix M)	mg/kg

*: This endpoint needs to be expressed also as µg/bee/day

Establish adult oral and contact LD50 for bumble bees (see Note 5).

Calculate the hazard quotient (HQ) between the application rate and the LD50 toxicity values (g ha^{-1} /LD50 in µg of active ingredient per bumble bee).

Assess possible longer term impacts on adult bumble bees (Note 6) using the endpoints of the LC50 study with *Apis* worker bees **as a surrogate** for bumble bees.

Calculate the exposure toxicity ratio (ETR_{adults}) of the amount of residues that may be ingested by bumble bees in 1 day and the LC50 value.

Assess possible impacts on bumble bee larvae (Note 6) using *Apis* larvae test endpoint **as a surrogate** for bumble bee larvae.

Calculate the exposure toxicity ratio (ETR_{larvae}) between the concentration of residues that may occur in the feed of a bumble bee larvae and the no observed effect concentration (NOEC).

Note: the above assessment should be made either for the treated crop, adjacent crop, following crops, weeds in the treated field or the field margin, which represent the highest exposure. As a conservative screening step, the scenario for weeds in the treated field might be considered. See Chapter 3 for further information.

Assess whether there is evidence for cumulative toxicity according to Haber's Law in the toxicity tests (see note 7).

if HQ (oral) < 5.5 and HQ (contact) < 1.76 and ETR_{adult} < 0.024/<0.0024 and if ETR_{larvae} < 0.01 and no evidence for cumulative toxicity go to 5

if HQ (oral) ≥ 5.5 or HQ (contact) ≥ 1.76 or ETR_{adult} ≥ 0.024/≥ 0.0024 or ETR_{larvae} ≥ 0.01 or evidence of cumulative toxicity go to 4

Please see Chapter 5 for a brief summary regarding the derivation of these trigger values.

4 Refinement of the risk assessment

It is assumed that at least one risk quotient has been breached and therefore further work is required before the use and associated product can be authorised. This further work can involve either the refinement of the toxicity and/or the refinement of the exposure. Refinement of the toxicity can take the form of carrying out either semi-field and/or field studies. Further information on possible approaches are provided in Chapter 4 and Appendices N and O.

As regards the refinement of exposure, this can be carried out by determining exposure estimates for the product and use under appropriate conditions. Further information is provided in Chapter 3 and associated Appendices. It should be noted that the refinement of the exposure may also include the use of risk mitigation measures (see Chapter 9).

If it is proposed to refine the risk assessment via the production of revised exposure estimate then it is necessary to re-run the assessment carried out at Point 2 above to ensure that the risk is acceptable. If it is proposed to carry out refined effects studies, it is essential to ensure that the exposure scenario is appropriate and reflects the exposure estimates determined in Chapter 3.

It is essential to determine whether the refined risk assessment will ensure that the Specific Protection Goals (see Chapter 2) are met or not.

The refined risk assessment should include an estimate of the uncertainty of the assessment (see step 4)

4 Assessment of uncertainty

Analyze uncertainties in the risk assessment as well as the underlying data to determine the uncertainty in the assessment and in particular whether the SPG will be met (see Chapter 10).

Notes

Note 1 Bumble bees can be exposed to pesticides both directly and indirectly. Direct exposure may result from spray of liquid formulations. Indirect exposure may result from systemic activity in plants. See the exposure flowchart for more detailed information. Examples of when exposure of bees is negligible: food storage in enclosed spaces, wound sealing and healing treatments and use in glasshouses without bumble bees as pollinators.

Note 2 The outcome of the honey bee assessment scheme can be used for deciding whether risk assessment for bumble bees also has to be carried out. A compound has to be assessed for bumble bees in case it was necessary in the honey bee scheme to revise the default exposure values or when an assessment of Higher Tier studies had to be carried out. Data for the non-target arthropods could also be used for assessing the potential insecticidal activity of a compound. For most of the compounds the two standard non target arthropods are tested (*Typhodromus pyri* and *Aphidius rhopalosiphi*). When the quotient of the application rate multiplied by a MAF factor and the LR50 is greater than 2 the compound could be considered as having insecticidal activity. In addition efficacy studies with other insects or studies carried out with insects in the screening process could be a source for assessing potential insecticidal activity.

Note 3 Note when to assess product or not. According to the data requirements for 1107/2009, formulation data on honey bees are stated as required . It may be possible to extrapolate data (toxicity endpoints) between similar formulations and also sometimes to estimate formulation toxicity from effects obtained in studies conducted with the technical active substance.

Testing of the formulation is required if:

1. the toxicity of a formulation containing one active substance cannot be reliably predicted to be either the same or lower than the active substance(s)
2. the product contains more than one active substance

As regards point (1), the toxicity to bees of an active substance may be increased by formulations containing significant quantities of organic solvents and/or surfactants. Therefore, the toxicity of formulations should be assessed using the standard laboratory toxicity studies conducted with the proposed or similar formulation. A case should be made if data on the formulation are not considered necessary, including a justification as to why the co-formulants are unlikely to increase the toxicity of the formulation compared to the active substance on its own.

As regards point (2), in principle the requirements for studies for formulations that contain more than one active substance are the same as for single active substance formulations. For a new formulation it would be expected that formulation studies should be submitted unless data from a similar product are available or can be accessed, or a well reasoned case for non-submission can be provided.

If formulation data are submitted on the toxicity of the two separate actives and a case made that the new formulation containing both active substances will not have a higher toxicity than the single active formulations, then there should be supporting evidence that there will not be synergistic or additive toxicity.

Currently available evidence indicates that synergistic effects between two or more active substances are quite rare; however additive effects are more common and may be expected where two (or more) active substances have the same effect on honey bees. Therefore, where the toxicological action in honey bees of component active substances are similar, it would be appropriate for applicants to provide formulation toxicity studies. Alternatively, it may be possible to calculate the formulation toxicity on the assumption of additive toxicity and hence reduce the need for additional testing (see Chapter 8 for further details).

Note 4 In the definitive version on regulatory requirements for active substances (SANCO, 2011) bumble bees as such are not mentioned.
There is a need to improve the testing protocols concerning bumble bees, in particular to better address the chronic risk to bumble bees and the identification and measurement of sub-lethal effects (e.g. effects on memory, learning capacity, orientation) to be used in the risk assessment. Pending the validation and adoption of new test protocols and of a new risk assessment scheme, all efforts shall be put in place to comprehensively address, with the existing protocols, the acute and chronic risk to bumble bees, including those on colony survival and development.
The tests shall provide the EC_{10}, EC_{20}, EC_{50} (or an explanation if they cannot be estimated) together with the NOEC. Sub-lethal effects, if observed, shall be reported

Note 5 *Bombus terrestris* is proposed as test species. Test protocols for this species are suggested in Appendix P.

Note 6 Appendix S gives practical advice how to calculate the amount of residues that may be ingested by an adult bee or the concentration to which bee larvae may be exposed. For the screening steps shortcut values and simplified equations may be used as reported in Note 4 of the risk assessment scheme for honey bees for spray applications, but the figures need to be multiplied with the adjustment factor of 5. For PECnectar and PECpollen for seed treatment for the target crop, the default of 1 mg/kg needs to be used.

Note 7 Either assume that honey bees are an adequate surrogate for bioaccumulative toxicity or replicate design of test but using bumble bees.

Note 8 At the moment no standardized guidelines are available for Higher Tier testing but protocols for semi-field and field studies are proposed in Appendix P. Endpoints measured in these tests are: bee mortality rate, queen production rate, progeny survival.

7.3. Risk assessment scheme for solitary bees

7.3.1. Risk assessment scheme for solitary bees for spray applications

The proposed risk assessment scheme for solitary bees is only in a preliminary phase. There is no reason to use a different type of scheme than that for honey bees. But before it will be possible to run this scheme additional research has to be done (see EFSA, 2012a, chapter 5).

4 Is exposure for solitary bees negligible (see Note 1)?

if yes, classify risk as negligible
if no, go to 2

2 Is the compound an insecticide, or an insect growth regulator or does the compound have insecticidal activity (see Note 2)?

if yes, go to 3
if no, go to 8

Remark: A risk assessment for solitary bees is only carried out for insecticides, insect growth regulators or compounds with insecticidal activity, in contrast to honey bees where the risk is assessed for each compound.

3 Assessment of the risk from sprayed applications

The following data are required on the toxicity of the active substance/product (Note 3) to adult bees (see note 4):

- acute oral toxicity to adult solitary bees
- acute contact toxicity to adult solitary bees
- chronic toxicity study for adult and larval honey bees as surrogate species (depending on the findings for honey bees, the study for larvae is either a study similar to the Aupinel method or a study similar to the Oomen method when the assessment for the brood care should also be taken into account (see honey bee scheme for more information)).

If the active substance is an insect growth regulator (IGR) a study according to Appendix M (Oomen study) should be performed because the compounds' mode of action will have the potential to affect the growth/development of insects and may also cause effects on adult bees.

The endpoints from these studies should be collated as follows:

Toxicity study	Endpoint
LD50 contact	µg/solitary bee
LD50 oral	µg/solitary bee
LC50 adult	mg/kg*
NOEC larvae (Appendix M)	mg/kg
NOEC bee brood (Appendix M)	mg/kg

*: This endpoint needs to be expressed also as µg/bee/day

Establish adult oral and contact LD50 for solitary bees (see Note 5).

Calculate the hazard quotient (HQ) between the application rate and the LD50 toxicity values (g ha^{-1} /LD50 in µg of active ingredient per solitary bee).

Assess possible longer term impacts on adult solitary bees (Note 6) using the endpoints of the LC50 study with *Apis* worker bees **as a surrogate** for solitary bees.

Calculate the exposure toxicity ratio (ETR_{adults}) of the amount of residues that may be ingested by solitary bees in 1 day and the LC50 value.

Assess possible impacts on solitary bee larvae (Note 6) using *Apis* larvae test endpoint **as a surrogate** for solitary bee larvae.

Calculate the exposure toxicity ratio (ETR_{larvae}) between the concentration of residues that may occur in the feed of solitary bee larvae and the no observed effect level (NOEL).

Note: the above assessment should be made either for the treated crop, adjacent crop, following crops, weeds in the treated field or the field margin, which represents the highest exposure. As a conservative screening step, the scenario for weeds in the treated field might be considered. See Chapter 3 for further information.

Assess whether there is evidence for cumulative toxicity according to Haber's Law in the toxicity tests (see note 7).

If HQ (oral) < 6.3 and HQ (contact) < 2 and ETR_{adult} < 0.027/<0.0027 and if ETR_{larvae} < 0.01 and no evidence for cumulative toxicity go to 5
If HQ (oral) ≥ 6.3 or HQ (contact) ≥ 2 or ETR_{adult} ≥ 0.027/≥ 0.0027 or ETR_{larvae} ≥ 0.01 or evidence of cumulative toxicity go to 4

Please see Chapter 5 for a brief summary regarding the derivation of these trigger values.

4 Refinement of the risk assessment

It is assumed that at least one risk quotient has been breached and therefore further work is required before the use and associated product can be authorised. This further work can involve either the refinement of the toxicity and/or the refinement of the exposure. Refinement of the toxicity can take the form of carrying out either semi-field and/or field studies. Further information on possible approaches are provided in Note 8 and Appendix Q.

As regards the refinement of exposure, this can be carried out by determining exposure estimates for the product and use under appropriate conditions. Further information is provided in Chapter 3 and associated Appendices. It should be noted that the refinement of the exposure may also include the use of risk mitigation measures (see Chapter 9).

If it is proposed to refine the risk assessment via the production of revised exposure estimate then it is necessary to re-run the assessment carried out at Point 2 above to ensure that the risk is acceptable. If it is proposed to carry out refined effects studies, it is essential to ensure that the exposure scenario is appropriate and reflects the exposure estimates determined in Chapter 3.

It is essential to determine whether the refined risk assessment will ensure that the Specific Protection Goals (see Chapter 2) are met or not.

The refined risk assessment should include an estimate of the uncertainty of the assessment (see step 4)

5 Assessment of uncertainty

Analyze uncertainties in the risk assessment as well as the underlying data to determine the uncertainty in the assessment and in particular whether the SPG will be met (see Chapter 10).

Notes

Note 1 Solitary bees can be exposed to pesticides both directly and indirectly. Direct exposure may result from spray of liquid formulations. Indirect exposure may result from systemic activity in plants. See the exposure flowchart for more detailed information. Examples of when exposure of bees is negligible: food storage in enclosed spaces, wound sealing and healing treatments and use in glasshouses without solitary bees as pollinators.

Note 2 The outcome of the honey bee assessment scheme can be used for deciding whether risk assessment for solitary bees also has to be carried out. A compound has to be assessed for solitary bees in case it was necessary in the honey bee scheme to revise the default exposure values or when an assessment of Higher Tier studies had to be carried out. Data for the non-target arthropods could also be used for assessing the potential insecticidal activity of a compound. For most of the compounds the two standard non target arthropods are tested (*Typhodromus pyri* and *Aphidius rhopalosiphi*). When the quotient of the application rate multiplied by a MAF factor and the LR50 is greater than 2 the compound could be considered as having insecticidal activity. In addition efficacy studies with other insects or studies carried out with insects in the screening process could be a source for assessing potential insecticidal activity.

Note 3 Note when to assess product or not. According to the data requirements for 1107/2009, formulation data on honey bees are stated as required. It may be possible to extrapolate data (toxicity endpoints) between similar formulations and also sometimes to estimate formulation toxicity from effects obtained in studies conducted with the technical active substance.

Testing of the formulation is required if:

1. the toxicity of a formulation containing one active substance cannot be reliably predicted to be either the same or lower than the active substance(s)
2. the product contains more than one active substance

As regards point (1), the toxicity to bees of an active substance may be increased by formulations containing significant quantities of organic solvents and/or surfactants. Therefore, the toxicity of formulations should be assessed using the standard laboratory toxicity studies conducted with the proposed or similar formulation. A case should be made if data on the formulation are not considered necessary; such a case should include a justification as to why the co-formulants are unlikely to increase the toxicity of the formulation compared to the active substance on its own.

As regards point (2), in principle the requirements for studies for formulations that contain more than one active substance are the same as for single active substance formulations. For a new formulation it would be expected that formulation studies should be submitted unless data from a similar product are available or can be accessed, or a well reasoned case for non-submission can be provided.

If formulation data are submitted on the toxicity of the two separate actives and a case made that the new formulation containing both active substances will not have a higher toxicity than the single active formulations, then there should be supporting evidence that there will not be synergistic or additive toxicity.

Currently available evidence indicates that synergistic effects between two or more active substances are quite rare; however additive effects are more common and may be expected

where two (or more) active substances have the same effect on honey bees. Therefore, where the toxicological action of component active substances in honey bees are similar, it would be appropriate for applicants to provide formulation toxicity studies. Alternatively, it may be possible to calculate the formulation toxicity on the assumption of additive toxicity and hence reduce the need for additional testing (see Chapter 8 for further details).

Note 4 In the definitive version on regulatory requirements for active substances (SANCO, 2011) solitary bees as such are not mentioned.
There is a need to improve the testing protocols concerning solitary bees, in particular to better address the chronic risk to solitary bees and the identification and measurement of sub-lethal effects (e.g. effects on memory, learning capacity, orientation) to be used in the risk assessment. Pending the validation and adoption of new test protocols and of a new risk assessment scheme, all efforts shall be put in place to comprehensively address, with the existing protocols, the acute and chronic risk to solitary bees, including those on colony survival and development.
The tests shall provide the EC_{10}, EC_{20}, EC_{50} (or an explanation if they cannot be estimated) together with the NOEC. Sub-lethal effects, if observed, shall be reported

Note 5 *Osmia cornuta* or *Osmia bicornis* (= *O. rufa*) are proposed as test species. Test protocols for these species are available in Appendix Q.

Note 6 Appendix S gives practical advice on how to calculate the amount of residues that may be ingested by an adult bee or the concentration to which bee larvae may be exposed. For the screening steps shortcut values and simplified equations may be used as reported in Note 4 of the risk assessment scheme for honey bees for spray applications.

Note 7 Either assume that honey bees are an adequate surrogate for bioaccumulative toxicity or replicate the design of test the but using solitary bees.

Note 8 At the moment no standardized guidelines are available for Higher Tier testing but protocols for semi-field and field studies are proposed in Appendix Q. Endpoints measured in these tests are: bee mortality rate, cell production rate, foraging and in-nest times, progeny survival.

7.3.2. Risk assessment scheme for solitary bees for solid applications

The proposed risk assessment scheme for solitary bees is only in a preliminary phase. There is no reason to use a different type of scheme than that for honey bees. But before it will be possible to run this scheme additional research has to be done (see EFSA, 2012a, Chapter 5).

In this context a solid application is defined as a Plant Protection Product that is applied as a solid or on a solid and hence honey bees are exposed to a solid rather than a spray or liquid. Examples of solid formulations are pellets, granules, baits, dusts and seed treatments (pelleted and non-pelleted). It does not include a solid formulation that is mixed with water and applied as a spray, for example water dispersible granules.

5 Is exposure for solitary bees negligible (see Note 1)?

if yes, classify risk as negligible
if no, go to 2

2 Is the compound an insecticide, or an insect growth regulator or does the compound have insecticidal activity (see Note 2)?

if yes, go to 3
if no, go to 8

Remark: A risk assessment for solitary bees is only carried out for insecticides, insect growth regulators or compounds with insecticidal activity, in contrast to honey bees where the risk is assessed for each compound.

3 Assessment of the risk from solid applications

The following data are required on the toxicity of the active substance/product (Note 3) to adult bees (see note 4):

- acute oral toxicity to adult solitary bees
- acute contact toxicity to adult solitary bees
- chronic toxicity study for adult and larval honey bees as surrogate species (depending on the findings for honey bees the study for larvae is either a study similar to the Aupinel method or a study similar to the Oomen method when the assessment for the brood care should also be taken into account (see honey bee scheme for more information)).

If the active substance is an insect growth regulator (IGR) a study according to Appendix M (Oomen study) should be performed because the compound's mode of action will have the potential to affect the growth/development of insects and may also cause effects on adult bees.

The endpoints from these studies should be collated as follows:

Toxicity study	Endpoint
LD50 contact	µg/solitary bee
LD50 oral	µg/solitary bee
LC50 adult	mg/kg*
NOEC larvae (Appendix M)	mg/kg
NOEC bee brood (Appendix M)	mg/kg

*: This endpoint needs to be expressed also as µg/bee/day

Establish adult oral and contact LD50 for solitary bees (see Note 5).
Calculate the hazard quotient (HQ) between the application rate and the LD50 toxicity values (g ha^{-1} ⁄LD50 in µg of active ingredient per solitary bee).

Assess possible longer term impacts on adult solitary bees (Note 6) using the endpoints of the LC50 study with *Apis* worker bees **as a surrogate** for solitary bees.
Calculate the exposure toxicity ratio (ETR_{adults}) of the amount of residues that may be ingested by solitary bees in 1 day and the LC50 value.

Assess possible impacts on solitary bee larvae (Note 6) using *Apis* larvae test endpoint **as a surrogate** for solitary bee larvae.
Calculate the exposure toxicity ratio (ETR_{larvae}) between the concentration of residues that may occur in the feed of solitary bee larvae and the no observed effect level (NOEL).

Note: the above assessment should be made either for the treated crop, adjacent crop, following crops, weeds in the treated field or the field margin, which represents the highest exposure. As a conservative

screening step, the scenario for weeds in the treated field might be considered. See Chapter 3 for further information.

Assess whether there is evidence for cumulative toxicity according to Haber's Law in the toxicity tests (see note 7).

If HQ (oral) < 6.3 and HQ (contact) < 2 and ETR_{adult} < 0.027/<0.0027 and if ETR_{larvae} < 0.01 and no evidence for cumulative toxicity go to 5
If HQ (oral) ≥ 6.3 or HQ (contact) ≥ 2 or ETR_{adult} ≥ 0.027/≥ 0.0027 or ETR_{larvae} ≥ 0.01 or evidence of cumulative toxicity go to 4

Please see Chapter 5 for a brief summary regarding the derivation of these trigger values.

4 Refinement of the risk assessment

It is assumed that at least one risk quotient has been breached and therefore further work is required before the use and associated product can be authorised. This further work can involve either the refinement of the toxicity and/or the refinement of the exposure. Refinement of the toxicity can take the form of carrying out either semi-field and/or field studies. Further information on possible approaches are provided in Note 8 and Appendix Q.

As regards the refinement of exposure, this can be carried out by determining exposure estimates for the product and use under appropriate conditions. Further information is provided in Chapter 3 and associated Appendices. It should be noted that the refinement of the exposure may also include the use of risk mitigation measures (see Chapter 9).

If it is proposed to refine the risk assessment via the production of revised exposure estimate then it is necessary to re-run the assessment carried out at Point 2 above to ensure that the risk is acceptable. If it is proposed to carry out refined effects studies, it is essential to ensure that the exposure scenario is appropriate and reflects the exposure estimates determined in Chapter 3.

It is essential to determine whether the refined risk assessment will ensure that the Specific Protection Goals (see Chapter 2) are met or not.

The refined risk assessment should include an estimate of the uncertainty of the assessment (see step 4).

5 Assessment of uncertainty

Analyze uncertainties in the risk assessment as well as the underlying data to determine the uncertainty in the assessment and in particular whether the SPG will be met (see Chapter 10).

Notes

Note 1 Solitary bees can be exposed to pesticides both directly and indirectly. Direct exposure may result from spray of liquid formulations. Indirect exposure may result from systemic activity in plants. See the exposure flowchart for more detailed information. Examples of when exposure of bees is negligible are: food storage in enclosed spaces, wound sealing and healing treatments and use in glasshouses without solitary bees as pollinators.

Note 2 The outcome of the honey bee assessment scheme can be used for deciding whether risk assessment for solitary bees also has to be carried out. A compound has to be assessed for solitary bees in case it was necessary in the honey bee scheme to revise the default exposure values or when an assessment of Higher Tier studies had to be carried out. Data for the non-target arthropods could also be used for assessing the potential insecticidal activity of a compound. For most of the compounds the two standard non target arthropods are tested (*Typhodromus pyri* and *Aphidius rhopalosiphi*). When the quotient of the application rate multiplied by a MAF factor and the LR50 is greater than 2 the compound could be considered as having insecticidal activity. In addition efficacy studies with other insects or studies carried out with insects in the screening process could be a source for assessing potential insecticidal activity.

Note 3 Note when to assess product or not. According to the data requirements for 1107/2009, formulation data on honey bees are stated as required It may be possible to extrapolate data (toxicity endpoints) between similar formulations and also sometimes to estimate formulation toxicity from effects obtained in studies conducted with the technical active substance.

Testing of the formulation is required if:

1. the toxicity of a formulation containing one active substance cannot be reliably predicted to be either the same or lower than the active substance(s)
2. the product contains more than one active substance

As regards point (1), the toxicity to bees of an active substance may be increased by formulations containing significant quantities of organic solvents and/or surfactants. Therefore, the toxicity of formulations should be assessed using the standard laboratory toxicity studies conducted with the proposed or similar formulation. A case should be made if data on the formulation are not considered necessary including a justification as to why the co-formulants are unlikely to increase the toxicity of the formulation compared to the active substance on its own.
As regards point (2), in principle the requirements for studies for formulations that contain more than one active substance are the same as for single active substance formulations. For a new formulation it would be expected that formulation studies should be submitted unless data from a similar product are available or can be accessed, or a well reasoned case for non-submission can be provided.
If formulation data are submitted on the toxicity of the two separate actives and a case made that the new formulation containing both active substances will not be any more toxic than the single active formulations, then there should be supporting evidence that there will not be synergistic or additive toxicity.
Currently available evidence indicates that synergistic effects between two or more active substances are quite rare; however additive effects are more common and may be expected where two (or more) active substances have the same effect on honey bees. Therefore, where the toxicological action in honey bees of component active substances are similar, it would be appropriate for applicants to provide formulation toxicity studies. Alternatively, it may be possible to calculate the formulation toxicity on the assumption of additive toxicity and hence reduce the need for additional testing, please see Chapter 8 for further details.

Note 4 In the definitive version on regulatory requirements for active substances (SANCO, 2011) solitary bees as such are not mentioned.
There is a need to improve the testing protocols concerning solitary bees, in particular to better address the chronic risk to solitary bees and the identification and measurement of sub-lethal effects (e.g. effects on memory, learning capacity, orientation) to be used in the risk assessment. Pending the validation and adoption of new test protocols and of a new risk assessment scheme, all efforts shall be put in place to comprehensively address, with the existing protocols, the acute and chronic risk to solitary bees, including those on colony survival and development.

The tests shall provide the EC_{10}, EC_{20}, EC_{50} (or an explanation if they cannot be estimated) together with the NOEC. Sub-lethal effects, if observed, shall be reported

Note 5 *Osmia cornuta* or *Osmia bicornis* (= *O. rufa*) are proposed as test species. Test protocols for these species are available in Appendix Q.

Note 6 Appendix S gives practical advice how to calculate the amount of residues that may be ingested by an adult bee or the concentration to that bee larvae may be exposed. For the screening steps shortcut values and simplified equations may be used as reported in Note 4 of the Risk assessment scheme for honey bees for spray applications, but the figures need to be multiplied with the adjustment factor of 5. For PECnectar and PECpollen for seed treatment for the target crop, the default of 1 mg/kg needs to be used.

Note 7 Either assume that honeybees are an adequate surrogate for bioaccumulative toxicity or replicate design of test but using solitary bees.

Note 8 At the moment no standardized guidelines are available for Higher Tier testing but protocols for semi-field and field studies are proposed in Appendix Q. Endpoints measured in these tests are: bee mortality rate, cell production rate, foraging and in-nest times, progeny survival.

8. Mixture toxicity and toxicity of formulated products with 2 or more active substances

The following parts of this paragraph are from either the Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009) or from the Scientific Opinion on the science behind the development of a risk assessment of Plant Protection Products on bees (EFSA, 2012a). In those two documents, in particular in the bee Opinion, more background information is provided.

In a recent review for the European Commission (Kortenkamp et al. 2009), the use of the concentration addition model was proposed as the concept of mixture toxicity that is most relevant for hazard characterisation and ultimately can be integrated into the legislative process for risk management purposes. The use of the concentration addition has also been discussed by Verbruggen and van den Brink (2010). There are two reasons that make the use of this model concept attractive for policy makers. First, the model concept is generally more conservative than the concept of response addition. Nevertheless, the magnitude of the differences at low levels of exposure between the two models is usually small and hence, the outcome will not be overly conservative. A second reason for the use of concentration addition is that the model concept can make use of existing data such as a NOEC, EC10 or EC50's by applying the concept of toxic units (TUs).

The concept of TUs has been recently reviewed by the three non food committees of the European Commission (the Scientific Committee on Health and Environmental Risks (SCHER), the Scientific Committee on Emerging and Newly Identified Health Risks (SCENHIR), the Scientific Committee on Consumer Safety (SCCS)) which defined TUs as "the ratio between the concentration of a mixture component and its toxicological acute (e.g. LC50) or chronic (e.g. long-term NOEC) endpoint". In addition, the toxic unit of a mixture (TUm) has been defined as the sum of TUs of each individual chemical of that mixture. The committees also noted that the TUs concept only refers to a specific organism representative of a group of organisms ecologically or taxonomically relevant for the ecosystem (e.g. algae, daphnids and fish for the freshwater ecosystem) but not to the ecosystem as a whole (SCHER/SCENIHR/SCCS, 2011).

Concentration addition (CA)

The following equation can be used for deriving a surrogate EDx, ECx, NOEC or NOEL value for a mixture of active substances with known toxicity assuming dose additivity:

$$EC_x \text{ (mix) or NOEC (mix)} = \left(\sum_i \frac{X(a.s._i)}{EC_x\_or\_NOEC(a.s._i} \right)$$

Where:

X(a.s.i) = fraction of active substance [i] in the mixture (please note that the sum Σ X(a.s.$_i$) must be 1)
ECx or NOEC(a.s.$_i$) = toxicity value for active substance [i].

Where the toxicity value of a formulated product with more than one active substance is available, this value should be compared with the predicted mixture toxicity assuming dose additivity. A different form of the equation is used.

$$\sum_i \frac{X(a.s._i)}{EC_x\_or\_NOEC(a.s._i)} = \frac{1}{EC_x\_or\_NOEC(mix)}$$

X(a.s.$_i$) = fraction of active substance [i] in the mixture (here: formulation)
EC_x or NOEC(a.s.$_i$) = acute toxicity value for active substance [i]

EC_x or NOEC(mix) = measured acute toxicity value for the mixture (here: formulation)

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity). This may be due to, e.g. further toxic co-formulants, toxicokinetic interaction or synergism/potentiation of effect. It may also reflect the inherent variability of toxicity testing. In all these cases, the use of the EC50 for the formulation (together with appropriate exposure estimates, see Step 4) is recommended for the first-tier assessment, because it cannot be excluded that such effects would also occur after exposure of animals to residues in the environment.
Dismissing the EC50 of the formulation from the risk assessment would only be acceptable at a Higher Tier if any observed greater toxicity in the test could be clearly and unambiguously ascribed to a factor that would not be relevant under environmental exposure conditions.
If, in contrast, the measured toxicity of a formulation is lower than predicted, the predicted mixture toxicity should be used in the first-tier risk assessment, together with appropriate exposure estimates.

For the First Tier it is assumed that all peaks will occur at the same moment and are not separated in time. In case the trigger value is not met in Higher Tiers the predicted exposure patterns can be taken into account (see for example calculations table xx).

Table 1: Example for a mixture of two compounds (all concentrations in µg/l). Values printed in red are above the trigger value of 0.1 and additional risk assessment should be considered.

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Concentration compound A	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2
Concentration compound B	0	0	0	2.3	1.2	0.6	0.3	0.1
Toxicity compound A	10	10	10	10	10	10	10	10
Toxicity compound B	8	8	8	8	8	8	8	8
Toxicity mixture	10	10	10	8.41	8.59	8.8	9	9.33
TER mixture	0.09	0.08	0.07	0.34	0.19	0.11	0.07	0.03

9. Risk mitigation options

9.1. Risk mitigation for honeybees

The risk assessment scheme for honeybees is in the first tier based on worst-case exposure situations. If a risk is found, refinement may be done with substance-specific data like residue trials and/or bee toxicity studies. However, for many exposure routes, mitigation measures are also a refinement option. This chapter first discusses the legal background of risk mitigation, the practice and uncertainties, and some definitions. Then an overview of all options available for the different exposure routes is given.

Risk mitigation – legal background

The only harmonized risk mitigation sentence aimed at bee risk mitigation is SPe8 from Annex V of 1999/45/EC, which is still relevant under 1107/2009/EC (see Article 65.1).
This sentence is more appropriate to mitigate risks from spray applications than from systemic soil/seed treatments and it does not cover all exposure routes. Therefore, other phrases are proposed below. Note that these phrases should be notified to the European Commission if they are used for authorisations (1107/2009 article 65.3)

Risk mitigation – practice and uncertainties

Ensure that all risk mitigation phrases are workable in practice and enforceable.

Always ensure that the risk mitigation phrase is seen by the relevant person. This is usually straightforward for spray formulations, where the risk mitigation can be stated on the product label. However it is more complicated for e.g. treated seeds. For measures relevant to the sowing process of treated seed, the risk mitigation phrases should be on the bag with treated seed or accompanying document and not (only) on the seed treatment product label; see 1107/2009 Article 49.4).
Also consider flowering plants grown from treated seed and sold to end users: if there are risk mitigation measures which are relevant for the field, e.g. waiting period for bee-attractive succeeding crops, these risk mitigation phrases should accompany the plants.

The risk mitigation phrases given below and the information on honey bee attractivity of crops (appendix G) are based on the agricultural situation and enforceability in the Netherlands. MS are asked to comment on the relevance for their own agricultural situation.

Definitions for terminology flower and flowering crop with respect to bee risks:

Definition flowering (bloom):
Flowers in which the stamen or pistils are visible.

Definition flowering crop - orchard:
An orchard is considered a flowering crop when more than 1% of the flowers in an orchard are flowering.

Definition flowering crop - field crops:

The crop is considered a flowering crop when more than two plants (crop and/or weed plants) per square meter are flowering .

Definition flowering crop – flower bulbs/bulb flowers:
A crop is in flower when more than 1% of the plants in a field is flowering. In Dutch agricultural practice this means that a crop is considered to be flowering when more than two plants per linear metre of a field are flowering.

9.2. Risk mitigation options for honeybees

9.2.1. Spray treatment

Determine the relevance of direct overspray of the crop with Appendix G, where for all crops it is indicated whether they are attractive to honeybees or not. This appendix takes both agricultural practice (does the crop flower in the field) and attractiveness of the flowers into account.

Direct:

If there is a direct risk via spray application on a flowering crop or flowering weeds, consider using parts of the harmonized risk mitigation phrase (SPe8, see ‘ background and uncertainties’ below) for bees for professional use:

Dangerous to bees./To protect bees and other pollinating insects do not apply on flowering crops./Do not use where bees are actively foraging./Remove or cover beehives during application and for (state time) after treatment./ Do not apply when flowering weeds are present./ Remove weeds before flowering./Do not apply before (state time).

Note that the sentence *Do not use where bees are actively foraging* covers direct overspray of bees foraging on honeydew.

For non-professional users, a simplified sentence is more appropiate:

Dangerous to bees and bumblebees. Do not apply on or near flowering plants and flowering weeds.

Determine the relevance of honeydew formation for the crop with Appendix E and determine the relevant sensitivity of aphids vs. honeybees. The concentration of a systemic compound that could circulate in the phloem and reach honeydew without harming aphids should, in principle, not be capable of harming bees foraging on the honeydew, unless the compound is highly selective towards non-aphid insects. If there is a risk via honeydew, consider adding a risk mitigation sentence to avoid formation of honeydew:

Aphids must be controlled in such a way that honeydew formation is excluded or do not spray when bees are foraging.

Off-field:

If there is a direct risk via spray application on a flowering margin or bordering crop, consider prescribing drift reducing measures:

Dangerous to bees./To protect bees and other pollinating insects, [specify risk mitigation measure, e.g. 90% drift reducing spray nozzles, a bufferzone of x m, …] must be used.

Indirect: systemics only.

Determine the relevance of exposure via nectar and pollen of the crop with appendix G, where for all crops it is mentioned whether they are attractive to honeybees or not.
If exposure is relevant, risk mitigation may prohibit flowering in the field.
Appliction may be restriction to post-flowering only. If pre-flowering is also requested, the last allowed application pre-flowering growth stage should be specified on the label (e.g. BBCH x, mouse-ear stage).

Determine the relevance of significant occurrence of weeds in the crop. If relevant, risk mitigation may prohibit flowering weeds in the field.

Determine the relevance of exposure via bee-attractive succeeding crops, considering e.g. the crop rotation scheme, Appendix G and the persistence of the substance/metabolites in soil. If exposure is relevant and a risk cannot be excluded in the normal rotation scheme, consider prescribing a waiting period for bee-attractive succeeding crops:

Because of the risk to bees, bee-attractive crops should not be sown or planted within a period of [x] after [application / sowing / planting in the field].

Determine the relevance of honeydew formation for the crop with Appendix E and determine the relevant sensitivity of aphids vs. honeybees. The concentration of a systemic compound that could circulate in the phloem and reach honeydew without harming aphids should, in principle, not be capable of harming bees foraging on the honeydew, unless the compound is highly selective towards non-aphid insects. If there is a risk via honeydew, consider adding a risk mitigation sentence to avoid formation of honeydew:

Aphids must be controlled in such a way that honeydew formation is excluded or do not spray when bees are foraging.

Please note that risk mitigation based on removing flowering weeds may lead to lack of food resources for bees in agricultural landscapes in particular during times when no flowering crops are available. This might have an impact on pollinators and consequently on pollination service and on biodiversity.

The view of stakeholders on this particular risk mitigation measure would be welcome.

9.2.2. Seed/soil treatment

Direct:

- In-field - bare soil so not relevant.
- Off-field. Dust drift on (bees flying on) weeds/bordering crops.

Determine the relevance of dust drift exposure on a flowering margin or bordering crop with Appendix K, This appendix takes into account whether the seed is sown outdoors or indoors, what type of machinery is used, and what type of seed coating is used, for a range of seed-treated crops. The appendix was written for the Netherlands and MS are asked to comment on the relevance for their own agricultural situation.

If a risk cannot be excluded, consider adding risk mitigation sentences:

… to reduce dust formation on the seed include sentence on seed treatment product label: *Treated seed should have a maximum dust level of [e.g. 0.75] g dust per [e.g.100.000 seeds] (Heubach-method).*

… to reduce dust drift during sowing include sentence on bag with treated seed:

Before sowing:
Do not transfer dust from bag into sowing machine

During sowing:
Do not sow during strong wind and sow the recommended amount of seed.
When using a pneumatic sowing machine, deflectors must lead the air stream towards or into the ground [or other recommendations relevant for the specific crop / sowing machine].

Indirect: systemics only

- Nectar/pollen of the crop –

Determine the relevance of exposure via nectar and pollen of the crop with Appendix G, where for all crops it is mentioned whether they are attractive to honeybees or not.
If exposure is relevant, risk mitigation may prohibit flowering in the field.

Determine the relevance of significant occurrence of weeds in the crop. If relevant, risk mitigation may prohibit flowering weeds in the field.

Determine the relevance of exposure via bee-attractive succeeding crops, considering e.g. the crop rotation scheme, appendix X and the persistence of the substance/metabolites in soil. If exposure is relevant and a risk cannot be excluded in the normal rotation scheme, consider prescribing a waiting period for bee-attractive succeeding crops:

Because of the risk to bees, bee-attractive crops should not be sown or planted within a period of [x] after [application / sowing / planting in the field].

Determine the relevance of honeydew formation for the crop with appendix E and determine the relevant sensitivity of aphids vs. honeybees. The concentration of a systemic compound that could circulate in the phloem and reach honeydew without harming aphids should, in principle, not be capable of harming bees foraging on the honeydew, unless the compound is highly selective towards non-aphid insects. If there is a risk via honeydew, consider adding a risk mitigation sentence to avoid formation of honeydew:

Aphids must be controlled in such a way that honeydew formation is excluded or do not spray when bees are foraging.

It is unclear if it is realistic to prescribe risk mitigation to avoid flowering weeds off-field, and/or formation of honeydew in succeeding crops.
The views of stakeholders on this particular risk mitigation would be welcome.

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3485 **10. Uncertainty analysis**

This chapter needs to be developed and will be included in the final Guidance Document. Proposals and views of stakeholders on the uncertainty analysis are welcome.

3486

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3705 **APPENDICES**

Name	Appendix Title
A	NOMENCLATURE FOR EFFECT SIZES
B	PROTECTION GOALS
C	MORTALITY OCCURRING IN A FIELD STUDY CONDUCTED ACCORDING TO EPPO 170 AND EXAMPLE FOR COMPARISON TO PROTECTION GOALS
D	RELEVANCE OF DUST FOR TREATED SEEDS
E	HONEYDEW
F	GUTTATION AND PROPOSED RISK ASSESSMENT FOR GUTTATION WATER
G	ATTRACTIVITY OF AGRICULTURAL CROPS TO HONEYBEES FOR THE COLLECTION OF NECTAR AND/OR POLLEN
H	LANDSCAPE-LEVEL EXPOSURE ASSESSMENT OF THE AVERAGE CONCENTRATION ENTERING THE HIVE
I	PESTICIDE RESIDUE LEVELS IN NECTAR AND POLLEN AND THE RESIDUE UNITE DOSES (RUDS)
J	PROTOCOL FOR PERFORMING FIELD STUDIES TO ASSESS A CERTAIN PERCENTILE OF THE CONCENTRATION IN POLLEN AND NECTAR IN A CERTAIN TYPE OF PLANTS IN THE AREA OF USE OF THE SUBSTANCE
K	ASSESSMENT OF SPRAY DRIFT AND DUST DRIFT DEPOSITION ONTO FIELD MARGINS AND ADJACENT FIELDS
L	ASSESSMENT OF THE PERCENTILE OF A SUBPOPULATION THAT CORRESPONDS TO A PRESCRIBED PERCENTILE OF THE TOTAL POPULATION
M	CHECKLISTS FOR EVALUATING LABORATORY STUDIES
N	CHECKLISTS FOR EVALUATING SEMI-FIELD STUDIES
O	HIGHER TIER EFFECTS STUDIES
P	TEST PROTOCOLS FOR BUMBLEBEES (*BOMBUS TERRESTRIS*)
Q	TEST PROTOCOLS SOLITARY BEES (*OSMIA CORNUTA* AND *OSMIA BICORNIS*=*O. RUFA*)
R	TEST CROPS TO BE USED
S	CALCULATION OF THE ORAL EXPOSURE WITH WORKING EXAMPLES
T	LITERATURE REVIEW ON DAILY MORTALITY RATE
U	TRIGGER VALUES

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A. NOMENCLATURE FOR EFFECT SIZES

Specific Protection Goals have been formulated based on ecosystem services according to the methodology outlined in the Scientific Opinion of EFSA (2010). With respect to honey bees, it is suggested to define the attributes to protect as survival and development of colonies and effects on larvae and honey bee behaviour as listed in regulation (EC) No 1107/2009. In addition, abundance/biomass and reproduction were also suggested because of their importance for the development and long-term survival of colonies. Pollination, hive products (for honey-bees only) and biodiversity (specifically addressed under genetic resources and cultural services) were identified as relevant ecosystem services.

The viability of each colony, the pollination services it provides, and its yield of hive products all depend on the colony's strength and, in particular, on the number of individuals it contains. It is therefore proposed to relate protection goals specifically to colony strength, which is defined operationally as the number of bees it contains, or colony size.

Based on expert judgement, the following nomenclature was defined for the magnitudes of detrimental impacts on colony, or 'effect sizes'.

Effect	Magnitude (reduction in colony size)
Large	>35%
Medium	15% to 35%
Small	7% to 15%
Negligible	3.5% to 7%

The variability in sizes among colonies prohibited defining effect sizes in terms of absolute reductions in the numbers of bees in a colony. Experts in the working group unanimously agreed that a proportional reduction in colony size of greater than one third would be likely to compromise the viability, pollinating capability and yield of any colony; this consideration was used to define an effect as 'large'. The magnitude of a negligible effect was defined with similar regard to biological considerations and also by reference to the potential for experimental detection, because a negligible effect must be statistically distinguishable from "small effects". The intermediate effect sizes were then defined arbitrarily at even intervals in the range between 'large' and 'negligible'.

These effect sizes will be used to refer exclusively to impacts on colony size. because (as will be shown below) other endpoints, such as mortality rates, may have quite different degrees of biological sensitivity. For example, a 35% change in mortality rates relative to background levels will have a relatively small impact on colony size (see analysis of model of Khoury et al. 2011 below) and would not be similarly considered a large effect. Correspondences will sometimes arise (e.g. the overall rate of background mortality among adult bees is c. 3.5% - Khoury et al. assume 15.4% mortality among foragers and 25% of adults are foragers, which implies overall rate is $15.4 \times 0.25 \approx 3.5\%$), but these are coincidental and will not arise across the broad range of effect sizes. The same reasoning means that similar non-correspondences are likely to apply to sublethal endpoints, such as behavioural aspects of performance or fecundity, except insofar as impacts on them cause proportional effects on colony size. However, it will be appropriate in many cases to use the terms (i.e. 'large', 'medium', etc.) to refer to effects on components of colony size, which are delineated by life stages. For example, a 35% reduction in the number of brood in a colony is appropriately referred to as a large impact because it is likely to translate eventually into a similar effect on overall colony size.

The effect sizes defined above have been defined principally by reference to honey bee colonies, but in the case of non-*Apis* bees, they will refer similar to colony-level impacts (other social bees, such as bumble bees) or to population sizes (solitary bees).

In reality, the detrimental effects of pesticides on colony size will be mediated through either mortality or fecundity or both. The effects of pesticides on fecundity are not yet well understood and cannot be properly explored here. However, it is possible to theoretically interrelate effect sizes and mortality by reference to the model of colony dynamics proposed by Khoury et al. (2011). The model Khoury et al. (2011) is focused on the effects of lifespan and mortality rates of forager bees on colony growth. Values for its parameters can be estimated from published observations predictions and the behaviour of the model is validated with experimental data of Ruepell et al. (2009), although the key predictions about the relationship between colony growth and forager mortality are not yet experimentally tested. As calibrated by Henry et al. (2012) the model is applicable for colonies in autumn and winter, but it can also be calibrated for colonies in spring and summer (Cresswell & Thompson, in press). According to these solutions to the model, autumn colonies are susceptible to decline caused by increased mortality of foragers (e.g. due to pesticide-induce navigation failure) but colonies in spring/summer are not.'

A theoretical basis for the magnitudes of large, small and negligible effects based on the model of Khoury et al. (2011).

In the honey bee colony, the development of newly hatched adult workers follows a consistent and well-understood pathway. The newly emerged adults are first hive bees, which undertake various duties such as feeding larvae, comb building and cleaning. After a period, hive bees progress to join the workforce of foragers and they normally continue in this role until death. In cases where there is an excess of foragers, bees can reverse their development and return to duties in the hive. The fundamental biology associated with this division of labour can be described mathematically by a simple model (Khoury et al. 2011; Figure A1).

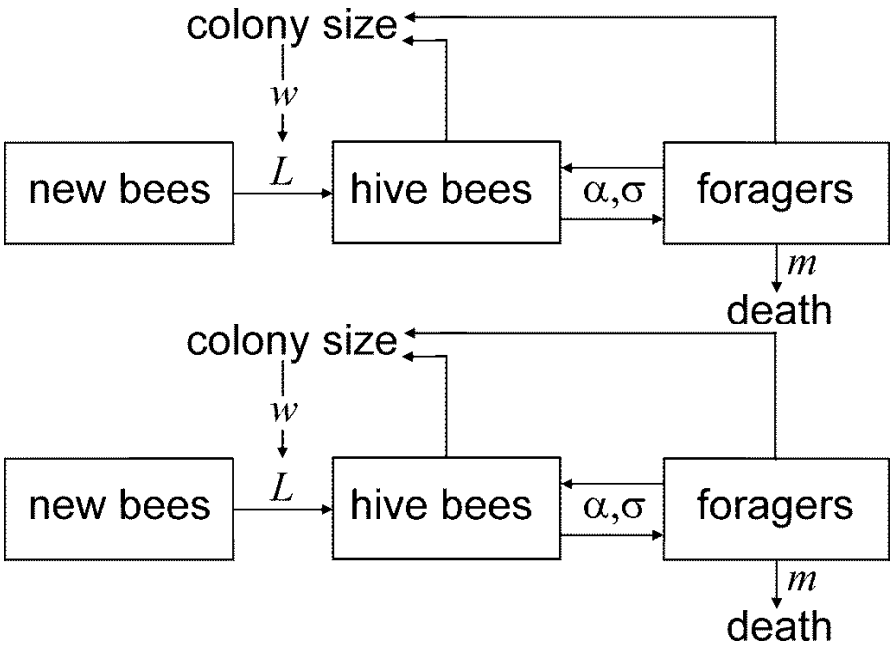

Figure A1: A simple description of the distribution of adult workers in a honey bee colony among stages of behavioural development (boxes: new bees, hive bees, foragers). Linking arrows indicate the possible pathways for progression and the nearby italicised parameters govern the daily rates of each transition.

Thus, the maximum daily rate at which hive bees are produced is *L* bees per day. However, this rate responds to colony size (smaller colonies have a lower capacity to produce hive bees) and the sensitivity of this size-dependence is governed by tuning *w*. Similarly, α and σ govern the rates of developmental transitions between hive bees and foragers, and *m* governs the daily *per capita* mortality rate.

In their analysis, Khoury et al. assumed that the rate of background mortality among foragers (i.e. deaths not due to pesticide exposure) was 15.4%, while hive bees did not suffer any mortality. The analysis below examines the impact on colony size of pesticide exposures that elevate the mortality rate by various multiples.

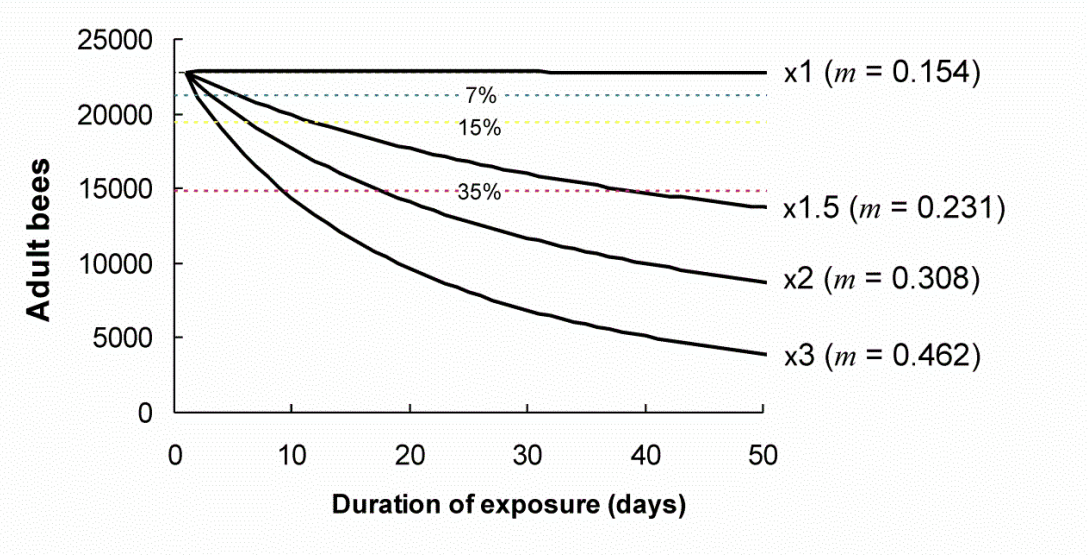

Figure A2: Behaviour of the model of a honey bee colony proposed by Khoury et al. (2011) with parameter values set as follows: N_0 = 22784, L = 2000, α = 0.25, σ = 0.75, w = 27000 and m set at various multiples of the background rate (Khoury et al. 2011). The y-axis shows the number of adult bees in the colony. In these calculations, the initial number of adult bees is set to equilibrate given background mortality among foragers (see trajectory labelled 'x1 m = 0.154'). Other curves show trajectories when elevated rates of mortality due to pesticide exposure are applied continuously (e.g. when an additional 15.4% of foragers are killed daily by pesticide mortality, then the mortality rate is 30.8% (see trajectory labelled 'x2 m = 0.308').

Multiple of background mortality	Negligible Reduction of colony size by ≤ 7%	Small Reduction of colony size by ≤ 15%	Medium Reduction of colony size by ≤ 35%	Viable after 50 days?
× 1.5 (m = 0.231)	6	13	40	Y
× 2 (m = 0.308)	3	7	18	Y
× 3 (m = 0.462)	2	4	10	N

Table A1: Extracts from Figure A2: number of days until effect (negligible, small, medium) under various levels of elevated forager mortality due to pesticide exposure (× 1.5 background, × 2, × 3) as determined by solutions to the model of Khoury et al. (2011). Colony viability is determined here by whether the colony contains at least 5000 adult bees after 50 days (5000 in often considered to be the minimum size suitable for successful overwintering).

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3824 **B. PROTECTION GOALS**

3825 Specific protection goals based on ecosystem services were suggested according to the methodology
3826 outlined in the Scientific Opinion of EFSA (2010). In consultation with risk managers in the SCoFCAH
3827 (Standing Committee on the Food Chain and Animal Health) the Specific Protection Goals for honey-
3828 bees were set as outlined below.

3829 The attributes to protect were defined as survival and development of colonies and effects on larvae and
3830 bee behaviour as listed in regulation (EC) No 1107/2009. In addition, abundance/biomass and
3831 reproduction were also suggested because of their importance for the development and long-term
3832 survival of colonies.

3833 The viability of each colony, the pollination services it provides, and its yield of hive products all depend
3834 on the colony's strength and, in particular, on the number of individuals it contains. It is therefore
3835 proposed to relate protection goals specifically to colony strength, which is defined operationally as the
3836 number of bees it contains (= colony size).

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3838 impacts on colony, or 'effect sizes'.

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3843 reduction in colony size of greater than one third would be likely to compromise the viability, pollinating
3844 capability and yield of any colony; this consideration was used to define an effect as 'large'. The
3845 magnitude of a negligible effect was defined with similar regard to biological considerations and also by
3846 reference to the potential for experimental detection, because a negligible effect must be statistically
3847 distinguishable from "small effects". The intermediate effect sizes were then defined arbitrarily at even
3848 intervals in the range between 'large' and 'negligible'.

3849 The effect sizes defined above have been defined principally by reference to honey bee colonies, but in
3850 the case of non-*Apis* bees, they will refer similar to colony-level impacts (other social bees, such as
3851 bumble bees) or to population sizes (solitary bees).

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3853 **Table B1:** Overview on combinations of magnitude of effects on forager mortality and time to reach
3854 point of where the colony may collapse (< 5000 bees in the hive) (for details see Appendix A):

Multiple of background mortality of forager bees	Negligible effect Reduction of colony size by	Small effect Reduction of colony size by	Medium effect Reduction of colony size by	Viable after 50 days?

	≤7%	≤15%	≤35%	
× 1.5 (*m* = 0.231)	6 days	13 days	40 days	Y
× 2 (*m* = 0.308)	3 days	7 days	18 days	Y
× 3 (*m* = 0.462)	2 days	4 days	10 days	N

It was agreed in the SCoFCAH to base the specific protection goal on a negligible effect on colonies. For example an increase in forager mortality by a factor of 1.5 compared to controls could be tolerated for 6 days (average factor over 6 days). From day 7 on the mortality rate would need to be back to control. An increase of a factor of 2 could be tolerated for 3 days and an increase of mortality of a factor of 3 for 2 days. After that period of time the mortality of foragers should not exceed background mortality. The effect on the colony should not exceed 7% compared to controls after 2 brood cycles. In the risk assessment (e.g. field studies) it needs to be ensured that the effects that are proposed for the Specific Protection Goals can be assessed. E.g. it needs to be ensured by the test design to detect an increase in mortality of more than a factor of 1.5 compared to controls with sufficient statistical power.

It is important to note that effects on colony should not exceed negligible effects also for products that are applied several times (according to the Good Agricultural Practice). Risk management options should be considered if the magnitude of effects exceeds “negligible” effects.

The overall level of protection also includes the exposure assessment goals. Decisions need to be taken on how conservative the exposure estimate should be and what percentage of exposure situations should be covered in the risk assessment. The first aspect of the spatial statistical population is the total area to be considered (e.g. the whole EU, one of the regulatory zones North-Centre-South or a Member State). In view of the terms of reference, we propose to consider each of the regulatory zones North-Centre-South as the total area for all Specific Protection Goals (SPGs). A second aspect of the spatial statistical population is the location of the spatial units (individual bees, colonies or populations) in the landscape in relation to the application of the substance. It is proposed that the risk assessment focuses at field scale to avoid ‘dilution’ of the spatial population with a large fraction of unexposed hives, for example.

It was decided that the exposure assessment should be done for each of the regulatory zones and it was suggested that representative scenarios should be developed in future

By defining a certain percentile exposure assessment goal (e.g. 90%) it is meant that 90% of all colonies at the edge of a treated field in one regulatory zone should be exposed to less than what is assessed in the risk assessment. For 10% of the colonies at the edge of a field in the regulatory zone the exposure could exceed what was assessed in the risk assessment. For these colonies the protection may not be achieved for substances which are highly toxic to bees (e.g. effects could exceed negligible effects). It was proposed to base the exposure estimates at the 90th percentile as is done for other groups of non-target organisms. However, there was also the suggestion to have a more conservative exposure assessment goal like for example the 95th percentile. The main concern was to be sufficiently conservative to avoid bee kill incidents. No final decision was taken by the SCoFCAH. The current version of the Guidance Document is based on the 90th percentile. If risk managers decide to choose a higher percentile after the public consultation period then the corresponding exposure values need to be changed in the final version of the GD.

The risk assessment scheme and associated trigger values enable an assessment that, if met, would ensure that exposure does not exceed a value that could lead to effects which are more than negligible in 90 % of sites (i.e. treated fields) where honey bee colonies are situated on the edge of treated fields. The trigger

values are set that an individual colony can tolerate an impact on foragers of y % effect over Z time or less. This will ensure that the protection goal related to in-field pollination services of crop plants is met.

It is unclear if honey production would be a more sensitive endpoint than effects on mortality or reduction of colony size. It may be more difficult to assess effects on honey production because there is a high variability depending on the site where the colony is located. Since only negligible effects on the colonies are acceptable the colony should stay as productive as a non-exposed one. However, considering the importance of honey production for beekeepers it is proposed to include honey production as a measurement endpoint in field studies.

C. MORTALITY OCCURRING IN A FIELD STUDY CONDUCTED ACCORDING TO EPPO 170 AND EXAMPLE FOR COMPARISON TO PROTECTION GOALS.

Presented below is a summary of the daily forager mortality that occurred in a regulatory field study and a comparison of the forager mortality rates to the protection goals. Please note that the study does not necessarily reflect the outcome of a good or of a representative field study. The data were used simply to illustrate the protection goals applied to mortality data from a field study.

The study was conducted on oilseed rape. Two active substances were tested as spray applications. One active substance was very toxic to bees and used as a toxic reference. The second substance (a new active substance - NAS) was of low toxicity to bees.
Dead bees were collected daily in dead bee traps starting from the day before treatment until 21 days after treatment. The factor of increase in mortality of foragers compared to the control was calculated for each day and the average factor of increase in daily mortality was calculated over 2 days, 3 days, 4 days, 6 days, 7 days, 10 days and 18 days.
The protection goal was defined as negligible effects (see Chapter 2 and Appendix B for further details). The average forager mortality compared to controls should not exceed the factors: 3 for 2 days, 2 for 3 days and 1.5 for 6 days.
As expected, the toxic standard clearly caused effects that exceed negligible effects (increase of average forager mortality by more than a factor of 3 for 2 days, a factor of 2 for 3 days and a factor 1.5 for 6 days). The active substance (NAS) did not affect forager mortality. The protection goal for the new active substance (NAS) with regard to forager mortality was met in the field study.

Table C1: Average number of dead bees per plot on each sampling date. Data collected via dead bee traps.

Days after application	Average number of dead bees			Factors of increase in forager mortality compared to controls			
	Control plot	NAS	Toxic std - reference	NAS	Toxic std.	NAS average	Toxic std. average
-1	24.75	13.25	19.00	0.54	0.77		
0	6.75	1.00	3.50	0.15	0.52		
1	712.75	4.25	5827.13	0.01	8.18		
2	8.50	0.00	970.00	0.00	114.12	0.00	61.15
3	339.50	3.50	427.75	0.01	1.26	0.01	41.18
4	95.00	2.25	174.75	0.02	1.84	0.01	31.35
5	80.75	0.75	89.25	0.01	1.11		
6	8.50	2.25	81.25	0.26	9.56	0.05	22.68
7	10.00	1.25	33.25	0.13	3.33	0.06	19.91
8	6.50	1.75	25.75	0.27	3.96		
9	11.00	1.25	35.50	0.11	3.23		
10	10.50	11.00	12.00	1.05	1.14	0.19	14.77
11	27.50	37.00	45.75	1.35	1.66		
12	7.25	6.25	19.25	0.86	2.66		
13	7.75	4.50	24.50	0.58	3.16		
14	4.75	3.50	22.25	0.74	4.68		
15	12.50	12.75	14.00	1.02	1.12		
16	4.00	0.50	8.75	0.13	2.19		
17	7.75	4.50	4.75	0.58	0.61		
18	5.50	9.25	2.50	1.68	0.45	0.49	9.13
19	26.75	4.25	14.00	0.16	0.52		
20	14.00	0.75	18.25	0.05	1.30		
21	11.25	1.75	11.75	0.16	1.04		

D. RELEVANCE OF DUST FOR TREATED SEEDS.

Most of this table is taken from SANCO/10553/2012 rev. 0, 8 March 2012, Guidance Document on the authorisation of Plant Protection Products for seed treatment (Annex I to Appendix VI). The last column is added to show relevance for off-field exposure of honeybees. The table is mainly based on seed treatment and sowing practice in the Netherlands.

Comments on relevance for other countries are welcomed. MS are also invited to add information on crops not yet included below.

Table D1: Representative coating practice and conditions of use of coated seeds

Crop	Direct sowing or transplanting	If direct sowing outdoors, type of driller [(a)]	Seed treatment technology [(b)]	Conclusion on dust formation (and potential risk for non-target organisms)
arable crops				
cereals - spring	Direct sowing	mostly mechanical and pneumatic seed drill equipment, pneumatic with vacuum principle upcoming	seed treatment facilities (fixed or mobile) and on farm treatment basic seed treatment / basic coating	Relevant
cereals - winter	Direct sowing	mostly mechanical and pneumatic seed drill equipment, pneumatic with vacuum principle upcoming	seed treatment facilities (fixed or mobile) and on farm treatment basic seed treatment / basic coating stickers more recently introduced more widely	Relevant
maize, sweet corn, sorghum	Direct sowing	90% vacuum principle	Professional treatment basic seed treatment direct on the seed (active ingredient can be present on the outside surface of the seed)	Relevant
oilseed rape	Direct sowing	mechanical and pneumatic seed drill equipment, pneumatic with vacuum principle upcoming	Professional treatment basic seed treatment / basic coating finishing powder to ensure flowability of seeds	Relevant
sunflower	Direct sowing	both mechanical and pneumatic with and without vacuum technique are possible	Professional treatment basic seed treatment / basic coating finishing powder to ensure flowability of seeds	
beet (sugar and fodder)	Direct sowing	Pneumatic or mechanical precision drilling equipment	Professional treatment pelleting, with active ingredient not on the outside of the seed but closed in by an inert layer; new development: filmcoating on top of the pellet	not relevant, due to pelleting and filmcoating (and mechanical drilling)

Crop	Direct sowing or transplanting	If direct sowing outdoors, type of driller [a]	Seed treatment technology [b]	Conclusion on dust formation (and potential risk for non-target organisms)
beans, peas	Direct sowing	Pneumatic (mainly vacuum technique) or mechanical precision drilling equipment	Professional treatment basic seed treatment / basic coating	Relevant
cotton	Direct sowing	Vacuum pneumatic drilling equipment	Professional treatment basic seed treatment / basic coating delinting process	Relevant
flax, poppy seed	Direct sowing	mostly mechanical seed drill equipment, pneumatic with vacuum principle upcoming	basic seed treatment / basic coating	Relevant
grasses, grasseed	Direct sowing	both mechanical and pneumatic (vacuum) are possible	basic seed treatment / basic coating	Relevant
alfalfa, caraway, green manure crops	Direct sowing	both mechanical and pneumatic (vacuum) are possible	no seed treatments	Not relevant (no seed treatments)
outdoor vegetables				
onion, carrot, radish	Direct sowing	Pneumatic precision drilling equipment	filmcoating/rotostat for insecticides	Not relevant for insecticides due to high quality coating; maybe relevant for other pesticides
leek	Most sowing in seed beds and transplanting later, approximately 10% direct sowing. Mostly sowing outdoors, some sowing indoors in trays.	Pneumatic precision drilling equipment	filmcoating/rotostat for insecticides	Not relevant for insecticides due to high quality coating; maybe relevant for other pesticides
asparagus	Sowing in seed beds, later transplanted.	yes	filmcoating/rotostat for insecticides	Not relevant for insecticides due to high quality coating; maybe relevant for other pesticides
chicory, endive, lamb's lettuce	Direct sowing	mainly coated seed, pneumatic ; also pelleted seeds, sown mechanically	filmcoating/rotostat for insecticides	Not relevant for insecticides due to high quality coating; maybe relevant for other pesticides
spinach	Direct sowing	mainly mechanically drilled, pneumatic equipment upcoming (both vacuum and gauge pressure principle)	basic coating, partly filmcoating, and sometimes toplayer	Relevant

Crop	Direct sowing or transplanting	If direct sowing outdoors, type of driller [a]	Seed treatment technology [b]	Conclusion on dust formation (and potential risk for non-target organisms)
beetroot	Direct sowing	Pneumatic precision drilling equipment	basic coating	Relevant
greenhouse vegetables				
lettuce, including lettuce-like (radichio rosso, endive, etcetera)	All these crops are only sown and raised to young plants indoors; later transplanted indoors or outdoors.	not applicable	pelleting, with active ingredient not on the outside of the seed but closed in by an inert layer	Not relevant due to indoor sowing
brassica, including head cabbages, Brussels sprouts, cauliflower, broccoli, Chinese cabbage, kale	All these crops are only sown and raised to young plants indoors; later transplanted indoors or outdoors.	not applicable	filmcoating/rotostat, and sometimes top layer	Not relevant due to indoor sowing
fruiting vegetables (tomatoes, cucumber, weet pepper, eggplant, etcetera)	Plant raising only indoors, later transplanted indoors or outdoors. In case of outdoor sowing (e.g. cucumber in Germany) vacuum systems are used.	Pneumatic precision drilling equipment	sometimes fungicide treatments	Not relevant due to indoor sowing
celeriac	Sown indoors, later transplanted outdoors.	not applicable		Not relevant due to indoor sowing
ornamentals				
several ornamental crops from seed	Cultivation both indoors and outdoors; many crops through plant raising indoors; limited crops directly sown outdoors.		filmcoating (high value seeds)	Not relevant for most crops due to indoor sowing; Relevant for some

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(a) Mechanical seed drill equipment does not work with air and therefore can not release air flows. With pneumatic seed drill equipment there are two principles: using the vacuum principle and using the gauge pressure principle. When using the gauge pressure principle there is no more air replacement (with potential dust) than with mechanical seed drill equipment. When using the vacuum principle seeds are put in the sowing row by vacuum and the excess air will come free. At conventional corn sowing machines, this exhaust air was directed upwards. Meanwhile, these machines (mostly) are modified: they have deflectors directing the exhaust air downwards to the soil. For vegetable vacuum seed drilling machines, the airflows already almost always were directed towards the soil.

(b) There is no complete one-on-one relationship crop - seed treatment: which method is used also depends on e.g. the type of pesticide used, the composition of that pesticide and whether multiple pesticides are used, seed type (smooth, rough, etc.), to a certain extent for which market the seed is treated, etc. Also, various terms are used. This table presents an indication. In general, the more valuable the seed is, the higher quality (and more expensive) seed treatment technology can be used. Furthermore: coating means stickers are used; in basic coating the pesticide can irregularly be distributed over the seed, in film coating a regular layer is spread over the seed (used for somewhat higher valuable seeds); a part of the market has on top of that a top layer (without active ingredient).

In general, doses are lower for fungicide treatments than for insecticide treatments, which means that less coating is needed for fungicide treatments, so there is less coating available for abrasion. On the other hand, a top layer is then not necessary.

E. HONEYDEW

Honeydew is a sugar sticky liquid, excreted by various insects including aphids, leafhoppers and some scale insects when they feed on plant sap. As nectar, honeydew derive by plant sap but it is not actively secreted by plant. For this reason honeydew production not only depends on crops, climatic and geographic conditions, as in nectar, but also by the dynamic population of the honeydew-producing insect. The plants producing honeydew are mainly conifers (genu *Abies*, *Picea*, *Pinus*, *Larix*) and several deciduous plants with no nectar in flowers (oak, beech, poplar) and with nectar (linden, willow tree, maple, chestnut, black locust, fruit trees). Several herbaceous crops and weeds can host honeydew-producing insects (alfalfa and sunflower). The honeydew-producing insects are all in the Hemiptera order including several species of the families: Flatidae, Psyllidea, Thelaxidae, Eriosomatidae, Lachnidae, Chaitophoridae, Callaphididae, Aphididae, Kermesidae, Coccidae (Persano Oddo et al. 1995). The flatid planthopper *Metacalfa pruinosa* is an invasive specie from America. In Europe, it was introduced accidentally in 1979 (Treviso province in Italy) and it is now present in Italy, Spain, Austria, Croatia, France, Slovenia, Switzerland, Serbia Montenegro, Czech Republic, Hungary, Greece, Turkey, Bulgaria, Bosnia Herzegovina, Slovakia, Albania and Romania. They produce large quantity of honeydew in several plants (more than 200 species): fruit trees, olive trees, grapevine, ornamental plants and herbaceous crops as maize, sunflower and soy (Santi and Maini, 2000). Host plant of this species varies from area to area.

Potentially all plants with a presence of honeydew-producing insects can be visited by bees to collect honeydew. However, the more important plants visited for honeydew by bees are listed in table 1. Honeybees collect honeydew mainly during late summer when there are few plants in bloom (few alternative sources) and in wild plants because the honeydew-producing insect populations are usually controlled in crops.

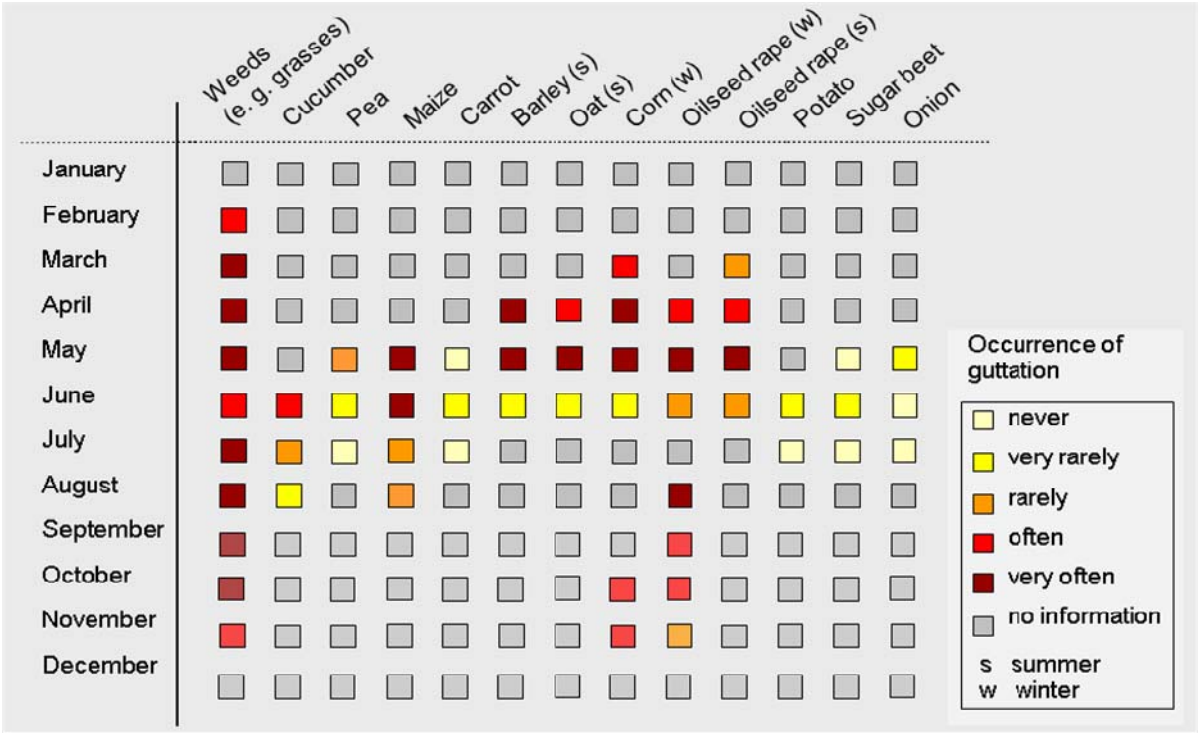
Table E1: List of plants visited by bees for honeydew (from Contessi, 2005)

Genus	Genus
Abies	*Mahonia*
Acer	*Nepeta*
Beta	*Picea*
Betula	*Pinus*
Castanea	*Populus*
Cercis	*Pyrus*
Cotinus	*Quercus*
Crepis	*Robinia*
Fagopyrum	*Salix*
Frangula	*Tamarix*
Juglans	*Tilia*
Juniperus	*Triticum*
Larix	*Tussilago*

The list is based on data from Italy. It is unclear if it is possible to extrapolate from the data representative for Italy to other regions in Europe. It would be welcome to receive data from other MSs on the plants from which honey dew is collected.

F. GUTTATION AND PROPOSED RISK ASSESSMENT FOR GUTTATION WATER

Most crops show guttation, some crops exsudate guttation droplets frequently, others rarely. For most crops, first guttation may be observed from first emergence up to flowering. In field trials in Germany in 2010-2011 sugar beets, onion and carrots showed guttation never or only on very rare occasions (0-25% of days), whereas most other crops showed guttation more often. Guttation cannot be fully excluded for any crop.



The effects of residues in guttation droplets may be investigated using worst case crops (e.g. maize) with high residues in the droplets and high potential exposure of bees due to high water demand of the colonies. Such studies may representative also for other crops that have lower guttation frequency and lower residues. If an effect study is undertaken, the exposure period with high residues must be covered (e.g. maize in spring, winter oilseed rape in autumn)

The potential risk of guttation is depending on the distance of the colonies to treated crops. The residues in guttation droplets vary for different actives, crops and growth stages but can in general be some magnitudes higher than systemic trace residues in nectar and pollen of seed treated crops. The attractivity of water is not comparable to the attractivity of nectar and pollen and forage distances will be shorter for water foraging due to energetic reasons. Nevertheless, bee colonies may be located next to or in the proximity of treated crops. As guttation issues have been investigated with special focus for a few years only, available conclusions on the current state of knowledge were considered for the proposal of a screening step for risk assessment.

Residues of systemic fungicides, herbicides and insecticides may be found in guttation droplets. As many different systemic actives of low to moderate toxicity to bees have been used for seed treatments and soil applications in the past and no effects on bees have been reported, it might be concluded that guttation has no unacceptable effects, e.g. increased mortality does not occur, for example for most of the fungicidal seed treatments. However for actives with high bee toxicity, the potential risk needs to be considered.

As the HQ approach is not applicable, in a first step to assess the potential risk, oral toxicity data e.g. LD_{50} values can be used for a calculation of the amount of liquid that would lead to an uptake of a lethal dose (e.g. approaching the oral LD_{50}). Other values e.g. NOEC values could also be used for a refined calculation. In this case, the LD_{50} is only used to demonstrate the potential magnitude of risk. In Table F1 such an example of a calculation is given. It illustrates that, for a substance with an LD_{50} of 100 ng/bee, 100 µl water would need to be consumed at a concentration of 1 ng as/µl in guttation droplets. At such concentrations, a risk would be unlikely. The data e.g. for clothianidin show that at a residue in guttation droplets of 1 ng/µl, a value found in seed treated maize or granular applications for approximately 4 weeks after emergence, only 3.7 µl of water would need to be consumed to achieve the LD_{50} of 3.7 ng/bee.

Table F1: Example for a calculation of the amount of solution that, if consumed would lead to an uptake of a lethal dose

Thiamethoxam		Clothianidin		Substance A		Substance B	
LD_{50} in ng/bee	5		3,7		50		100
Guttation droplets residues ng/µl	consumption µl/bee	Guttation droplets ng/µl	consumption µl/bee	Guttation droplets ng/µl	consumption µl/bee	Guttation droplets ng/µl	consumption µl/bee
0,01	500	0,01	370	0,01	5000	0,01	10000
0,05	100	0,05	74	0,05	1000	0,05	2000
0,1	50	0,1	37	0,1	500	0,1	1000
0,5	10	0,5	7,4	0,5	100	0,5	200
1	5	1	3,7	1	50	1	100
1,5	3,33	1,5	2,47	1,5	33,33	1,5	66,67
2	2,5	2	1,85	2	25	2	50
3	1,67	3	1,23	3	16,67	3	33,33

The approach presented in this Appendix is a first starter to address this exposure route and further work is required. The view of MSs and proposals would be welcome.

G. ATTRACTIVITY OF AGRICULTURAL CROPS TO HONEYBEES FOR THE COLLECTION OF NECTAR AND/OR POLLEN

This list contains an overview of most agricultural crops in the Netherlands. The list indicates for each crop whether it is attractive to honeybees for the collection of nectar and/or pollen. This is based on crop properties and agricultural practice in the Netherlands and may not be (completely) relevant for other countries.

Therefore, in the commenting round MS are invited to comment on the relevance of this list for their countries.

Good Agricultural Practice is assumed. If a crop does not flower during normal production, it is indicated as not attractive to honeybees (example: cabbage crops (e.g. cauliflower)).
It may also occur that a crop does flower in the field, but is not foraged on by honeybees for nectar and/or pollen. These crops are also indicated as not attractive to honeybees (example: potatoes).

Within a crop category or subcategory there may be differences, e.g. when a crop does in principle flower and is attractive to honeybees, but in some cases flowering is avoided for agricultural reasons. An example is the reproduction culture of strawberries where flowering does not occur. Nevertheless the crop subcategory strawberries is indicated as attractive to honeybees in the list since in the production culture of strawberries, flowering does occur.

The cultivation category of the ornamentals contains a large variety of crops. For this category it is assumed that non-flowering species are not attractive to honeybees while flowering species are attractive to honeybees (both for protected and unprotected crops; see the risk mitigation chapter for mitigation options to avoid entering of honeybees in greenhouses).

A number of crops, among which prunus, elder, willow, pumpkin, hollyhock, peony, sunflower, and a number of beans, amoung which broad bean (*Vicia*), produce nectar from extrafloral nectaries (nectar glands outside the flower). A number of flowering plants (e.g. cornflower, sunflower), produce extrafloral nectar on the flower bud, already before the plants flowers. Exposure to products harmful to honeybees should be avoided in these cases. Most of these crops are already indicated as attractive to honeybees in the list.

Please note that a crop field may be attractive to honeybees even if the crop is indicated as not attractive to honeybees in this list. This may be due to flowering weeds or honeydew. See the exposure chapter.

In some crops (e.g. carrots, chicory (root growing)) which usually do not flower and are therefore indicated as not attractive to honeybees, some individual plants may flower. These flowering plants need to be removed in case there are more than two flowering plants per square meter (see definition of flowering in risk mitigation chapter).

Honeybees fly in the period of February till October. Outside this period, crops that are indicated as attractive to honeybees can be treated without restrictions with regard to honeybees.

The crop hierarchy is based on the ‘Definitielijst toepassingsgebieden gewasbeschermingsmiddelen’ (DTG lijst, versie 2.0, Ctgb juni 2011). Stakeholders from beekeeping organisations, agricultural sector and research were involved in drafting the list.

Table G1: Attractivity of agricultural crops to honeybees for the collection of nectar and/or pollen

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
1. Arable crops	1.1	Potatoes		-	Seed potatoes	No	
					Ware potatoes	No	
					Starch potatoes	No	
	1.2	Beetroot		-	Sugar beets	No	
					Fodder beets	No	
	1.3	Cereals	1.3.1	Winter cereals	Winter wheat	No	
					Winter barley	No	
					Winter rye	No	
					Triticale	No	
					Spelt	No	
					Canary grass	No	
			1.3.2	Spring cereals	Spring wheat	No	
					Spring barley	No	
					Spring rye	No	
					Oats	No	
					Teff	No	
			1.3.3	Other cereals		No	
	1.4	Maize			Silage maize	Yes	for pollen
					Grain maize	Yes	for pollen
					Corn cob mix	Yes	for pollen
					Corn cob silage	Yes	for pollen

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
	1.5	Pulses	1.5.1	Dry-harvested peas	Marrowfat peas	Yes	
					Yellow peas	Yes	
					Grey pea	Yes	
					Green peas	Yes	
					Lentils	Yes	
					Maple pea	Yes	
					Brown Marrowfat	Yes	
					Sugar snaps	Yes	
					Chickpeas	Yes	
			1.5.2	Dry-harvested beans	Brown bean	Yes	
					Yellow bean	Yes	
					Pinto bean	Yes	
					White bean (haricot)	Yes	
					Soya bean	Yes	
	1.6	Grass seed crops	1.6.1	Ryegrass	English ryegrass	No	
					Italian ryegrass	No	
					French ryegrass	No	
					Westerwold ryegrass	No	
					Hybrid ryegrass	No	
					Other ryegrasses	No	
			1.6.2	Fescue	Red Fescue	No	
					Sheep’s Fescue	No	
					Tall Fescue	No	
					Other fescues	No	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
			1.6.3	Bluegrass	Kentucky bluegrass	No	
					Fowl bluegrass	No	
					Wood bluegrass	No	
					Meadow fescue	No	
					Other bluegrasses	No	
			1.6.4	Other grasses	Timothy-grass	No	
					Cock's-foot	No	
					Colonial bent	No	
					Crested dog's-tail	No	
					Tufted hair-grass	No	
					Junegrass	No	
					Other grass seed crops	No	
	1.7	Oil-bearing seeds		-	Poppy seed	Yes	
					Caraway	Yes	
					Linseed	Yes	
					Mustard seed	Yes	
					Rapeseed	Yes	
					Evening primrose	Yes	
					Sunflower	Yes	
					Camelina	Yes	
					Crambe	Yes	
					Other oil-bearing seeds	Yes, when flowering occurs in the field	
	1.8	Fibre crops		-	Hemp	No	

Cultivation categories, application sectors.	Crop categories, areas of application	Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
				Flaxseed (flax = flaxseed and linseed)	Yes	
				Nettle	No	
				Other fibre crops	Yes, when flowering occurs in the field	
	1.9 Green fertiliser crops	1.9.1	Leguminous green fertilisers	Clover	Yes	
				Lupin	Yes	
				Serradella	Yes	
				Common vetch	Yes	
				Sanfoin	Yes	
				Field beans	Yes	
				Other leguminous green fertilisers	Yes	
		1.9.2	Grass family green fertilisers	Rye	No	
				Ryegrass	No	
		1.9.3	Brassicaceae green fertilisers	Oil radish	Yes	
				Rapeseed	Yes	
				Yellow mustard seed	Yes	
				Rape kale	Yes	for seed production
				Marrow-stem kale	No	
		1.9.4	Other green fertilisers	Phacelia	Yes	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
					Corn spurrey	Yes	
					Marigold (*Tagetes*)	Yes	
					Sticky nightshade	Yes	
					Sudan grass	No	
	1.10	Fodder crops	1.10.1	Leguminous fodder crops	Clover	Yes	
					Alfalfa	Yes	
					Common vetch	Yes	
					Sanfoin	Yes	
					Field beans (for ensilaging)	Yes	
					Field mustard	No	
			1.10.2	Other fodder crops		Yes, when flowering occurs in the field	
	1.11	Other arable crops	1.11.1	-	Chicory (roots)	No	
					Wild chicory	No	
					Buckwheat	Yes	
					Hops	No	
					Common madder	Yes	
					Elephant grass	No	
2. Cultivated grassland	2.1	Fodder grassland		-	Pastureland	No, unless flowering weeds are present	This is the case when more than two flowering weeds per square meter are present

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
					Mowing grassland	No, unless flowering weeds are present	This is the case when more than two flowering weeds per square meter are present
	2.2	Grass sod				No	
3. Fruit crops	3.1	Large fruits Only refers to production of unharvested fruits	3.1.1	Pomes	Apples	Yes	
					Pears	Yes	
					Quince	Yes	
					Medlar	Yes	
					Other pomes	Yes	
			3.1.2	Drupes	Cherries (both sweet and sour)	Yes	
					Plum	Yes	
					Apricot	Yes	
					Peach (incl. Nectarine)	Yes	
					Other drupes	Yes	
	3.2	Small fruits	3.2.1	Strawberries		Yes	except production culture
			3.2.2	Berries	Currant (red, white and black)	Yes	
					Gooseberry	Yes	
					Blueberry (incl. Cowberry)	Yes	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
					Cranberry (incl. Fenberry and American Cranberry)	Yes	
					Mulberry	Yes	
					Rose hips	Yes	
					Kiwiberry	Yes	
					Elderberry	Yes	
					Other berries	Yes	
			3.2.3	Grapes	Table grape	Yes	
					Wine grape	Yes	
			3.2.4	Blackberry and raspberry family (*Rubus* spp.)	Blackberry	Yes	
					Raspberry (incl. Tayberry and Wineberry)	Yes	
					Dewberries	Yes	
	3.3	Nuts		-	Hazelnut	Yes	
					Chestnut	Yes	
					Walnut	No	
	3.4	Other fruits		-	Fig	No	
					Kiwi	Yes	
4. Vegetable crops	4.1	Leafy vegetables	4.1.1	Lettuce (*Lactuca* spp.)		No	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
			4.1.2	Endive	Endive	No	
			4.1.3	Spinach family	Spinach	No	
					Chard	No	
					Orache	No	
					Purslane	No	
			4.1.4	Other leafy vegetables	Chicory (forced cultivation)	No	
					Garden cress	No	
					Watercress	No	
					Lamb's lettuce	No	
					Rocket	No	
					Sea lavender	No	
	4.2	Pulses	4.2.1	Bean with pod	Bush green beans	Yes	
					Bush common bean	Yes	
					Waxpod bean	Yes	
					Climbing green beans	Yes	
					Climbing common bean	Yes	
					Snap bean	Yes	
					Runner bean	Yes	
					Yardlong bean	Yes	
			4.2.2	Podless beans	Broad bean	Yes	
					Lima bean	Yes	
					Flageolet bean	Yes	
			4.2.3	Pea with pod	Legume/pod	Yes	
					Asparagus pea	Yes	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
					Sugar snap	Yes	
			4.2.4	Pea without pod	Green pea/garden pea	Yes	
					Marrowfat pea	Yes	
			4.2.5	Vegetable sprouts	Bean sprouts (Mung bean sprouts)	No	
					Alfalfa	No	
					Other vegetable sprouts	No	
	4.3	Fruiting vegetables	4.3.1	Fruiting vegetables of *Cucurbitaceae* with edible skin	Gherkin	Yes	
					Courgette	Yes	
					Cucumbers	Yes	
			4.3.2	Fruiting vegetables of *Cucurbitaceae* with non-edible skin	Pumpkin family	Yes	
					Melon	Yes	
					Watermelon	Yes	
			4.3.3	Fruiting vegetables of *Solanaceae*	Aubergines	Yes	
					Tomato	Yes	
					Sweet pepper	Yes	
			4.3.4	Fruiting vegetables of *Malvaceae*	Okra	Yes	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
	4.4	Cabbages	4.4.1	Heading cabbages	Heading cabbage	No	
					Sprouts	No	
			4.4.2	Cauliflower family	Cauliflower	No	
					Broccoli	No	
			4.4.3	Loose leaf cabbage family	Chinese cabbage	No	
					Kale	No	
			4.4.4	Stalk cabbage	Kohlrabi	No	
	4.5	Root vegetables and tubers	4.5.1	Radish family	Cultivated radish	No	
					Black/white radish	No	
			4.5.2	Root vegetables *(Umbelliferae)*	Carrots	No	
					Skirret	No	
					Hamburg root parsley	No	
					Parsnips	No	
			4.5.3	Other root vegetables and tubers	Turnip	No	
					Swede	No	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory		Crops/Objects	Attractive to honeybees	Remarks
					Jerusalem artichoke	Yes	
					Chinese artichoke	No	
					Sweet potato	Yes	
					Beetroot	No	
					Celeriac	No	
					Salsify	No	
					Horseradish	No	
					Yam	No	
	4.6	Onion family	4.6.1.	Onions	Seed onions	No	
					First year bulb onion	No	
					Second year bulb onion	No	
					Silverskin	No	
					Picklers	No	
			4.6.2	Shallots	Seed shallot	No	
					Bulb shallot	No	
			4.6.3	Scallions	Scallion (incl. Welsh onion, spring onion, escallion)	No	
			4.6.4.	Garlic	Garlic	No	
	4.7	Stalk vegetables		-	Asparagus (white and green asparagus)	Yes	
					Stalk celery	No	
					Cardoon	No	
					Rhubarb	No	
					Florence fennel	No	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory	Crops/Objects	Attractive to honeybees	Remarks
				Leek	No	
				Artichoke	No	
				Sea kale	Yes	
	4.8	Other vegetable crops		Sweet corn	Yes	
5. Fresh or dried herbs	5.1	Aromatic herbs	-	Basil	No	
				Chives (incl. garlic chives)	No	
				Savoury	Yes	
				Lemon balm	Yes	
				Dill	Yes	
				Tarragon (Russian and French Tarragon)	Yes	
				Hyssop	Yes	
				Chervil	No	
				Coriander	Yes	
				Parsley	No	
				Lovage (Lovage leaves)	No	
				Marjoram	Yes	
				Oregano (Wild marjoram)	Yes	
				Mint	Yes	
				Burnet	Yes	
				Rosemary	Yes	
				Sage	Yes	
				Thyme	Yes	
				Fennel	Yes	
				Leaf Celery (stalk celery)	No	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory	Crops/Objects	Attractive to honeybees	Remarks
				Sorrel	No	
				Other aromatic garden herbs	Yes, when flowering occurs in the field	
	5.2	Aromatic root crops	-	Lovage root	No	
				Angelica	Yes	
				Burnet Saxifrage root *(Pimpinella saxifraga)*	No	
				Hamburg root parsley	No	
				Other aromatic root crops	Yes, when flowering occurs in the field	
	5.3	Medicinal herbs	-	Indian tobacco (*Lobelia inflata*)	No	
				Wooly foxglove (*Digitalis lanata*)	No	
				Heartsease *(Viola tricolor*)	No	
				German chamomile	Yes	
				Purple coneflower (*Echinacea*)	Yes	
				Pot marigold (*Calendula officinalis*)	No	
				Other medicinal herbs	Yes, when flowering occurs in the field	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory	Crops/Objects	Attractive to honeybees	Remarks
	5.4	Medicinal root crops	-	Valerian	Yes	
				Ginseng	No	
				Purple coneflower root (*Echinacea*)	Yes	
				Other medicinal root crops	Yes, when flowering occurs in the field	
	5.5	Seed herbs	-	Caraway	Yes	
				Poppy seed	Yes	
				Other seed herbs	Yes, when flowering occurs in the field	
6. Mushroom crops	6.1	Edible mushrooms		Champignon mushroom	not applicable	
				Oyster mushroom	not applicable	
				Other mushrooms	not applicable	
7. Ornamental crops	7.1	Flower bulb and Flower corm crops	7.1.1	Flower bulbs and Flower corms (cultivation for reproduction of amaryllis, dahlia, gladiolus, hyacinth, lily, narcissus, tulip, iris, crocus, other flower bulbs and corms)	Yes, when flowering occurs in the field	
			7.1.2	Bulb flower and Corm	Yes, when	

Cultivation categories, application sectors.	Crop categories, areas of application	Crop subcategory	Crops/Objects	Attractive to honeybees	Remarks
			flower Flower cultivation of amaryllis, dahlia, gladiolus, hyacinth, lily, narcissus, tulip, iris, crocus, other flower bulbs and corms	flowering occurs in the field	
	7.2	Floriculture crops	Pot plants (including annual bedding plants)	Yes, when flowering occurs in the field	
			Cut flowers (including summer flowers, dried flowers, bulb flowers and corm flowers)	Yes, when flowering occurs in the field	
			Forced shrubs	Yes, when flowering occurs in the field	
			Cut green	No	
	7.3	Tree nursery crops	Avenue trees	Yes, when flowering occurs in the field	
			Climbing plants	Yes, when flowering occurs in the field	
			Roses (including rose stocks and outdoor roses)	Yes	
			Conifers	No	
			Ornamental shrubs	Yes, when flowering occurs in the field	

Cultivation categories, application sectors.	Crop categories, areas of application	Crop subcategory	Crops/Objects	Attractive to honeybees	Remarks
			Christmas trees	No	
			Heather	Yes	
			Forest trees and hedging plants	Yes, when flowering occurs in the field	
			Fruit trees and shrubs (including Fruit tree stocks)	Yes	
	7.4	Perennial crops		Yes, when flowering occurs in the field	
	7.5	Flower seed crops		Yes	
	7.6	Marsh and Water plants		Not applicable	
	7.7	Plant breeding crops and basic seed production for arable, vegetable and fruit crops, herbs and ornamental crops.		Yes	Most of these crops are attractive to honeybees
8. Public green spaces	8.1	Grass vegetation	Lawn (including grass sods)	No, unless flowering weeds are present	This is the case when more than two flowering weeds per square meter are present
			Playing field (including grass sods)	No, unless flowering weeds are present	This is the case when more than two flowering weeds per square meter are present

Cultivation categories, application sectors.	Crop categories, areas of application	Crop subcategory	Crops/Objects	Attractive to honeybees	Remarks
			Sports field (including golf courses and grass sods)	No, unless flowering weeds are present	This is the case when more than two flowering weeds per square meter are present
			Grassy verges	No, unless flowering weeds are present	This is the case when more than two flowering weeds per square meter are present
	8.2	Woody plantings	Avenue and border trees	Yes, when flowering occurs in the field	
			Shelter belts, windbreaks and protective hedgerows	Yes, when flowering occurs in the field	Depending on the species and the pruning practice
			Other woody plantings (forest trees and verge plantings)	Yes, when flowering occurs in the field	Depending on the species and the pruning practice
	8.3	Herbaceous plantings		Yes, when flowering occurs in the field	
9. Forestry	9.1	Deciduous trees		Yes, when flowering occurs in the field	
	9.2	Coniferous trees		No	
10. Plant	10.1	Temporarily uncultivated	Deforestation area	Not applicable	

Cultivation categories, application sectors.	Crop categories, areas of application	Crop subcategory	Crops/Objects	Attractive to honeybees	Remarks
free area		terrain			
			Temporarily uncultivated land.	Yes, when flowering occurs in the field	
			Buffer areas of fields	Yes, when flowering occurs in the field	This is the case when more than two flowering weeds per square meter are present
	10.2	Permanently uncultivated land	Closed surfaces (hardened surface without joins, e.g. asphalt, concrete)	Not applicable	
			Half open surfaces (Surfaces made of paving, blocks or slabs, with joins (e.g. paving stones on pavements and roads, dual-layer porous asphalt [ZOAB])	Not applicable	
			Open surfaces (Poured or water-permeable material (e.g. gravel, shells or grass concrete tiles)	Not applicable	
			Unmetalled	Not applicable	
11. Water courses	11.1	Bank (dry or otherwise)		Not applicable	
	11.2	Dry ditches		Not applicable	

Cultivation categories, application sectors.	Crop categories, areas of application		Crop subcategory	Crops/Objects	Attractive to honeybees	Remarks
	11.3	Water courses carrying water			Not applicable	
	11.4	Maintenance paths for water courses			Not applicable	
	11.5	Ponds			Not applicable	Littoral plants are frequently foraged on
12. Reed and osier crops				Osier (dry and wet crops)	Not applicable	
				Reed		
13. Refuse heaps					Not applicable	
14. In and around the house, private home environment	14.1	Ornamental garden			Yes, when flowering occurs in the field	
	14.2	Vegetable gardens			Yes, when flowering occurs in the field	
	14.3	House plants			Not applicable	
	14.4	Container plants			Yes	
	14.5	Lawns			No, unless flowering weeds are present	This is the case when more than two flowering weeds per square meter are present

Cultivation categories, application sectors.	Crop categories, areas of application	Crop subcategory	Crops/Objects	Attractive to honeybees	Remarks
	14.6	Pastures		No, unless flowering weeds are present	This is the case when more than two flowering weeds per square meter are present
	14.7	Open surfaces (e.g. gravel, shells)		Not applicable	
	14.8	Half-open surfaces (e.g. paving stones on pavements and roads)		Not applicable	
	14.9	Closed surfaces (e.g. concrete)		Not applicable	
	14.10	Unmettaled terrain		Not applicable	
15. Disinfectants			Agricultural and horticultural equipment, tools and materials (On condition that combatting plant pathogens is claimed, otherwise biocide.)	Not applicable	

H. LANDSCAPE-LEVEL EXPOSURE ASSESSMENT OF THE AVERAGE CONCENTRATION ENTERING THE HIVE

Landscape-level exposure assessment model

Let us consider a foraging area of a hive that consists of N different fields. The average concentration in the hive (PEC_{hive}) can then as a first approximation be estimated with

$$PEC_{hive} = \frac{\sum_{n=1}^{N} f_n a_n PEC_n}{\sum_{n=1}^{N} f_n a_n} \quad \text{(Eqn H1)}$$

where f_n is the attractiveness factor of the crop in field n, a_n is the surface area of field n and PEC_n is the concentration in nectar and pollen in field n. The definition of f_n can be illustrated with the example of a foraging area consisting of two fields of equal size, one grown with *Phacelia* and one grown with pumpkin. Let us further assume that $f_{Phacelia} = 10$ and $f_{pumpkin} = 1$. Eqn H1 reduces in this case into

$$PEC_{hive} = \frac{10PEC_{Phaselia} + PEC_{pumpkin}}{11} \quad \text{(Eqn H2)}$$

So the attractiveness factor is a quantitative measure of the attractiveness of different crops and can best be defined in relation to a reference crop (e.g. pumpkin as was done in the example of Eqn H2). This factor can be measured by counting the number of foraging bees within a surface area of e.g. 1 m^2 at the same time in different fields within the foraging area. Typical values are 25 m^{-2} for *Phacelia* and 3 m^{-2} for a flowering pumpkin crop (these numbers would then correspond to $f_{Phacelia} = 8.333$ and $f_{pumpkin} = 1$, taking pumpkin as the reference crop; we use in the example 10 instead of 8.33 to keep the numbers simple).

Let us consider the most normal situation for the exposure assessment: use of a certain substance in a single crop in a foraging area. Let us further define φ as the fraction of the crop treated with this substance (e.g. because there are different products used for the same pest) and A_g as the total surface area grown with crop g (so the sum of all a_n values of the fields grown with the same crop g). In such a case, Eqn F1 reduces to

$$PEC_{hive} = \frac{f_x A_x \varphi PEC_x}{\sum_{g=1}^{G} f_g A_g} \quad \text{(Eqn H3)}$$

where f_x is the attractiveness factor of the treated crop, A_x is the total surface area of crop x in the foraging area, PEC_x is the concentration in nectar or pollen in the treated crop, G is the total number of attractive plants in the foraging area, f_g is the attractiveness factor of plant g. If there are attractive plants that are no crops (e.g. weeds in field margins), these can of course also be included in the sum in the denominator of Eqn H3.

Based on Eqn H3 we can define Φ as the 'foraging dilution factor' for crop x and this hive as:

$$\Phi = \frac{PEC_x}{PEC_{hive}} = \frac{f_x A_x \varphi}{\sum_{g=1}^{G} f_g F_g} \quad \text{(Eqn H4)}$$

If Φ is for example 0.3, this means that the average concentration in pollen or nectar entering the hive is 0.3 times the concentration in pollen or nectar from fields treated with this substance.

Effect of the foraging surface area on the risk assessment

The foraging surface area of a hive is not exactly known so it is useful to know which role this surface area may play in the risk assessment. Any risk assessment for organisms is based on two types of exposure assessment: one for the exposure in the effect study and one for the exposure that will occur in the field resulting from the use of the Plant Protection Product (Boesten et al., 2007). Let us first consider the exposure in the field. Let us consider the use of a substance in oil seed rape applied at a rate of 1 kg/ha and the resulting concentration in the nectar entering the hives at the edges of treated field. Let us assume the following scenario: (1) 25% of the surface area in the landscape is grown with oil seed rape, (2) this substance is applied to half of the oil seed rape fields, (3) there are no other attractive plants in the landscape, (4) the concentration in the nectar of treated fields is 1 mg/kg. Eqn H3 gives then a PEC_{hive} of 0.5 mg/kg because only 50% of the oil seed rape surface area is treated (φ = 0.5). The size of the foraging surface area has no effect on the PEC_{hive} in this scenario because we assume that the land use does not change.

Let us now consider exposure in the higher-tier field study. Let us consider therefore the following simplified example: the highest-tier Regulatory Acceptable Concentration for the hive (RAC_{hive}) was based on a field study with a hive at the edge of a 1-ha *Phacelia* field that was treated with the substance and in which no unacceptable effects were observed. If the concentration in nectar entering the hive was measured in the field study, we do not need any assumptions on the foraging surface area. So in this case such assumptions play no role in the risk assessment.

However, if this concentration was not measured (as is the case in many current dossiers), the RAC_{hive} has to be calculated from Eqn F1. Let us assume the same landscape scenario: 25% of surface area is grown with attractive oil seed rape plants (now untreated) with 1 ha of a *Phacelia* field treated at a rate of 1 kg/ha close to the hive. We assume that the concentration in the nectar of the *Phacelia* is again 1 mg/kg. Let us assume $f_{Phacelia}$ = 10 and f_{OSR} = 1. For a total foraging area of 10 ha, Eqn H1 gives then RAC_{hive} = 10/(10+2.5) = 0.80 mg/kg. However, for a total foraging area of 100 ha, Eqn H1 gives RAC_{hive} = 10/(10+25) = 0.29 mg/kg. Figure H1 illustrates this strong dependence of the RAC_{hive} of the foraging surface area. We consider a foraging radius of 1 km to be a defensible minimum value for a hive. This corresponds to about 3 km^2, so 300 ha. Figure H1 indicates that it is well possible that the exposure in such a *Phacelia* effect study is considerably lower than in a realistic field exposure scenario.

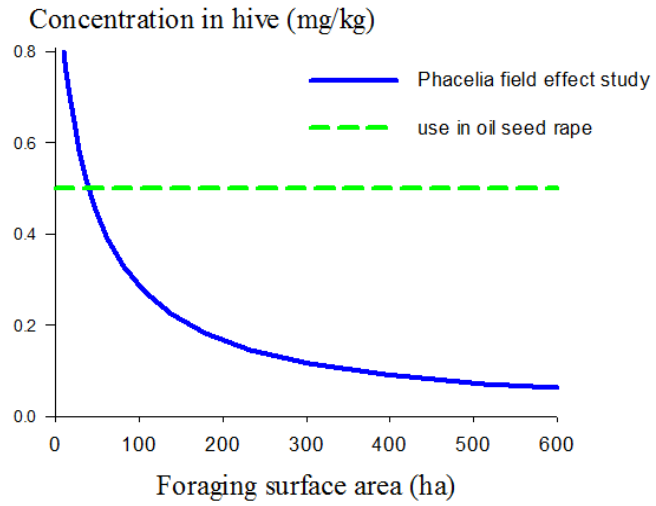


Figure H1: Concentration in the nectar entering the hive as a function of the foraging surface area as calculated with Eqn H1 for an application in oil seed rape and for an application in a *Phacelia* field effect study. It was assumed that the PEC in the treated Phacelia and oil seed rape fields was 1 mg/kg.

Figure H1 shows that the RAC_{hive} decreases with increasing foraging surface area for field studies in which the concentrations in pollen and nectar have not been measured. The lower the RAC_{hive}, the more conservative the risk assessment will be. So to be able to use such studies, consensus needs to be achieved on a realistic upper limit of a foraging surface area of a hive. Moreover, the surface area of attractive crops within this foraging surface area during the field effect study needs to be assessed. This will in general not be an easy task. It seems therefore advisable to measure the concentrations in nectar and pollen entering the hive in future field effect studies.

I. PESTICIDE RESIDUE LEVELS IN NECTAR AND POLLEN AND THE RESIDUE UNITE DOSES (RUDS)

Three sources of data were considered to compile a data set for RUD (residue unit dose) values.

- Appendix G of the EFSA Opinion (EFSA, 2012a)
- Table 1.5, Table 1.6 and Table 1.8 of the external scientific report (EFSA, 2012c)
- The data in the excel sheet compiled for the EFSA statement (EFSA, 2012d). Detailed data were not published in the statement, therefore references are provided for these data in Table 2 of this appendix.

Moreover a very few data that were erroneously left out from one or the other data base, were used here. In order to avoid double counting, the references of the studies in the data bases were checked and overlapping data were considered only once here. Where necessary, further details of the relevant studies, where available to EFSA, or the original study reports were consulted for further information or correction (e.g. several RUD values for thiamethoxam and CGA322704 were reported in Table G11 of the Opinion (EFSA, 2012a), but ignored here since they were based on results of < LOD). In some cases different RUD values from the same origin were reported in two different data sets (e.g. one based on average of subsamples while the other on the highest value). Where reliable information was available, the worst case (e.g. the highest measured) residue value was used for the RUD calculations. From a study, sometimes more than one value was derived when more than one trial was conducted within a study. A stand-alone trial was defined when one or more of the following factors were different from other trials: plant, test site, time of the trial, application rate, pre-treatment of the soil. When several measurements of residues for the same matrix were available within a trial, only the highest value was used for the RUD calculation. In some cases the only differences were in the time of application with a few days difference. In these cases the data from the trial with the worst case value was only considered further.

Two reported values were derived from greenhouse studies. It was considered that the residues determined in this studies cannot be combined with the residues investigated in field or semi-field trials, therefore, these greenhouse data were not used in the data analysis and are not reported here (all other values originate from open field trials).

Where the residue detected in a trial was reported to be between the limit of quantification (LOQ) and the limit of detection (LOD), as a worst case assumption, the residue was considered to be equal to the LOQ for the calculations. When the exact value measured between the LOD and the LOQ was reported than this reported value was used in the calculations.

In cases when toxic metabolites were also identified in nectar or pollen, the residue levels were summed with the residue level of the parent and the RUD values were derived from this combined value. It should be noted that in these cases, the highest reported values were always used. Results from subsamples were not considered separately, which may mean that the combined residue originates from different subsamples (but from the same trial). Since metabolites were investigated only for a few parent molecules, this was only done in a limited number of cases; only for thiamethoxam where metabolite CGA322704 (=clothianidin) was summed with parent thiamethoxam. This approach is considered as a worst case approach, especially in cases where residue levels equal with the LOQ were considered in the calculations, while the actually measured levels were below the LOQ (as explained above). Olefine- and the monohydroxy metabolites of imidacloprid were not detected in the available studies, therefore not considered here. Metabolites of clothianidin TZMU and TZNG were also not considered in the RUD calculations, since these molecules are more than thee order of magnitude less toxic to bees[8] than the parent clothianidin. A single value is available for the metabolite CGA322704. In this trial the parent compound was not detected.

The compiled RUD values derived from foliar spray applications are reported in Table I1 of this Appendix, while the RUD values derived from seed dressing applications are reported in Table I2.

[8] Based on the acute oral LD_{50} values as reported in the DAR of clothianidin (Belgium, 2003)

3990 Regarding seed dressing (Table I2), two sets of data were calculated. One is based on the seed loading
3991 and the values refer to the theoretical seed dressing rate of 1 mg a.s./seed, and the other set of data is
3992 based on application rate expressed in applied mass per area. These later values refer to the theoretical
3993 application rate of 1 kg a.s./hectare. All values in Table I1 refer to the theoretical application rate of 1
3994 kg a.s./hectare.
3995 The cumulative distributions of the RUD values are visualised in Figures I1 to I6 of this Appendix.
3996
3997 **Table I1:** RUD values referring to an application rate of 1 kg a.s./hectare derived from foliar spray
3998 applications

Compound	Crop	RUD (mg/kg) pollen	RUD (mg/kg) nectar	Reference	Data source
acephate + methamidophos	raspberry	-	20.7	Fiedler, 1987	esr
acephate + methamidophos	cherry	-	4.1	Fiedler, 1987	esr
acephate + methamidophos	apple	-	11.3	Fiedler, 1987	esr
acetamiprid	rape	14.8		Rexer, 2010, S10-01355	
acetamiprid	rape	3.4		Rexer, 2010, S10-01355	
azoxystrobin	rape		5.8	Schatz, Wallner, 2009	op
boscalid	rape		1.0	Schatz, Wallner, 2009	op
boscalid	rape		6.4	Schatz, Wallner, 2009	op
boscalid	rape	52.4	2.9	Wallner, 2009	op/esr
captan	apple	9.5		Kubik et al. 2000	esr
carbaryl	alfalfa	0.2	-	Stanger and Winterlin, 1975	esr
carbendazim met.	rape	-	1.3	Schatz, Wallner 2009	op
carbofuran	maize	0.0(1)	-	Data from DAR	op
carbofuran	alfalfa	10.5	-	Moffett et al., 1986	esr
carbofuran	alfalfa	4.1	-	Moffett et al., 1986	esr
chlorantraniprole	phacelia	43.0	0.6	Dinter et al., 2009	esr
cypermethrin	rape	43.1	-	Fries and Wibran, 1987	esr
difeconazole	apple	0.8	-	Kubik et al., 2000	esr
difeconazole	apple	0.2	-	Skerl et al., 2009	esr
dimethoate	lemons	-	1.4	Waller et al., 1984	esr
dimoxystrobin	rape	-	1.7	Schatz, Wallner 2009	op
endosulfan	mustard	4.2	3.5	Choudhary and Sharma, 2008	esr/op
endosulfan	mustard	4.1	3.1	Choudhary and Sharma, 2009	esr/op
ethylparathion	sunflower	3.4	-	Cox et al., 1986	esr
flufenoxuron	phacelia	18.3	-	Data from DAR	op
flufenoxuron	phacelia	90.5(2)	2.0	Data from DAR	op
flufenoxuron	phacelia	8.0	-	Data from DAR	op
flufenoxuron	grape	1.5	-	Data from DAR	op
fluvalinate	rape	-	12.5	Schatz, Wallner 2009	op
fluvalinate	apple	1.8	-	Haouar et al., 1990	esr
gamma-cyhalothrin	rape	21.3	2.3	Barth et al., 111048020 B	op
iprodione	rape	-	5.7	Schatz, Wallner 2009	op
iprodione	cherry	0.3(3)	-	Kubik et al., 1999	esr
lambda-cyhalothrin	mustard	22.3	11.4	Choudhary and Sharma, 2008	esr/op
lambda-cyhalothrin	mustard	21.5	11.1	Choudhary and Sharma,	esr/op

Compound	Crop	RUD (mg/kg) pollen	RUD (mg/kg) nectar	Reference	Data source
				2009	
metconazol	rape	-	3.7	Schatz, Wallner 2009	op
methyl-parathion	alfalfa	2.0	-	Moffett et al., 1986	esr
methyl-parathion	alfalfa	2.1	-	Moffett et al., 1986	esr
methyl-parathion	alfalfa	11.8	-	Johansen and Kious, 1978	esr
methyl-thiophanate	cherry	1.2	-	Kubik et al., 1999	esr
monocrotofos	alfalfa	0.5	-	Stanger and Winterlin, 1975	esr
PP321 (pyrethroid)	rape	40.0	-	Fries and Wibran, 1988	esr
procymidon	strawberry	0.04		Kubik et al., 1992	esr
prothioconazole	rape	-	0.1	Schatz, Wallner	op
prothioconazole	rape	-	2.8	Wallner, 2009	op/esr
spiromesifen	mustard	9.3	6.5	Choudhary and Sharma, 2008	esr/op
spiromesifen	mustard	8.1	6.3	Choudhary and Sharma, 2009	esr/op
Sum TP+C	rape		2.3	Schatz, Wallner 2009	op
teflubenzuron	rape	21.7	0.9	Data from DAR	op
teflubenzuron	rape	149.8	-	Data from DAR	op
thiacloprid	rape	-	0.5	Schatz, Wallner 2009	op
thiacloprid	apple	0.9	-	Skerl et al., 2009	esr
thiophanat-methyl	rape	-	1.0	Schatz, Wallner 2009	op
vinclozolin	cherry	4.1	-	Kubik et al., 1992	esr

Number of data	37	28
Lowest value	0.0002	0.1429
Median value	4.2	3.0
90th % value	43.0	11.3
95th % value	60.0	12.1
Highest value	149.8	20.7

Legend: -: no value or no reliable value for RUD calculation
op: EFSA Opinion (EFSA, 2012a)
esr: External Scientific Report (EFSA, 2012c)

Notes: (1): The exact value is 0.0002417 mg/kg
(2): The value was considered unrealistic by the study authors based on the fact that the results of the other subsamples of the same trial gave considerable lower residue concentrations. No other reasoning was given, therefore, as a worst case assumption, this value was considered here.
(3): 2 applications were performed

Table I2: RUD values referring to an application rate of 1 mg/seed or 1 kg a.s./hectare derived from seed dressing applications

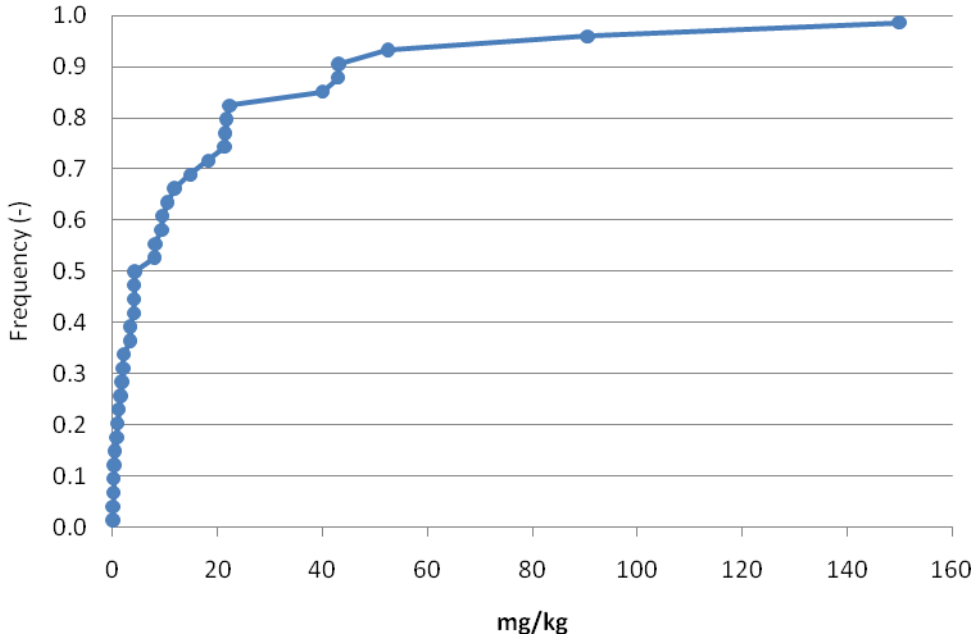
Compound	Crop	RUD (mg/kg) based on seed dressing rate		RUD (mg/kg) based on app-lication rate		Reference[1]	Data source
		pollen	nectar	pollen	nectar		
CGA322704	rape	-	0.056	-	0.056	L	op
clothianidin	rape	-	-	-	0.111	1	op

Compound	Crop	RUD (mg/kg) based on seed dressing rate		RUD (mg/kg) based on application rate		Reference[1]	Data source
		pollen	nectar	pollen	nectar		
clothianidin	rape	-	-	0.093	0.200	2	op
clothianidin	rape	0.002	0.002	0.020	0.020	7	op
clothianidin	rape	-	-	0.082	0.173	9	op
clothianidin	rape	-	-	0.066	-	10	op
clothianidin	rape	-	-	0.034	0.020	11	op
clothianidin	rape	-	-	0.071	0.088	12a	op
clothianidin	rape	-	-	0.093	0.037	12b	op
clothianidin	sunflower	0.011	-	0.122	-	3	op
clothianidin	sunflower	0.010	-	0.114	-	4	op
clothianidin	maize	-	-	0.083	-	Nikolakis et al., 2009	op
clothianidin	maize	-	-	0.115	-	8	op
clothianidin	maize	-	-	0.054	-	8b	op
clothianidin	maize	0.008	-	-	-	Staedtler T., 2009	st
clothianidin	maize	0.004	-	-	-	Ch. Maus et al, 2005 (E 319 2902-6)	st
clothianidin	maize	0.004	-	-	-	Ch. Maus et al, 2006 (E 319 2902-6)	st
clothianidin	maize	0.003	-	-	-	Ch. Maus et al, 2007 (E 319 2903-7)	st
clothianidin	maize	0.003	-	-	-	Ch. Maus et al, 2007 (E 319 2903-7)	st
clothianidin	rape	0.086	0.074	-	-	Cutler and Scott-Dupree, 2007	esr
clothianidin	rape	-	0.05	-	-	Wallner, 2009	esr
clothianidin	maize	0.007	-	-	-	Kruype, Hunt et al., 2012	esr
imidacloprid	rape	-	-	0.156	0.017	11	op
imidacloprid	maize	0.006	-	0.056	-	5	op
imidacloprid	maize	0.006	-	0.056	-	6	op
imidacloprid	rape	-	-	0.149	0.149	7	op
imidacloprid	rape	-	-	0.069	0.069	8	op
imidacloprid	rape	-	-	-	0.159	9	op
imidacloprid	sunflower	0.036	-	-	-	Laurent and Rathahao, 2003	esr
imidacloprid	maize	0.002	-	-	-	Bonmatin et al., 2005	esr
imidacloprid	sunflower	0.004	-	-	-	Bonmatin et al., 2005	esr
imidacloprid	sunflower	0.015	-	-	-	Bonmatin et al., 2003	esr

Compound	Crop	RUD (mg/kg) based on seed dressing rate		RUD (mg/kg) based on application rate		Reference[1]	Data source
		pollen	nectar	pollen	nectar		
imidacloprid	maize	0.003	-	-	-	Bonmatin et al., 2003, 2007	esr
thiamethoxam	rape	0.263	0.131	0.162	0.081	F	op
thiamethoxam	sunflower	0.006	-	0.039	-	H	op
thiamethoxam	sunflower	0.013	--	0.145	-	I	op
thiamethoxam	rape	-	-	0.242	-	Hargreaves N., 2007 (T003253-05-REG)	st
thiamethoxam	maize	0.002	-	-	-	Kruype et al., 2012	esr
thiamethoxam	maize	0.013	-	-	-	AFSSA 2007	esr
thiamethoxam + CGA322704	rape	0.2875	-	0.148	-	M	op
thiamethoxam + CGA322705	rape	0.05	0.005	0.033	0.032	O	op
thiamethoxam + CGA322706	maize	0.022	-	0.213	-	Hecht-Rost S., 2007 (20051149/F1-BZEU)	st
thiamethoxam + CGA322707	maize	0.005	-	0.047	-	Hecht-Rost S., 2007 (20051149/F1-BZEU)	
thiamethoxam + CGA322708	maize	0.015	-	0.155	-	Hecht-Rost S., 2007 (20051149/F2-BZEU)	st
thiamethoxam + CGA322709	maize	0.012	-	0.130	-	Hecht-Rost S., 2007 (20051149/F2-BZEU)	
thiamethoxam + CGA322710	maize	-	-	0.079	-	Hargreaves N., 2007 (T003256-05-REG)	st
thiamethoxam + CGA322711	maize	-	-	0.045	-	Hargreaves N., 2007 (T003256-05-REG)	st
thiamethoxam + CGA322712	rape	-	-	0.574	-	Hecht-Rost S., 2007 (20051040/F2-BZEU)	st
Number of data		28	6	30	14		
Lowest value		0.0020	0.0024	0.0201	0.0166		
Median value		0.0077	0.0528	0.0879	0.0751		
90th % value		0.0608	0.1026	0.1667	0.1687		
95th % value		0.2007	0.1169	0.2288	0.1822		
Highest value		0.2875	0.1313	0.5739	0.2000		

Legend: -: no value or no reliable value for RUD calculation
op: EFSA Opinion (EFSA, 2012a)
esr: External Scientific Report (EFSA, 2012c)
st: EFSA statement (EFSA, 2012d)

Note: [(1)]: Where a letter or figure appears in the column, see for reference in the data source



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4024 **Figure I1:** Cumulative frequency distribution of peak RUD values derived from the application rate
4025 (mass/area) for pollen after spray applications. RUD values refer to application rate of 1 kg/hectare.

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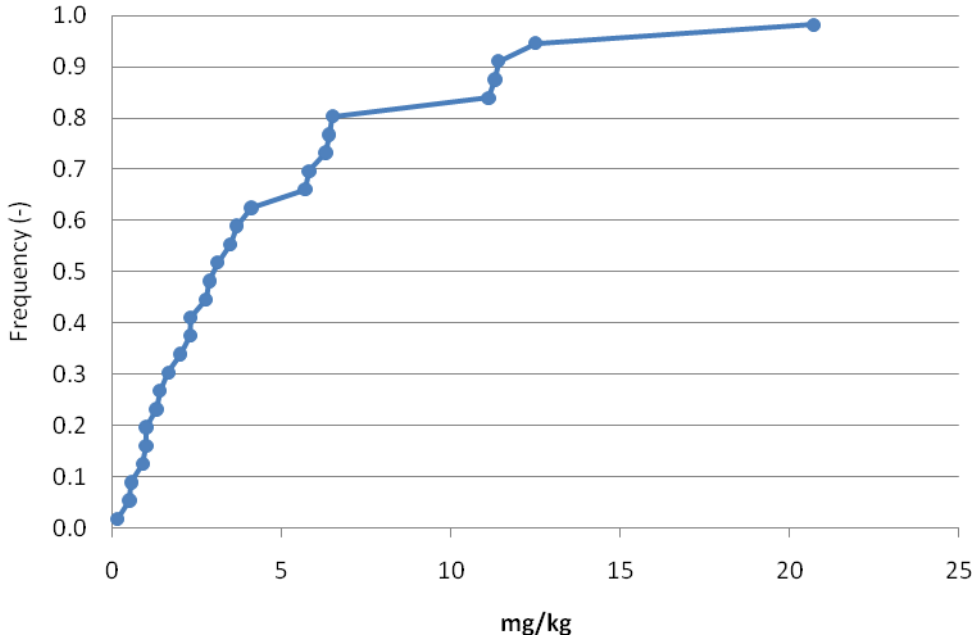

4027
4028 **Figure I2**: Cumulative frequency distribution of peak RUD values derived from the application rate
4029 (mass/area) for nectar after spray applications. RUD values refer to application rate of 1 kg/hectare.
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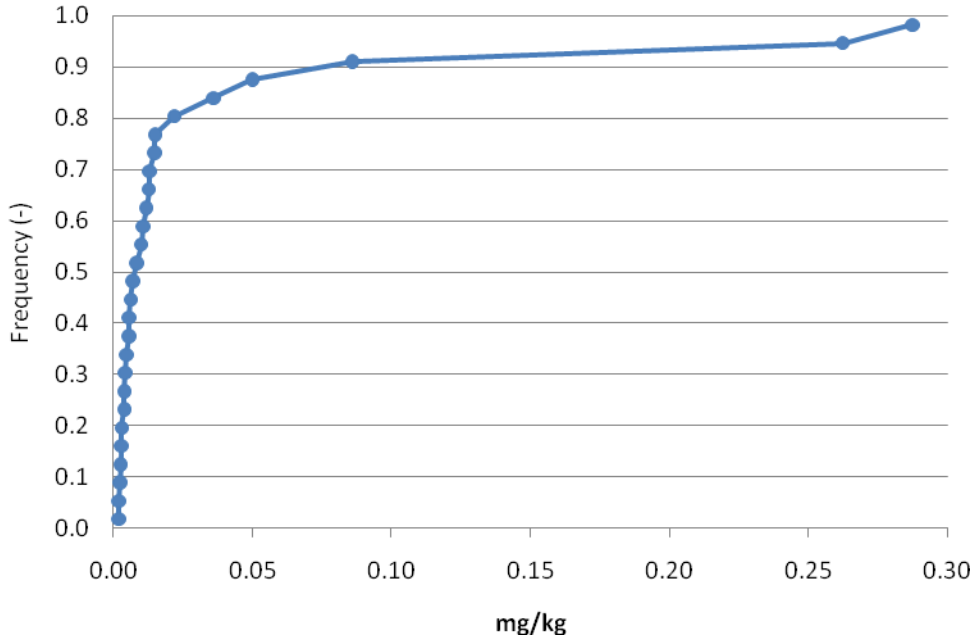

Figure I3: Cumulative frequency distribution of peak RUD values derived from the seed loading rate for pollen after seed applications. RUD values refer to application rate of 1 mg/seed.

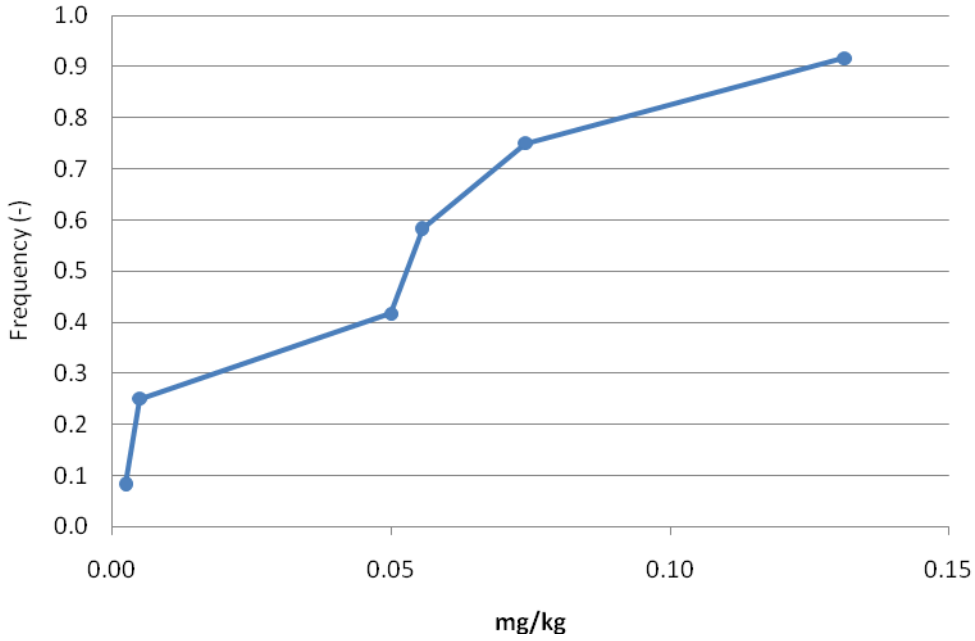

Figure I4: Cumulative frequency distribution of peak RUD values derived from the seed loading rate for nectar after seed applications. RUD values refer to application rate of 1 mg/seed.

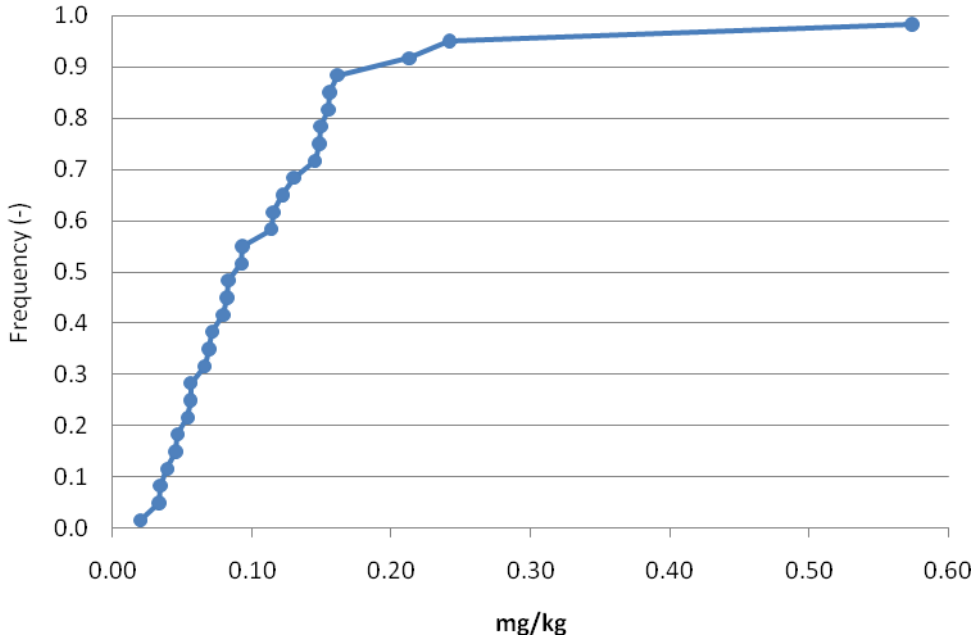

Figure I5: Cumulative frequency distribution of peak RUD values derived from the application rate (mass/area) for pollen after seed applications. RUD values refer to application rate of 1 kg/hectare.

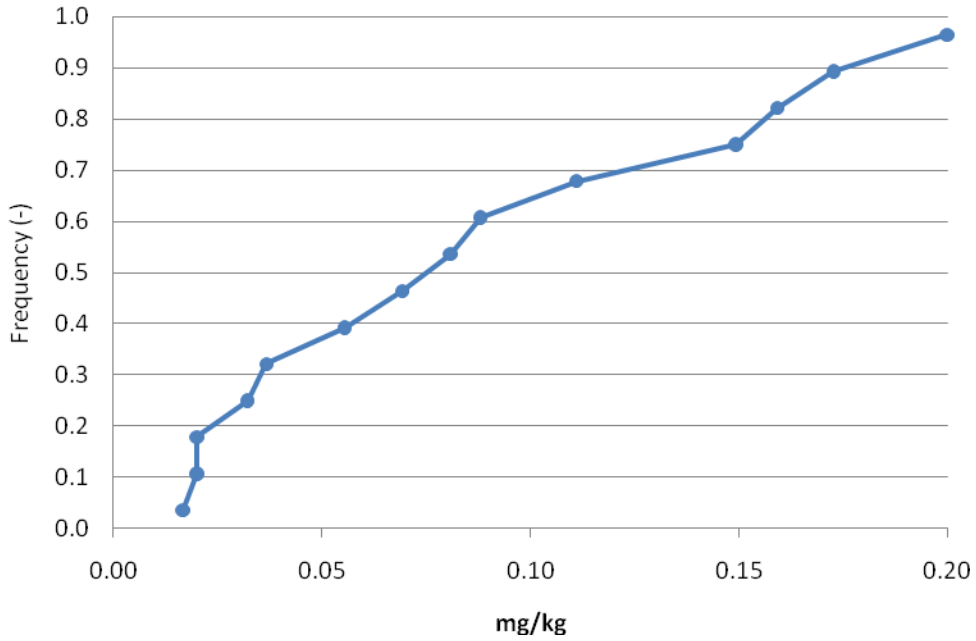

Figure I6: Cumulative frequency distribution of peak RUD values derived from the application rate (mass/area) for nectar after seed applications. RUD values refer to application rate of 1 kg/hectare.

J. PROTOCOL FOR PERFORMING FIELD STUDIES TO ASSESS A CERTAIN PERCENTILE OF THE CONCENTRATION IN POLLEN AND NECTAR IN A CERTAIN TYPE OF PLANTS IN THE AREA OF USE OF THE SUBSTANCE.

In a number of the exposure flow charts there is a higher-tier option to assess the concentration in nectar and pollen under realistic field conditions. This is the case for the flow charts:
--- the treated crop after spray applications, seed treatments or granule applications (Figures 2 and 9)
--- permanent crops in the year after spray applications or granule applications (Figure 6)
--- succeeding annual crops after spray application, seed treatments or granule applications in the treated crop (Figure 7).

The aim of such experiments is to assess a certain spatial percentile of the peak concentration in nectar and pollen for the area of use of a substance for a certain use of application (e.g. spraying of a dosage of 0.5 kg/ha in cherries two weeks before flowering). The procedure is to measure these concentrations at a number of locations which is the most direct assessment of these concentration that is possible.
In view of time limitations we are unable to provide guidance at a very detailed level. Therefore we recommend to use the principles provided in earlier guidance documents on related subjects (DG Agriculture, 1997; OECD, 2007, 2009; DG SANCO, 2009, 2011) keeping of course the aim of the study in mind.

DG SANCO (2009) proposes the following residue definition for monitoring and risk assessment for honey: the sum of parent and all metabolites included in the residue definition for monitoring in plants and animal products. Since not much experience has been gained until now, it is proposed to adopt this proposal. The sensitivity (i.e. limit of quantification and detection) of the analytical methods that are used in the residue studies should be checked in order to ensure that they are low enough to detect residue levels that exert toxic effects to honeybees.

Sampling times depend on the purpose of the study. In case of spray or granule applications before flowering of the plant, sampling can start of course only after flowering has started. In case of spray or granule applications during flowering, sampling has to start one day before application of the substance and has to be performed immediately after application and 1, 3, 6 and 10 days after application. In case of measurements in permanent crops one year after application or in succeeding annual crops or in case of 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.

The selection of the locations and the number of locations has to be tailored to the purpose of the study, i.e. to assess a certain spatial percentile in the area of use of the substance. In general the locations should be distributed over the area of use. The number of locations should ensure that the required percentile is assessed with enough certainty and this should be demonstrated with a statistical analysis. E.g. in case of a 90^{th} percentile we propose to perform studies at least five randomly selected locations in the area of use of the substance and to derive the 90^{th} percentile from the frequency distribution of this sample population (the highest of five ranked values is the 90^{th} percentile). The statistical analysis should assess the confidence interval of the required spatial percentile. The required certainty is of course also related to the margin of safety that is available in this tier in the flow chart. E.g. if the Regulatory Acceptable Concentration (RAC) in nectar is 1.0 mg/kg and measurements at five locations distributed over the area of use (perform to assess a 90^{th} percentile) show nectar concentrations of 0.01, 0.03, 0.05,0.07 and 0.09 mg/kg, then the details of the statistical analysis will hardly matter. However if the measurements give 0.1, 0.3, 0.5, 0.7 and 0.9 mg/kg, then these details will of course matter. So for wide safety margins, a large uncertainty in the spatial percentile may be no problem whereas this uncertainty needs to be analysed in detail for small safety margins.

This guidance refers to concentrations in nectar and pollen for the different types of plants. As described in Section 3.1.6, this is based on a conservative approach not considering the dilution of these concentrations in the hives. In view of our recommendation to include this dilution in the exposure assessment in the foreseeable future, notifiers may consider to limit measurements not only to the concentrations in the plants but to include also measurements in hives located at the edge of treated fields.

K. ASSESSMENT OF SPRAY DRIFT AND DUST DRIFT DEPOSITION ONTO FIELD MARGINS AND ADJACENT FIELDS

Introduction

In this Guidance Document deposition of sprays and dust outside the treated field (field margins or adjacent crops) has to be assessed at several places. This appendix describes how this should be done.

Based on EFSA (2004) we use the following terminology:
- drift is the process by which liquid or solid particles are carried out of the treated area by wind or the air stream of the application equipment,
- spray drift is drift of liquid particles applied via a spray boom,
- dust drift is drift of solid particles released during non-spray applications (seed treatments or granules).

The target of the exposure assessment for the field margin is the average deposition onto attractive plants in the whole field margin of a treated field because there are a priori no reasons to assume that foragers from a hive at the edge of the treated field would preferably forage more on contaminated parts of the field margin than on non-contaminated parts (e.g. because they were upwind during application). Similarly the target for the adjacent crop is the average deposition onto the whole adjacent crop field because there are a priori no reasons to assume that foragers from a hive at the edge of the treated field would preferably forage more on the contaminated strip of the adjacent crop that is closest to the treated field.

Both spray and dust drift deposition decreases with the distance from the treated field. So the downwind width of the margin or the adjacent field will influence the average deposition. We propose tentatively a width of 2 m for the field margin and of 50 m for the adjacent field and consider these to be conservative values. We recommend to underpin or refine these 2 and 50 m by geostatistical analyses.

We use the geometry as shown in Figure K1 as a conceptual model for the effect of the wind angle on the average deposition: field margins will usually surround the whole field and an adjacent crop will usually be only on one side of the treated field. We recommend to perform geostatistical analyses to underpin or refine this simplified geometry.

Figure K1: Simplified geometries of (left) a combination of treated crop and a field margin and (right) a combination of a treated crop and an adjacent crop.

In the EU assessment of the spray drift deposition onto field margins for non-target terrestrial organisms, the first 1 and 3 m of the off-field area is ignored for field and fruit crops, respectively. This is based on risk management considerations. However in our assessment of the spray and dust drift deposition in the field margin it is not defensible to ignore these first 1 and 3 m because the bees do not know that they should avoid sampling of these plants.

Spray drift deposition

Field margins

Assessment of the spray drift deposition onto field margins is needed for the flow chart in Figure 4. This section describes how this should be done.

Spray drift deposition is strongly influenced by the spray drift equipment, the wind angle and the wind speed at the time of application (van de Zande et al., 2012). Spray drift deposition measurements are usually carried out downwind of treated fields along lines whose angle with the wind direction is less than 30^o, so considering only 60^o of the in total 360^o. Deposition upwind can be considered negligibly small (180 of the 360^o) and deposition onto the remaining 120^o downwind will be smaller than for the directions whose angle with the wind direction is less than 30^o (Van de Zande et al., 2012). So the average deposition on field margins surrounding a rectangular field will be between 1/6 and ½ of deposition measured in directions whose angle with the wind direction is less than 30^o. As a best guess we propose to assume 1/3 (average of 1/6 and ½). This best guess needs of course further underpinning or refinement. Therefore we recommend to perform a modelling study in which the spray drift deposition onto field margins is simulated as a function of a stochastic wind angle and a stochastic wind speed from which the 90^{th} percentile spray deposition case can be derived (see van der Zande et al., 2012, for an example of such a study for spray deposition on surface water). This modelling study should also consider the effect of repeated applications because these probably influence the assessment of the 90^{th} percentile case (van der Zande et al, 2012).

Candolfi et al. (2001) recommended to use spray drift tables by BBA (2000) for spray deposition on field margins. These tables give deposition percentages as a function of distance from the treated field for field crops, fruit crops, grapevine, hops and vegetables. There are tables for a single application and 2-3-4-5-6-7 applications. The deposition percentages decrease with the number of applications. Van de Zande et al. (2012) made stochastic calculations on spray drift deposition onto surface water considering a stochastic wind angle and a stochastic wind speed. They showed that a decrease of the 90^{th} percentile deposition with the number of applications will only occur if the concentrations of the different applications sum up. They showed furthermore that if these concentrations do not sum up (because of rapid dissipation of the substance), the deposition percentage should increase with the number of applications because more applications give more possibilities of obtaining unfavourable meteorological conditions with respect to spray drift. Concentrations in nectar and pollen in plants show usually rapid dissipation after spray applications (EFSA, 2012a). So the decreasing drift deposition with increasing number of applications as recommended by Candolfi et al. (2001) seems not defensible; instead the drift deposition should increase with the number of applications.

Furthermore the drift deposition tables from BBA (2000) were based only on measurements in Germany and there have been significant developments in the field of harmonisation of drift deposition in the EU (Huijsmans & van de Zande, 2011). Therefore we recommend to improve the estimates of deposition of spray drift by analysing all spray drift data available within the EU. In this analysis also the effect should be considered that the plants in field margins and of the adjacent crop

may catch more drift than bare soil (most drift deposition measurements are carried out on bare soil or in a short crop).

In the absence of better alternatives, we propose for the time being the following procedure for default conservative spray drift depositions onto the field margins in boxes 1 and 3 of Figure 4: both for single and repeated applications take the spray drift deposition figures by Candolfi et al. (2001) for a single application at distance of 1 m for downward spray applications (in field crops) and at a distance of 3 m for sideward and upward applications (in fruit crops and grapevine) and multiply these figures with 1/3 to account for the effect of the wind angle on the deposition. This gives 0.9% for field crops, 10% for early fruit, 5% for late fruit, 0.9% for early grapevine, 3% for late grapevine, and 6% for hops. Given all the complications described above, we are at this moment unable to assess whether this interim solution is on the conservative or optimistic side for single or repeated applications but it is our best guess at this moment.

Adjacent crops

Assessment of the spray drift deposition onto adjacent crops is needed for the flow chart in Figure 5. This section describes how this should be done.

For the adjacent crops the geometry in Figure K1 shows that the effect of the wind angle leads to another type of statistics. For the field margin, the wind angle has no effect on the average deposition because the field margin surrounds the whole field so the angle does not matter. However, if the adjacent crop is upwind during application, there is no deposition at all. If this crop is downwind, then the wind angle may vary 180^{o} whereas the measurements are usually carried out for the 60^{o} with the highest deposition (angle with wind direction less than 30^{o}; see previous section). So for the adjacent crop the wind angle leads to a probability density function of deposition values (of which 50% are zero values considering only a single application). So if we use such measurements as a basis for the average drift deposition on the whole adjacent field, we have to be aware that these figures represent only the highest 60^{o} of the 360^{o} that are possible, so the highest 16%, ie above the 84^{th} percentile when considering the wind angle as the only stochastic variable.

To assess the exposure of the 90^{th} percentile hive, a stochastic modelling study is needed considering a stochastic wind angle and a stochastic wind speed similar to the approach described for the field margins. As indicated in Section 3.2.6, the 90^{th} percentile hive may be linked to a 50^{th} percentile spray drift case (e.g. if a relevant attractive crop is present only at the border of 20% of treated fields). So the modelling study has to calculate the full frequency distribution and a table should be generated from this from which the desired percentile spray drift deposition can be derived. The modelling study has to include repeated applications because these influence such frequency distributions (Van de Zande et al., 2012).

Box 1, 2 and 7 of the flow chart for adjacent crops (Figure 5) need default conservative spray drift deposition figures. In the absence of better information, we propose to use for the time being both for single and repeated applications the spray drift deposition figures by Candolfi et al. (2001) for a single application. For adjacent fields thus the average deposition over the first 50 m was to be derived from these figures. This resulted in 0.3% for field crops, 7% for early fruit, 3% for late fruit, 0.5% for early grapevine, 1.4% for late grapevine and 4% for hops.

As for the field margins, we are at this moment unable to assess whether this proposed interim solution is on the conservative side or on the optimistic side. However, the deposition is likely to be much less than that for the field margins because (i) the average over 50 m is less than the deposition onto a 2-m wide field margin and because (ii) only a fraction of the treated fields has downwind adjacent attractive crops at the time of application. So the spray drift assessment for the treated crop is much less critical than that for the field margins (in the short term; in the long term it may be the opposite as described in See Section 3.2.8).

Dust drift deposition

Field margins

Seed treatments

Assessment of the dust drift deposition onto field margins is needed for the flow charts in Figures 10 and 11 for the seed treatments. This section describes how this should be done.

The deposition of dust drift is the result of (i) emission and (ii) transport through the air and deposition onto the plants. So there are two questions to be addressed: (i) which factors influence dust emission from the application equipment, and (ii) which factors influence dust deposition onto the plants in the field margins ?

The dust emission is strongly influenced by (i) the sowing equipment, (ii) use of deflectors in case of pneumatic sowing, (iii) the abrasiveness of the seed coating and the granules as determined in the Heubach test, (iv) the concentration of active ingredient in the dust released in the Heubach test (EFSA, 2012a). Mechanical sowing gives much less emission than pneumatic sowing. In case of pneumatic sowing, use of deflectors decreases the emission strongly. The higher the amount of dust released in the Heubach test, the higher the emission of dust. The higher the concentration of the active ingredient in this dust, the higher the emission of the active ingredient.

Dust deposition is strongly influenced by (i) wind angle, (ii) the 'filtering capacity' of the crop. The effect of the wind angle is obvious: there will be little deposition upwind and much deposition downwind. The larger the filtering capacity the higher the deposition in the crop will be. The effect of the wind speed on the deposition is as yet unclear.

The draft SANCO Guidance Document for seed treatments provided the following conservative default dust deposition (mass of substance per surface area of the field margin expressed as percentage of the mass of substance applied per surface area of treated field): 7% for maize, 3% for oil seed rape, 4% for cereals and 0.01% for sugar beets.
The above procedure is likely to generate concentrations in nectar and pollen that are higher than the 90^{th} percentile of the specified spatial population (i.e. the hives at the edge of field grown with attractive crops that are next to and downwind of treated fields) because the wind angle is restricted to $\pm$ 30^{o} so only 30^{o} of the 180^{o} corresponding to all the downwind possibilities. Therefore we recommend to perform studies using calibrated physical models in which the dust deposition onto attractive adjacent crops is simulated as a function of wind speed and wind angle (see EFSA, 2004, for examples of such model calculations for deposition of dust on surface water). Stochastic simulations with such models can then be used to obtain a more realistic assessment of the 90^{th} percentile deposition (e.g. by multiplying the results of the proposed well-defined experiments with an appropriate factor). See van der Zande et al. (2012) for an example of a similar stochastic simulations for spray drift deposition on surface water.

In the simulation studies recommended above, also the variation between different Heubach-AI should be included (if possible) and the overall desired X^{th} percentile should be assessed considering the combined effects of variability in the Heubach-AI and wind angle and windspeed because only this combination will describe exposure of the total spatial population of hives adequately. So the simplified approach to use only the Heubach-AI value to assess the percentile ([i] in boxes 4 and 5 of Figure 10, [ii] in box 4 of Figure 11 and [iii] in boxes 6 and 7 of Figure 12) should be seen as a conservative approach which can be made more realistic when science in this field progresses.

4334 **Granule applications**
4335
4336 Assessment of the dust drift deposition from granule applications onto plants in field margins is
4337 needed in box 2 of the flow chart in Figure 13. This section describes how this should be done.
4338
4339 Also for the granule applications, the dust emission is strongly influenced by the application
4340 equipment: a spinning disc gives considerably less emission than a boom spreader (EFSA, 2004).
4341
4342 We propose to based the default conservative dust depositions from granules on simulations by EFSA
4343 (2004) for worst-case depositions onto surface water. The highest value reported by EFSA (2004) was
4344 3.2% of the dose (deposition defined as the mass of substance deposited divided by the surface area of
4345 water and dose defined as mass of substance applied per surface area of treated field). We propose to
4346 multiply with 10 to account for the filtering capacity of the plants in the field margin These factors 10
4347 and 3 are preliminary figures that should be underpinned by further research. So in combination this
4348 gives that the resulting deposition should be multiplied with 10/3. So we get 3.2 × 10/3 = 11% for the
4349 default dust deposition for granules.
4350
4351
4352 **ADJACENT CROPS**
4353
4354 **Seed treatments**
4355
4356 Assessment of the dust drift deposition from seed treatments onto adjacent crops is needed in the flow
4357 chart in Figure 12. This section describes how this should be done.
4358
4359 Based on the measurements of dust deposition as a function of distance to the treated field as shown in
4360 Figures J3 and J5 of EFSA (2012a), we propose as a conservative assumption that the dust deposition
4361 declines exponentially with distance to the treated field and that the deposition at 20 m distance is 50%
4362 lower than at the edge of the treated field. It then can be calculated that the average deposition on a 50
4363 m wide adjacent field is 48% of the deposition at the edge of the treated field. So we propose to use for
4364 the conservative dust depositions in box 3 of Figure 12 the figures provided in the draft SANCO
4365 Guidance Document for seed treatments (7% for maize, 3% for oil seed rape, 4% for cereals and
4366 0.01% for sugar beets) multiplied with 0.48; this gives 3.4% for maize, 1.4% for oil seed rape, 1.9%
4367 for cereals, 0.005% for sugar beets and 3.4% for other crops.
4368
4369 These conservative estimates for adjacent fields are higher than those for field margins which is in
4370 contrast to the spray applications where the deposition in the field margin is expected to be much
4371 higher than in the adjacent field (over its full width). This difference is caused by the difference in
4372 decline of deposition with increasing distance to the treated field: this decrease is much sharper for
4373 spray drift than for dust drift.
4374
4375 Also field measurements on dust deposition are commonly carried out for directions that differ no
4376 more than 30^{o} from the wind direction. As described in Section 3.2.5, we have eliminated the upwind
4377 wind directions already in the selection of the X^{th} percentile in box 6 of Figure 12. So the problem left
4378 here is to assess how these field measurements should be used. As described before, the target is the
4379 average concentration over the full width of the adjacent field, so from the field measurements the
4380 average deposition over 50 m have to be derived. Then there is the problem left that the target is the
4381 X^{th} percentile of all downwind adjacent attractive crops and the selected sowing equipment while we
4382 have already taken the X^{th} percentile of the Heubach-AI values. So here we have the problem of
4383 finding a percentile X of a quantity that is a function of two variables ((i) Heubach AI and (ii) the
4384 combination of wind angle and wind speed) which have each their probability density functions. To
4385 solve this problem, we need information on the probability density functions of the two variables and
4386 their interaction which is not readily available. Therefore we propose as a conservative interim
4387 solution to use simply the measured average deposition over 50 m width of the adjacent field directly.

As indicated in box 7 of Figure 12, this still has to be multiplied with a factor 10 for the catchment effect of the crop (this is not considered here).

Granule applications

A conservative default dust drift deposition value for granule applications and adjacent crops is needed in the flow chart in Figure 14. This section describes how this is derived.
We propose to based the default conservative dust depositions on the 3.2% derived from EFSA (2004) in section C-3.1.2. We propose to multiply with 10 to account for the filtering capacity of the plants in the field margin and to multiply with 0.48 get the average deposition onto the first 50 m. So we get 3.2 × 10 × 0.48 = 15% for the default dust deposition of granules onto adjacent crops in Figure 14.

Admittedly, an average 15% deposition over a width of 50 m of the adjacent crop seems a very conservative value. Therefore we recommend to collect and analyse all available data on dust deposition of granules onto plants in adjacent crops in order to reduce this conservative default value. A too high conservative default value is of course not a fundamental problem for the risk assessment: it will only lead to more higher-tier field experiments and thus to more efforts for notifiers and authorities than necessary.

SUMMARY OF CONSERVATIVE DEFAULT DEPOSITION PERCENTAGES

The summary of the conservative default deposition percentages to be used for the different combinations of application technique and types of plants in Table K1 shows that the granule applications have the highest default values. This reflects the very limited information that was available to us for this application technique.

Table K1: Conservative default deposition percentages for spray drift and dust drift to be used for the different combinations of application technique and types of plants.

	Plants in field margin	**Adjacent crop**
Spray applications (spray drift)	0.9% for field crops 10% for early fruit 5% for late fruit 0.9% for early grapevine 3% for late grapevine 6% for hops	0.3% for field crops 7% for early fruit 3% for late fruit 0.5% for early grapevine 1.4% for late grapevine 4% for hops
Seed treatments (dust drift)	2.3% for maize 1.0% for oil seed rape 1.3% for cereals 0.003% for sugar beets	3.4% for maize 1.4% for oil seed rape 1.9% for cereals 0.005% for sugar beets
Granule applications (dust drift)	11% for all crops	15% for all crops

L. ASSESSMENT OF THE PERCENTILE OF A SUBPOPULATION THAT CORRESPONDS TO A PRESCRIBED PERCENTILE OF THE TOTAL POPULATION.

Let us consider a statistical population of a certain quantity Z. Let us assume that we can divide this population in n subpopulations which are ranked based on their Z values in such a way that all Z values of subpopulation 1 are smaller than those of subpopulation 2, all Z values of subpopulation 2 are smaller than those of subpopulation 3, etc.

Let us assume that we want to know the 90th percentile of Z by sampling only one of these subpopulations (for efficiency reasons). The question is then what percentile of the subpopulation should be assessed to obtain this overall 90th percentile. For example, if the subpopulation covers all values between the 85th and the 95th percentile, then it will be clear that we need the 50th percentile of the subpopulation to obtain the overall 90th percentile. This scaling procedure can be generalised to the following equation:

$$X = 100\,\frac{90 - x_{low}}{x_{high} - x_{low}} \qquad \text{(Eqn L1)}$$

where X is the percentile of the subpopulation corresponding to the overall 90th percentile, x_{low} is the percentile of the total population corresponding with the lowest value of the subpopulation and x_{high} is the percentile of the total population corresponding with the highest value of the subpopulation. So for the above example, $x_{low} = 85$ and $x_{high} = 95$, so $X = 50$ indeed.

Often the 90th percentile will be located in the subpopulation with the highest Z values. For such cases it is interesting to write X as a function of the percentage of Z values that is present in this subpopulation which is further called F. So F is defined as $F = 100 - x_{low}$ and $x_{high} = 100$. This gives the following expression for X:

$$X = 100\,\frac{F - 10}{F} \qquad \text{(Eqn L2)}$$

Figure L1 shows that X increases with F and that it becomes of course 90 if F approaches 100 (so the subpopulation becomes the full population). If F is smaller than 10%, then X has no meaningful value anymore because the subpopulation consists of less than 10% of the values of Z, so the 90th percentile is then determined by another subpopulation. Figure L1 can be illustrated by considering the easy case of F= 20, so the subpopulation of the highest values is 20% of the total population. In such case the Eqn L2 and Figure L1 give X = 50 which is the expected value: if only the highest 20% of all values are considered, then the 50th percentile of these highest 20% should give the overall 90th percentile.

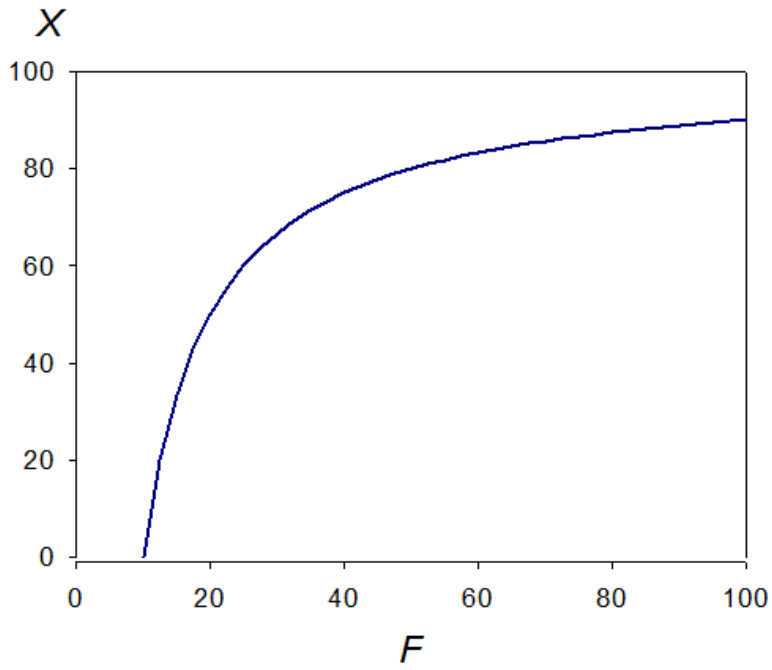

Figure L1: The relationship between *X* and *F* as described by Eqn L2.

M. CHECKLISTS FOR EVALUATING LABORATORY STUDIES

Laboratory tests for honey bees (Adult)

Acute oral and contact toxicity test

Acute oral and contact toxicity of the test compounds to adult honey worker bees are assessed in laboratory following the OECD guidelines 213 and 214 or the EPPO 1/170 (4). In these tests, bees are exposed to a single dose of the compound by feeding a contaminated sugar solution or by topical application. A suitable range and number of concentration should be used to provide a regression line and calculate the LD_{50}. It is important that the OECD guidelines are complied with in detail and the following improvements from EFSA Opinion (2012a) are considered:

- the observation period have to be always 96 hours and extended if the mortality continues to rise until the test is valid (control mortality ≤ 10%);

- all sub-lethal effects have to be reported in quantitative way. Any symptoms of intoxication observed in bees during laboratory toxiciological tests are recording together with their duration, time of onset, severity and number of affected bees at each dosage level. Examples of neurotoxicity symptoms are: uncoordinated movement, trembling, tumbling, hypo/hyper-responsiveness and hypo/hyperactivity, abnormal movements of legs or wings. Specific tests (PER test – Proboscis extension reflex) in laboratory or in field (homing ability - see section of Gerard for field study) have to be conducted in the Higher Tier in case of neurotoxic effects.

- the following variables need to be controlled and always noted: the age of the individuals tested, the nutritional and health status of colonies from which the bees were collected for testing, the subspecies of the bees, the temperature and the humidity during the test.

- the endpoint from this studies should be: LD50 contact (µg/bees) and LD50 oral (µg/bees) at 48h.

Chronic oral toxicity test

In EFSA Opinion (2012a) it was highlighted that the single acute exposure scenarios are not representative of the exposure of foragers or in-hive honey bees for compounds which may persist for more than a single day in the environment, or in nectar and/or pollen returned to the hive. Because there is insufficient evidence that toxicity following extended exposures can be reliably predicted from acute oral LD50 data, a chronic oral toxicity test is recommended. This is performed by conducting a toxicity test in which newly eclosed worker honey bees are fed *at libitum* with treated sucrose for 10 days.

Oral extended exposure studies should be undertaken for both the active ingredient and the product and any observed sub-lethal effects should be reported as for acute toxicity test.

The chronic oral toxicity test should be conducted in compliance with a protocol for extending exposure adapted from Decourtye et al (2005), Suchail et al. (2001) and Thompson (p.c.).

Experimental conditions: Adult honey bees or young emerged honeybees are used to run the test. They should be from a single strain in order to provide a similar status regarding origin and healthy. At least 10 bees are kept in holding cages with a syrup feeder. During the test, the cages are placed in

incubators or in a controlled room at 25 ± 2°C and with Relative Humidity higher than 50%. For each test product, five concentrations are selected so as to range from 10 to greater than 100% mortality with no more than 2 fold dilutions between doses. A preliminary test can be carried out with a concentration range of factor 10 in order to determine the choice of the appropriate concentrations.

A control with bees fed with only sugar solution is included in each test. Test solutions should be stored in the fridge at 0-10°C until required for dosing. From three to five replicates of the cages with each test dose are used to constitute a test. Three replicates of the test (3x3) can be performed during different periods of the bee season.

Mode of treatment: Immediately prior to treatment each group of bees in its cage is anaesthetised by placing the cage into a beaker filled with carbon dioxide gas. Any bees which were visibly damaged are excluded from the study. The bees will be anaesthetised with carbon dioxide immediately before dosing and gently tipped out onto filter paper and counted into the cage (drones were discarded). Each group of 10-20 newly eclosed worker bees is offered a known weight of a given concentration (or controls as above) for 10 days, the dose being measured into the feeder each day (1-2 ml per cage). Every day the feeders are removed and weighed and replaced with fresh feed so that bees has continuous access to the treated feed throughout the study. The dose consumed is determined by comparison of the weight of the dose remaining in the feeders with the initial weight of the feeders and weight of a known volume of the test solutions. The individual daily consumption was corrected by the surviving bees.

Data assessment and reporting: Observations of mortality and behaviour are recorded at daily intervals up to 10 days. The data is used to determine both the LC50 (mg/kg) and NOEC (mg/kg) and to investigate whether there are any indications of cumulative effects according to Chapter 4.1. Test is valid if the mortality in the control group is less than 15%.

Laboratory tests for honey bees (Brood)

Aupinel test

A honey bee larvae toxicity test is performed in the First Tier for any substance that can reach the hive via pollen or nectar. A test method based on the *in vitro* rearing method of honey bee larvae (Aupinel et al. 2005) is proposed for brood risk assessment following the Aupinel methodology (Aupinel et al. 2007). This test is run under laboratory conditions and permits to control exactly individual exposure providing quantitative oral toxicity data. It is designed for *in vitro* treatments of active substances or formulated pesticides. Larvae at the L1 stage are fed with standardized amounts of artificial diet. Test products are incorporated into the food at the different concentrations within an appropriate range in order to compute the end points: LD50, LC50, NOAEL and NOAEC. In Aupinel protocol, the reference product is dimethoate but a more relevant water-soluble active substance is recommended (EFSA, 2012a). This method also allows assessing several sublethal effects such as prepupal weight, duration of development, adult morphology and behavior. The method can be used either to study acute effects by applying contaminated diet to one particular instar, or to investigate chronic effects by each day providing the larvae with the test substance. The chronic dosing study is more relevant to the exposure of larvae in the hive than a single acute dose and this test design is recommended for pesticide risk assessment. This method has already been ring-tested (Aupinel et al, 2009) by 7 laboratories from 6 countries and validated: < 15% mortality in the control at D6 and successful workers adults eclosion in at least the control group. Currently, it is proposed for a validation at OECD. The endpoints of this study should be: LC50 larvae (mg/kg), NOEL (mg/kg).

Rearing procedure: The rearing method is described in details in Aupinel et al. (2005) or in the BeeBook (in preparation) and summarized in Figure M1. Larvae have to be collected in an healthy colony with no visible clinical signs. No treatment has to be applied in the hive within the 4 weeks

preceding the beginning of experiments and the test should be carried out with summer larvae. The experimental unit is a 48 larvae plate. From a comb the young larvae are transferred into individual rearing cells with a grafting tool. The larvae are fed once a day (except day 2) with a micropipette. Diet composition, temperature and humidity during the test vary according to larvae age (Figure M1, Table M1). Before adult emergence (at D15), each plate is transferred into an emergence box with *ad libitum* food and checked for longevity.

Mode of treatment: For each tested product, 5 concentrations (1 plate/concentration) should be used in order to provide a regression line and the LC50. A control (1 plate) and a reference treatment with dimethoate or a more relevant water-soluble active substance (1 plate) must be included.

One test has a minimum of three replicates with one different larvae origin and new tested solutions for each replicate. The test pesticide is preferably dissolved in water. If it is not soluble in water at the experimental concentrations, it is possible to use another solvent such as acetone. In that case, it is necessary to prepare a second control feed with diet containing the solvent at the same concentration as the treated samples. In the chronic toxicity test, larvae are treated every day (except D2) with the diets containing the preparation to test at a constant concentration.

When dimethoate is used as toxic reference it should be mixed with the three diets at the constant concentration of 20,000 µg/kg diet. The treatment procedures are described in details in Aupinel et al. (2005) or the BeeBook (in preparation).

Data assessment and results: Larva mortality is checked every day and systematically removed for sanitary reasons. The larval mortality rate is noted at D7 (immobile larva or a larva which does not react to the contact) and the pupal mortality is noted at D22 (non emerged bees).

The test is considered valid if: in control samples, larval mortality (number of dead larvae/48), pupal mortality (number of dead pupae at D22/number of alive pre pupae at D7) and adult mortality (number of dead emerged bees at D22/total number of emerged bees) are lower or equal to 15% (for the assessment of a LD50 or a LC50) or 20% (for the assessment of a NOAEL or a NOAEL).

In case dimethoate is used as standard toxic, the mortality rate must be higher than or equal to 50% at D7. The calculated LD50 and LC50 must be in each case between the two extreme tested doses.

LC50 is calculated from percentage of mortalities after an adjustment according to the Abbott formula. The NOAEL and NOAEC are the highest dose and concentration respectively, which do not induce mortality significantly higher than that observed in controls. This analysis will be done by using a Chi2 test.

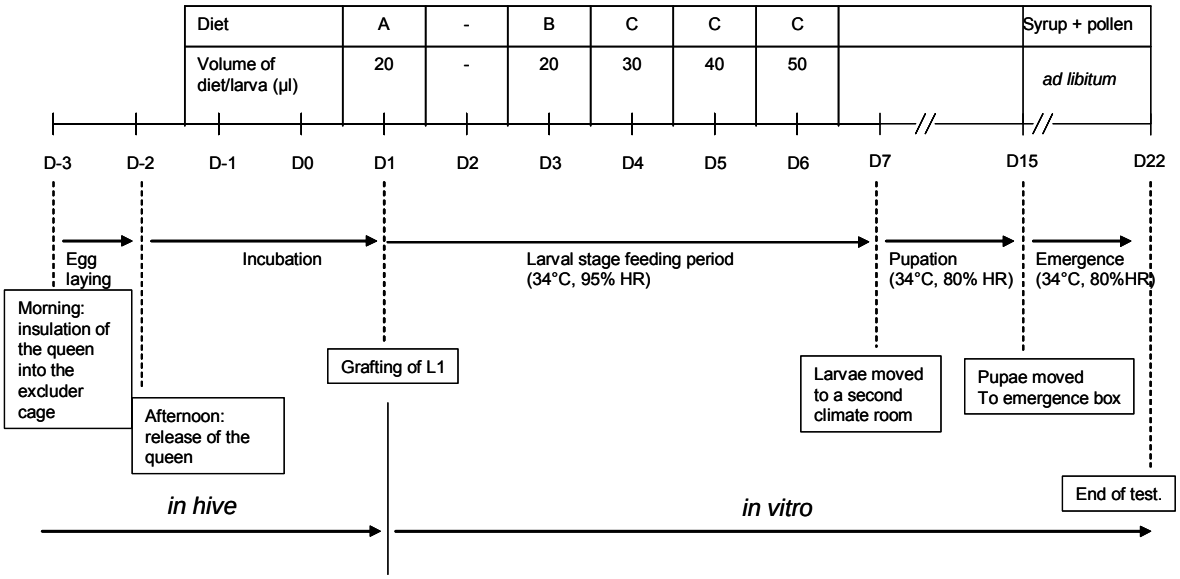

Figure M1: Steps of an *in vitro* test

Table M1: Composition of the diets provided to larvae (Aupinel et al, 2005)

Diet	**A**	**B**	**C**
Royal jelly (%)	50	50	50
Yeast extract (%)	1.0	1.5	2.0
D glucose (%)	6.0	7.5	9.0
D fructose (%)	6.0	7.5	9.0
Dry matter (%)	29.6	33.1	36.6

Oomen tests (Brood)

The Oomen test is designed for investigation of effects following oral exposure especially of oral exposure of bee brood. The endpoints are the mortality at 7 days and just prior to emergence, together with assessments of brood deformities in pupae extracted just prior to emergence. This test may be run under semi-field or field conditions and permits to assess the effects after exposure to defined concentration of active substance in the sugar solution fed to bee colonies. It is described in the laboratory section as feeding of defined concentrations and e.g. a dose-response testing is possible; thus it is considered as an intermediate test between first and Higher Tier testing. Brood rearing and brood care is conducted by the nurse bees of the bee colony. The test may be designed for formulated

pesticides but may also be used for of active substances. Presumably all larval stages and also in hive bees are exposed to the test solution, as stores – especially nectar stores- should be reduced to a minimum while ensuring the colony has enough stores to just prevent starvation. Due to in-hive feeding of the sugar solution, even lower nectar/honey stores need to remain in the colonies compared to semi-field tests with bee attractive crops, as bees may access the food also during rainy or cold weather conditions.

Test procedure: Set-up in semi-field conditions should in general follow EPPO 170. In field conditions, study should be conducted in an environment with negligible natural nectar/honey flow. Colonies in field conditions should be of natural size (full size colonies) according to season (e.g. in early spring at least 10.000- 15.000 bees) and the region. Colonies in semi-field should be adapted to semi-field conditions (smaller colonies, see Appendix N on semi-field tests for details) but additional pollen feeding in the hive or in the tunnel may be necessary to prevent starvation of pollen. Further standard measurements which are necessary in semi-field or field tests with colonies, e.g. diagnose of bee diseases and status of colony health, assessment of colony development and food stores in hives, assessment of weather conditions should be conducted as described in the EPPO Guidelines and in the semi-field and field section of this document. As a minimum, 3 replicates per treatment concentration are recommended.

Mode of treatment: The test solution is made of sucrose sirup mixed with the test item and fed daily to the bees, as toxic standard Fenoxycarb is recommended. Feeding sucrose solution during the exposure period should be extended from a single dose feed on one day to feeding contaminated solution daily for 9 days to ensure that all larval stages are exposed. Usually test products are fed at a concentration recommended for a high-volume use.

Data assessment and results: The duration of the study should be at least 28 days after start of feeding (DAF) and first assessment of different brood stages to ensure all larval stages are assessed and that new eggs are laid into the cells after successful hatch of one brood cycle. Individual cells should be assessed on DAF +5 ±1, DAF +10 ±1, DAF +17 ±1, DAF +22 ±1, DAF +28 ±1 (DAF 0: Day of first feeding of the test item). Measurements of dead adult bees and dead bee larvae should be assessed daily using dead bee traps.

The development, the mortality of different brood stages and hatching success are assessed in regular intervals by assessment of brood development of all stages, egg, larvae, pupae. For this purpose at least 200 eggs, at least 200 young larvae and at least 200 old larvae should assessed, preferably using digital brood assessment. The development of pupae should be assessed by extracting additional pupae on another comb, just prior to emergence to assess morphological abnormalities and weight of pupae. Although the implications of decreased pupal weight are not fully understood there are obvious implications of lower weights on fitness and longevity. Once before start of feeding (control) and at DAF+ 13 ±1 for old larvae, DAF+ 15 ±1 for young larvae and DAF+17 ±1 for eggs, 50 pupae each should be taken for weighing from the test colonies. As pupae are removed at the last assessment for each stage (just prior to expected emergence) to determine morphological effects, the actual growth stage (from colour of the body and wing pads) and the weights of pupae should also be assessed to determine any adverse effects on development, e.g. delayed development.

N. CHECKLISTS FOR EVALUATING SEMI-FIELD STUDIES

For semi-field testing (cage, tunnel or tent tests) in principle the approach as described in EPPO 170 (4), the OECD 75 brood Guidance Document (OECD, 2007), and the Oomen et al. (1992) test is considered appropriate. Semi-field studies aim at assessing the level of effects that may be expected on bees exposed to the product under realistic use conditions when the target crop has been treated. The exposure is worst-case and more intensive than in the field (bees/colonies confined and forced to forage on the treated crop) and potential mortality is easy to assess. Next to the standard information required by the guidelines in the following section, several further recommendations are provided to enhance the quality of the tests. Semi-field testing should be designed to address and reproduce the route(s) of exposure of bees and the maximum level of exposure expected by these routes, as a result of a spray or of the presence of residues in flowers (nectar/pollen). For all test systems in the semi-field, it is necessary that all categories of bees are thoroughly exposed and proof of exposure and consumption of the test item needs to be provided for all categories of bees. For accurate quantification of exposure, semi-field studies may provide suitable and reproducible information on residue levels both for sprayed products and also for residues following seed treatments or soil applications with systemic compounds. Modifications of the guidelines or test methods depending on study aim may be necessary and should be justified.

Test crop and preparation of the colonies

The use of small colonies is required in the semi-field methodology compared to field tests due to limited forage area. For semi-field testing colonies should be of similar size and the strength adapted to forage area but as large as possible. It is recommended to use bigger colonies but at least 6000 adult bees and 3 to 4 brood combs (at least 15.000 brood cells), containing a high amount of capped brood and to start, if possible, studies early in the season. Major modifications of the colonies shortly before application should be avoided. At least 4 replicates per treatment are recommended.

The level of stores within the colonies should be reduced to a minimum before the start of the trial. As and effective forage area > 60 m², preferably > 80 m² are recommended. In principle, *Phacelia* or a highly bee attractive crop, e.g Winter oilseed rape should be used as a test crop for assessing the effects of spray applications. Nevertheless, e.g. for systemic compounds, identification of a surrogate (worst-case) test crop may be more difficult, where the test crop should be one for intended use. For assessing the effects of crops which might have low numbers of flowers per m² (e.g. zucchini) a worst-case flowering crop like *Phacelia tanacetifolia* is recommended to be used for testing potential risks assuming worst-case exposure. For sprayed products, semi-field tests may be used for demonstration of acceptable or unacceptable effects in a semi-field test using a worst-case flowering crop, in some cases also standard crops (i.e., wheat) which have been made artificially attractive through a sugar solution and treated at the maximum application rate.

The colonies should be healthy at the beginning of the experiment, e.g. free of clinical signs of significant brood diseases such as American Foul Brood and European Foul Brood. As most of the European colonies, even strong ones, contain infectious agents, it is not possible to use colonies that are completely free of them. Regarding the mite *Varroa destructor*, present in almost all European colonies, the level of infestation of the control and test colonies should be as low as possible. During and after the exposure period up to termination of the study, infestation of Varroa should be monitored at regular intervals. During and after the experiment, the health of the colonies should be evaluated for the whole range of bee diseases (including Nosema, acarine and the main viruses, e.g. through molecular screening).

Assessments

Standard assessments which should be observed in semi-field tests are flight activity as well foraging behaviour on the treated crop and potential behavioural abnormalities (e.g. according to CEB 230), observations of behaviour of bees at hive entrance, observations of behaviour of colonies (e.g. aggressive) as well as daily assessments mortality on linen sheets in the crop and daily assessments mortality in front of hives. A detailed description and categorization of all observed behavioral abnormalities should be provided. Colony assessments should include brood development (all stages, egg, larvae, pupae), morphological abnormalities of the brood and appearance of brood nest, Colony development as well as the mortality in the bottom of the hives, nectar and pollen stores, and the diagnose of bee diseases.

For all tests it is recommended that the OECD Guidance Document is extended to assess adverse effects on all 3 stages of brood. There are significant advantages to interpretation if the effects of pesticides on eggs, young larvae and old larvae are assessed, so this should be included in assessments of effects on brood in all studies. For OECD 75 and Oomen et al. the development of at least 100 eggs, 100 young larvae and 100 old larvae per colony should be used, preferably by the use digital imaging instead of acetate sheets. The contents of all cells including deformities in pupae should be assessed as well as weight of pupae before and after treatment to determine any adverse effects on development, e.g. delayed development.

Depending on the study aim, further endpoints e.g. specific behaviour, homing behaviour, homing ability or the weight or lifespan of hatching bees can be addressed in all studies for investigation of special effects. Residue analyses must be performed on the nectar and pollen brought back to the colonies in the treatment and the control. More detailed residue sampling of foraging bees and in hive (e.g. nectar/pollen/wax/larvae/bees/propolis) may be required in some cases; as some assessments may be difficult to conduct in one tunnel different tunnels may be needed for further special investigations (e.g. high frequency of residue sampling in hive; due to frequent colony disturbance increased mortality). Consideration should be given to extending studies where significant exposure is likely to occur over a period longer than a single brood cycle, e.g. systemic or highly persistent residues.

Reporting

Results should be analysed with appropriate statistical methods, information on statistical power of the method is required. Statistical evaluation is needed for mortality and of the flight intensity before and after treatment. Specific statistical analysis for bee trials in semi-field and field conditions is still under development. In general it is recommended to follow the OECD guidelines (OECD, 2006) until that further specific guidance on the appropriateness of methods and statistical evaluation for bee trials is elaborated.

Furthermore, all further interpretation needed for the interpretation of a study e.g. details on study substance, application, climate conditions, crop stage, crop development during study should be reported.

Further guidance on semi-field studies is given in Appendix O.

O. HIGHER TIER EFFECTS STUDIES

FIELD STUDIES

BACKGROUND

Outlined below is guidance on how to determine the potential effects of a pesticide on honey bees under field conditions. The guidance is split into two parts, one for applications via spray and one for application of solids. If a field study is to be undertaken it is important to ensure that the 90th percentile PEC is determined beforehand and that this is achieved in the study. If adequate exposure is not achieved, the field study will be of limited use. Please see Chapter 3 for guidance on how to determine appropriate exposure levels. Please also see Section 'Study methodology for field study' (c) below regarding how this information will be used in validating and hence using a field study in the risk assessment. It should be noted from Section 'Study methodology for field study' (c) below that it may be necessary to carry out a semi-field study (see Section 2 for details) in order to determine the appropriate exposure. Please note that exposure will be determined by residues in pollen and nectar in the hive and hence this will be used to demonstrate whether the field study's exposure was appropriate for making a risk assessment.

There are two sets of assessment endpoints for field studies and these are as follows:

- **Primary assessment endpoints**: forager mortality, colony strength (number of bees), over-wintering success, honey production
- **Secondary assessment endpoints**: behavioural effects

The primary assessment endpoints link directly to the Specific Protection Goals outlined in Chapter 2.

In order to address concerns raised in EFSA, 2012a regarding the limited ability of field studies to adequately assess adverse effects on behaviour of bees, and in particular effects on orientation and homing ability of bees, it is proposed that a **homing study** should be carried out. Such a study can be carried out as part of the field study. Details as to how to carry out such a study are provided in the Section 'Methodology for homing study' below.

Observations of the secondary assessment endpoints (behavioural effects) will be used to help explain any effects observed on the primary assessment endpoints. Even in the event that these observations suggest detrimental impacts, this cannot be used as the sole basis for a regulatory decision because effects on secondary endpoints do not in themselves threaten the Specific Protection Goals (SPG). For example, if there is no effect on colony strength and/or overwintering survival or mortality, but there is an effect on foraging behaviour this will not over-ride an assessment's conclusion of 'acceptable risk' when based on a lack of effects on colony strength, over-wintering success and forager mortality.

In principle, the same concepts apply to both spray and solid applications but some practical differences are better handled separately and so schemes for field studies of both modes of application are presented below.

METHOD FOR APPLICATIONS VIA A SPRAY

Assessment methodology for field study for applications applied via a spray

Presented below is an outline as to how the primary and secondary assessment endpoints can be determined:

The ***primary assessment endpoint*** of ***colony strength*** can be determined by using the Liebefeld Method (Imdorf et al., 1987). This method estimates the adult bee population and the amount of brood present in the colony. The adult bee population is assessed by visual estimation of the percentage of comb surface covered by bees. Each percentage value is then transformed into a number of bees according to the size of frame. In order to control some of the intrinsic variation among colonies, it is proposed to determine the number of adult bees at the beginning of the experiment and at the end of the exposure (after at least two brood cycles). A methodology for carrying this out is provided by Costa et al., 2012. It is proposed to use a similar approach to determine over-wintering survival.
The ***primary assessment endpoint*** of ***mortality of foragers*** needs to be determined. This can be done via the use of dead bees traps placed at the entrance of the colony as well as via the use of collecting sheets placed around the colonies. It is appreciated that this method will underestimate total mortality. Alternative methods are available, for example the quantitative measure of returning foraging honey bees via the use of marking individual bees, and these can be used if preferred.
The ***primary assessment endpoint*** of ***honey production*** can be determined by estimating the amount (in terms of weight) of honey produced in the colonies compared to that produced by the control colonies.
The ***secondary assessment*** **endpoint** of ***behavioural effects*** can be determined using the following approaches:
The **behaviour of foragers** on flowers should be assessed both qualitatively and quantitatively. In order to determine the level of exposure of nectar and pollen foragers, the foragers should be counted on the test and control crops, at different moments of the day, during a significant period of time, and throughout the experiment (see, for example Karise et al., 2007). The number of data collected should be sufficient for allowing statistical treatment[9]. The behaviour of nectar and pollen foragers should be observed, at least once a day. In particular, it is important to check that the honey bees are able to make the pollen pellet and to collect nectar.

In addition to behaviour on flowers, there should be a consideration of the following:

- **Presence signs:** this parameter refers mainly to motionless bees on the flower and to bees on the whole plant but not on the flower.
- **Cleaning signs:** observation and counting of the bees that clean themselves in two ways: (a) limited cleaning of legs and antennae, (b) overall cleaning (the whole body is brushed with middle or hind legs). These observations should be made for at least a few seconds and sometimes for several minutes for one bee.
- **Clinical intoxication signs:** Bees hang from leaves or from flowers by one or two legs. Sometimes bees are motionless, sometimes they clean themselves. Any such honey bee is supposed to fly away when pushed by the experimenter's finger and is counted as 'hanging bee'. When the bee falls and lays down, it is counted as a 'falling bee'. Paralysis and disordered wings or legs or disturbed movements - cramping or shaking bees, regurgitation stomach content.

Study methodology for field study

(a) Definition of terms

- **'Field'**: a contiguous area of crop with a single chemical regime - either treated or untreated (control) with the pesticide, i.e. it is appropriate to refer to a 'control field'.

- **'Site'**: a location in the region for which the applicant seeks permission to use the pesticide. The site may include one or more fields, i.e. a site may include both control and treated fields.

(b) Principles

[9] It is appreciated that currently there is a lack of guidance on appropriate statistical techniques.

The following principles are considered key to carrying out a field study:

a. The field test must emulate the appropriate exposure of honey bees to the pesticide as used in agricultural practice – see below.
b. Bee colonies that are exposed to the pesticide in the field must be compared to control colonies that are not exposed or exposed to only a negligible degree (i.e. where the exposure is less than the lowest achievable LOD.)
c. In order to show that the hives are affected consistently by the exposure, the test include more than one hive in both exposed and control treatments.
d. In order to demonstrate that the pesticide's effects (if any) apply to sites/landscapes in general, the test must include more than one study site.
e. The test must be conducted without conscious or unconscious bias.
f. The test must be sufficiently powerful to detect the maximum effects allowed under the protection goals.

(c) Appropriate exposure

The key to achieving a valid study is ensuring adequate exposure. As stated above, the study must be designed to ensure that residues will be in line with the exposure assessment. In order to ensure adequate exposure, the Applicant may consider either carrying out multiple studies at various rates, or applying the pesticide at a sufficient rate to ensure that residues in both pollen and nectar are appropriate so that they are at least as high as the concentrations determined in the exposure section – see Chapter 3.

An ideal field study will be one where the bees forage almost exclusively on the target crop and where the nectar and pollen in the flowers contain residues at least equivalent to the 90th percentile that has been generated from previous studies. It should be noted that if the HQ-contact is the only risk quotient that is breached, then it may not be appropriate to carry out a field study at increased rates as this will not reflect reality. In such circumstances, it is recommended to carry out a semi-field study only.

Views are requested on the proposal to rely on a semi-field study when the only risk quotient to be breached is the HQ-contact.

In the exposure assessment (see Chapter 3), it is assumed that the residues in the pollen and nectar in flowers are equal to the residues in pollen and nectar in the colonies. This assumption has been made due to the lack of data to indicate how the residues in flowers compare to the residues in the colonies. Instead, residues in colonies could be lower due to factors such as compound degradation and metabolism by the bees themselves. Whilst residues in pollen and nectar of the treated plant can be compared to residues from previous studies used to determine the 90th percentile exposure value (as outlined in Appendix J of Chapter 3) there is no similar threshold to establish that the exposure of the colony in a field study has been adequate to investigate a 90th percentile scenario. For example, it may be that an undesirable dilution of residues has occurred due to honey bees foraging on flowers other than those of the treated crop. Thus it could be unclear whether an observation of a low level of residues in the colony is as expected after appropriate exposure or whether, instead, foraging bees have avoided the treated field. Applicants can justify the adequacy of the exposure by demonstrating that a similar differential exists between the concentration of residue in flowers and colonies in semi-field trails where exclusive foraging on treated flowers is enforced by (for example) an enclosure.

It is recommended to:

1. Carry out studies to determine the range of residues of the active substance in the pollen and nectar of flowers of the treated crop. See Appendix J for further information.

2. This information will first be to refine the First Tier risk assessment (see Risk Assessment Schemes). If as a result, risk quotient(s) are breached, then it is recommended to carry out semi-field studies (see below for details) or implement suitable risk mitigation measures (see Chapter 3). The semi-field studies can be used to determine both the effect of the pesticide as well as to establish the differential (if any) in the concentrations of residues in pollen and nectar of flowers versus in the colony when exclusive foraging on treated flowers is enforced. Samples of pollen and nectar from the colony should be taken to ensure that the peak residues have been determined, or that the residue data match the toxicity study in terms of duration, i.e. 48 hours. In practice, this is likely to be achieved at two days post spraying. The residue information can be used to estimate the ratio between residues in flowers with those in the colony. This information is used to generate an adjustment factor for compound degradation and metabolism of the active substance. This 'metabolism adjustment factor' will be used to validate the adequacy of the exposure achieved in field studies if/when undertaken.

3. In designing a field study it is essential to take note of the above information and hence ensure that exposure within the colony is appropriate. In practice, this may mean adjusting the application rate in order to ensure adequate exposure. It should be noted that the concentration achieved in in-hive residues in the field study has to be at least as high as the concentration achieved in the semi-field study. The Applicant may therefore consider either carrying out multiple studies at various application rates, or applying the pesticide at high(er) rates to ensure that residues in both pollen and nectar in the flower and colonies of the field study are appropriate so that they meet the concentrations determined in the exposure section.

4. Residues in pollen and nectar from both the treated (and control) flower and hive stores should be determined during the field study.

5. Once completed, the residues in both flowers and hive stores need to meet or exceed the 90th percentile estimates produced as a result of the exposure assessment. In order to achieve this, an applicant should collect all the residue data from pollen and nectar in flowers from the residue and effects field study. The datasets should be kept separate – i.e. there should be one dataset for pollen and one for nectar. To account for the differential in concentrations between flowers and the in-hive residues, apply the 'metabolism adjustment factor' determined from the ratio between floral and in-hive residues in the semi-field studies (if appropriate) to the separate datasets and form a distribution by pooling these resulting numbers with the in-hive residues obtained from the semi-field studies. These data are then used to determine the 90th percentile of in-hive residue levels against which the in-hive residues from the field study will be compared.

It is also possible to use existing datasets to establish the distribution required in point (5).

If following the above procedure, the in-hive residues under field conditions were either not achieved or achievable, then the Applicant needs to provide evidence to justify that the exposure achieved is nevertheless in line with the exposure assessment. For example, low in-hive residues may be realistic if under field conditions bees normally collect only small proportions of their pollen from the target crop[10].

Design of a field study

Choice of crop

[10] In order to measure the proportion of pollen coming from the treated and control plants compared to pollens coming from other plants in the foraging area, pollen traps should be provided in some test and control hives, for further pollen analysis. This pollen analysis should not be limited to the observation of the pollen pellets colour, but should include the identification of the pollen grains under the microscope (palynology).

The choice of crop that can be used for this study is up to the Applicant. It may be possible to carry out this study with the proposed crop outlined on the label but alternatively it may be possible to use a highly attractive model plant (e.g. *Phacelia tanacetifolia* or oilseed rape) and extrapolate the study findings to a range of crops. The key issue in selecting a suitable crop is to ensure that it is attractive to honey bees and that the residues, and hence the exposure to honey bees, is environmentally relevant and at least as high as predicted in the exposure section.

Number of colonies

The number of test and control colonies must be high enough to account for the normal inter-colony variability and allow statistical analyses (Principle c and f).

Conventionally, a statistical test has adequate power when there is 80% confidence that the experiment detects an effect of the specified magnitude, if it exists. For example, roughly speaking, it requires treatment groups of n = 13 to detect an effect whose magnitude is similar to the standard deviation of the individual measurements with 80% confidence in a one-sided Student's t-test (i.e. when the treatment with the lower mean is specified in advance; one-sided tests are appropriate here because only the detrimental effect of the pesticide is sought).

The Specific Protection Goal (SPG) requires the experiment to detect a >7% detrimental effect on colony size and it is reasonable to expect that the average colony will differ by at least about 7% from the mean value of colony strength in the control group (colony growth rate is likely to be a relatively noisy variable even when the initial colony size and quality is tightly controlled), which means that the standard deviation of the measurements is equivalent to the magnitude of the effect sought. It will be the Applicant's responsibility to show that the experiment had the required statistical power (Principle f),

Currently, it is not possible to recommend a precise number of colonies that need to be tested. EFSA would welcome thoughts on this issue, as well as indication of the number of colonies considered appropriate. For more details on how to calculate the required number of colonies to detect a certain magnitude of effects at a given coefficient of variation is given in the example below.

To measure the effect (X) of pesticides on a bee hive several measures are under discussion, e.g. the difference of numbers of adult bees before and after application ($X=\Delta A$) / the difference in number of brood before and after application ($X=\Delta B$).

We would assume a multiplicative effect, which can be transformed by the logarithmic function into an additive one:

Hives without exposure: $\ln(X_C) = \mu + \varepsilon$ (Control)

Hives with pesticide exposure: $\ln(X_E) = \mu + \rho + \varepsilon$ (Exposed)

with:
- μ Logarithmic mean effect in control group
- ρ Logarithmic treatment effect
- ε Stochastic error, assumed: $\sim N(0,\sigma^2)$
- σ^2 Between hive variation (all other conditions are fixed)

4999 In reality many other factors will influence the result and give additional variation τ^2, these are the
5000 type and condition of the field, topography of the landscape etc. We would consider the mean effect as
5001 random:

5002 μ Random mean effect, assumed: ~ $N(\nu,\tau^2)$

5003 The global model is therefore:

5004 Hives without exposure: $\ln(X_C) = \nu + \varepsilon$ (Control)

5005 Hives with pesticide exposure: $\ln(X_E) = \nu + \rho + \varepsilon$ (Exposed)

5006 with: ν Logarithmic mean effect in control group

5007 ρ Logarithmic treatment effect

5008 ε Stochastic error, assumed: ~ $N(0,\sigma^{2+}\tau^2)$

5009 $\sigma^2 + \tau^2$ Total variation (between hives and fields)

5010 The regulatory condition should be justified for all fields and should be expressed in relation to the
5011 overall mean ν:

5012
$$E(X_C) = \exp(\nu) \cdot \exp\left(\tfrac{1}{2}(\sigma^2 + \tau^2)\right)$$
$$E(X_E) = \exp(\nu) \cdot \exp(\rho) \cdot \exp\left(\tfrac{1}{2}(\sigma^2 + \tau^2)\right)$$

5013
$$E(X_E)/E(X_C) = \exp(\rho) \geq 0.925$$
$$\Rightarrow \ln\left(E(X_E)/E(X_C)\right) = \rho \geq \ln(0.925) = -0.0253$$

5014
5015 To calculate the sample size to observe this difference we use a simple t-test on the logarithmic
5016 transformed observation (on independent samples of controls and treatment groups) and the
5017 approximation for the null hypothesis of no increase after treatment. To detect a decrease in colony
5018 size of at least 7% the following approximate formula can be used.

5019
$$N = \frac{(z_\alpha + z_\beta)}{\rho^2/(\sigma^2 + \tau^2)}$$

5020 N Number of independent pairs of observations (treated and untreated fields)

5021 α Significance level of the t-test

5022 z_α α-quantile of standard normal distribution N(0,1)

5023 1-β Power of the t-test to observe minimal effect

5024 z_β β-quantile of standard normal distribution N(0,1)

5025 ρ Logarithmic treatment effect

5026 $\sigma^2 + \tau^2$ Total variation (between hives and fields)

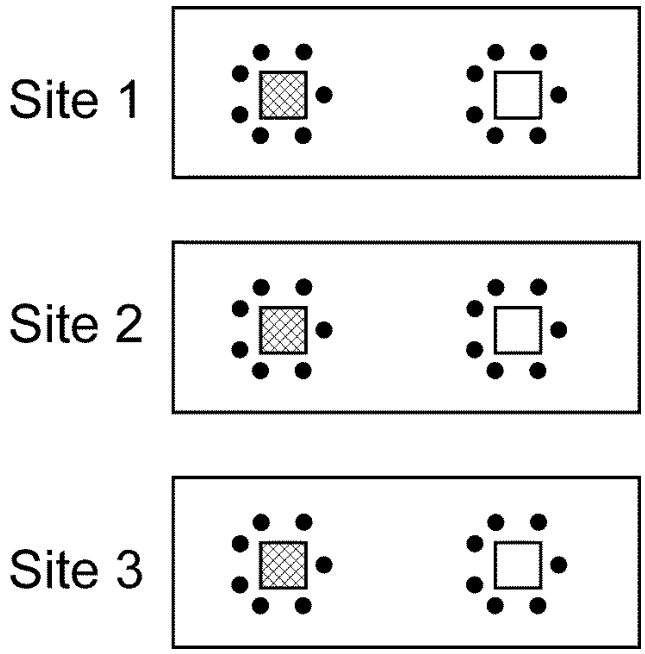

Figure O1: Hypothetical design of experiment to test the effect of exposure to a pesticide on honeybee colonies. Each hive is denoted by ● Treated fields are shown crosshatched squares and untreated fields by open squares. The diagram does not show the exact locations of individual hives – the symbols are only to show the overall number of colonies associated with each field.

This implies that N pairs of fields should be tested to conclude on the effect. In reality several (n) hives will be used at only one (treated or untreated) field. This test design reduces the number of fields, but increases the total number of hives needed to reach the requested power:

$$N = \frac{(z_\alpha + z_\beta)\left[1 + (n-1)\frac{\tau^2}{\sigma^2+\tau^2}\right]}{n \cdot \rho^2 / (\sigma^2 + \tau^2)}$$

N	Number of independent pairs of observations (treated and untreated fields)
α	Significance level of the t-test
z_α	α-quantile of standard normal distribution N(0,1)
$1-\beta$	Power of the t-test to observe minimal effect
z_β	β-quantile of standard normal distribution N(0,1)
ρ	Logarithmic treatment effect
$\sigma^2 + \tau^2$	Total variation (between hives and fields)
τ^2	Variation between fields
n	Number of hives per field

Given an example with a coefficient of variation between hives of CV_H= 15% ($\Rightarrow \sigma^2=\ln(CV^2_H +1)$ = 0.022), between fields of CV_F= 5% ($\Rightarrow \tau^2$=0.0025) and a number of hives per field of n=7. The number of pairs of fields is then N=14 (or 98 pairs of hives in total). Would only one hive per field used in the experiment, then 60 pairs of fields (or hives) are needed.

For the same input parameters (coefficient of variation) but an effect size of 50% (increase in forager mortality rate by a factor of 1.5) the number of fields is then N=2 (or 14 pairs of hives in total).

These formulas give an approximation of the number of hives needed to test the difference of effect size between control and treatment of 7% (colony size) and 50% (forager mortality) to significance level α=5% and a power of β=80%. For a concrete study design, the calculation must be adjusted to the individual situation.

Size of treated field

In order to ensure appropriate exposure (Principle a), the treated and control fields should each be at least 2 ha in area and otherwise large enough to provide sufficient flowers to support exclusive foraging by the experimental hives. In order to ensure that honey bees forage principally from the experimental fields (Principle a), sources of nearby alternative forage should be sparse during the field test. It is appreciated that this size cannot prevent foragers who do not visit the test field from bringing pollen and nectar from untreated flowers to the hive.

Colony size and health

At the beginning of the experiment, all colonies (treated and controls) must be in the same state (population size, health status). In order to ensure exposure of honey bees to the nectar and pollen from treated flowers, most of the frames containing food stocks should be removed from the colony before the beginning of the experiment to a level that just prevents starvation but allows sufficient stores for survival. It is acknowledged that this operation is difficult as it could cause a weakening of the colonies and it should only be conducted by experienced beekeepers.

All colonies should be of equal strength initially and then allocated to treatment (control, exposed) at random (Principle e). Applicants should ensure that genetic variation is properly controlled. Ideally, the experimental colonies should initially comprise sister queens and identical numbers of adult workers taken from a common stock. To improve statistical power, steps should be taken wherever possible to minimise variation among colonies, including ensuring uniform initial colony composition before the colonies are allocated randomly between the control and treated fields at each site.

For testing a pesticide on a given crop, the most realistic conditions are to use colonies having the same level of development as the other colonies in this region at the time of year when they forage on the respective crop.

Generally, the normal size of a colony during the spring and summer seasons, is between 20000 (spring) and 60000 or more (June - July) individuals, depending on the climate region. A colony of 10000 individuals corresponds to the beginning of its development at the end of the over-wintering period in Europe when it starts rapid expansion in the early spring.

The colonies should be healthy at the beginning of the experiment, e.g. free of clinical signs of significant brood diseases such as American Foul Brood (AFB) and European Foul Brood (EFB). As most of the European colonies, even strong ones, contain infectious agents, it is not possible to use colonies that are completely free of them. Regarding the mite *Varroa destructor*, present in almost all European colonies, the level of infestation of the control and test colonies should be as low as possible. During and after the experiment, the health of the colonies should be evaluated for the whole

range of bee diseases (including *Nosema, acarine* and the main viruses, e.g. through molecular screening).

Number of sites and location of field

The sites should be representative of the region(s) for which authorization is sought. As regards location of the control and treated fields within a single site, it is recommended that they should be as similar as possible in terms of size and surrounding landscape.

The distance between the tested and the control colonies must be sufficient for preventing cross-foraging between treated and control plots. If there is an overlap in the foraging area of the control and tested colonies, the presence of significant residues in control hives could threaten the validity of the study. In particular, if the control bees can forage in the treated field, the controls colonies will fail principle (b) above and conversely, the honey bees from the treated field could forage on the untreated crop and hence the resulting residue will be less than required by the exposure assessment. Information presented in EFSA (2012a) indicates that a distance of 2-3 km between the treated and control colonies cannot fully guarantee the absence of an overlap between the foraging area of the control and tested colonies. Therefore, it is proposed to choose areas presenting similar environmental conditions, where possible at least 4 km away apart. If necessary, the fields may each be situated on a unique site.

At each site that contains a pair of fields, the location of the control and treated fields should be decided at random (principle e).

Duration of study

The colonies used in the experiments (including controls) should be monitored for a time covering the entire flowering period and beyond. The study should last at least two brood cycles (42 days) to ensure that a significant proportion of brood is exposed to residues stored within the colony.

For those pesticides that are persistent in hive products, it is recommended that monitoring should be maintained for a time after the wintering period as contaminated honey and pollen stores could be consumed during winter (honey) and after the wintering period (honey and pollen).

For long-term study, including the over-wintering phase, the treated and control colonies should be placed in an area far from fields in intensive agriculture in order to avoid a new exposure to pesticides. All experimental colonies should be set up together at the same post-treatment location where no further pesticide exposure is expected (i.e. no flowering crops present), so that they are not exposed to different location-specific factors.

Determination of exposure

Residue analyses

Residue analyses must be performed on the nectar and pollen in both the treated and control fields. These analyses should have two goals: (1) to check that the bees from the treated fields have been exposed to the pesticide; and (2) to check that the bees at the control fields have not been exposed to the pesticide from either the treated field or another one. If a biologically significant level of residues is detected in the flowers and/or colonies at a control field then it is not appropriate to include that field in the risk assessment. In addition, residues in nectar and pollen in the colonies should be determined. All the residue analyses should be realized with the lowest possible LOD and LOQ.

METHOD FOR APPLICATIONS FOR A PESTICIDE APPLIED VIA A SOLID

A field study with a pesticide applied as a solid may be triggered for two reasons:

- The potential risk from deposition of dust on to adjacent crops/weeds and directly on foraging bees when they are flying over or near the sowed field, or
- The presence of the active substance in pollen and nectar of the treated crop, weeds, or adjacent crops.

The design of these field studies will be fundamentally the same as outlined above, but will differ in the following respects:

Exposure via dust

If a risk from dust is predicted, then it is proposed that a study as outlined above for sprays is conducted, however it is essential that the exposure is in line with that determined in semi-field studies (see above) and Chapter 3.

Exposure via the presence of the active substance in the pollen and nectar (e.g. systemic compounds)

If a risk is predicted via this route, it may be possible to address this as outlined above ensuring that the concentrations in pollen and nectar are in line with those determined in semi-field studies (see above) and the Chapter 3. It will be important to ensure that the exposure profile in terms of duration is considered; for example in plants grown from treated seed residues may occur for the duration of flowering, hence bees will be exposed for many days possibly weeks. In these circumstances, it may be appropriate to use the crop of concern rather than a model species, ensuring that the residues in pollen and nectar are at least as high as those predicted in Chapter 3.

In carrying out a study as outlined in the two sections above it is important to consider Section 'study methodology for field study' above and in particular point 2. For solids, samples of pollen and nectar from the colony should be taken to ensure that the residue data match the toxicity study in terms of duration, i.e. 10 days; it is considered that in practice this means at peak bloom.

SEMI-FIELD STUDIES

BACKGROUND

Outlined below is guidance on how to determine the potential effects of a pesticide on honey bees under semi-field conditions. As for field studies, the guidance is split in to two parts, one for applications via spray and one for application of solids. If a semi-field study is to be undertaken it is important to ensure that the 90th percentile exposure is determined beforehand and that this is achieved in the study. If an adequate exposure is not achieved, the semi-field study will be of limited use. Please see the Chapter 3 to determine appropriate exposure levels.

Considering the Specific Protection Goals outlined in Chapter 2, it can be concluded that the key assessment endpoints from semi-field studies should be:

- colony strength, over-wintering capacity, honey production, behavioural effects, forager mortality

Small colonies are used in the semi-field studies and hence assessment of realistic impacts on colony strength and over-wintering capacity may be potentially difficult. Similarly, it is difficult to determine effects on honey production. Due to these issues, it is proposed that other endpoints, for example flight activity, foraging behaviour, behavioural abnormalities, observations of behaviour of bees at

colony entrance, observations of behaviour of colonies (e.g. aggressive) as well as daily assessments of adult mortality (e.g. counts of dead bees on linen sheets in the crop and in front of hives) should be determined.

Providing residues in pollen and nectar are considered to be at least as high as predicted as a result of the exposure assessment (see Chapter 3) and no adverse effects were observed under semi-field conditions, then it is proposed that no effects are likely under field conditions. In this case, a full-scale field study may be obviated except that a homing study should first be carried out to check that there are no unacceptable impacts due to navigation failure at realistic foraging distances. The homing study is necessary in order to address concerns raised in EFSA (2012a) regarding the limited ability of field studies to adequately assess potential adverse effects on behaviour of bees, and in particular effects on orientation and a subsequent effect on the ability of bees to return to the colony.

METHOD FOR APPLICATIONS VIA A SPRAY

As for field studies, it is proposed that the same methodologies should be used for semi-field studies under various modes of pesticide application.

Assessment methodology for semi-field study

(a) Definition of terms

- **'Plot'**: an area of crop with a single chemical regime - either treated or untreated (control) with the pesticide, i.e. it is appropriate to refer to a 'control plot'.

- **'Site'**: a location in the region for which the applicant seeks permission to use the pesticide. The site may include one or more plots i.e. a site may include both control and treated plots.

(b) Principles

The same principles as presented above are considered appropriate for semi-field studies as well.

(c) Exposure

Key to any study is ensuring adequate exposure. As stated above, the semi-field study must be designed to ensure that residues will be as predicted in the exposure assessment. In order to ensure adequate exposure, the Applicant may consider either carrying out multiple studies at various rates, or applying the pesticide at a high rate to ensure that residues in both pollen and nectar are appropriate so that they meet the concentrations determined in the exposure section – see Chapter 3.

In order to carry out a valid semi-field study, it is recommended to:

1. Carry out a number of studies in order to determine the residue of the active substance in the pollen and nectar of flowers of the treated crop. See Appendix J for further information.

2. This information will be used as a Higher Tier in the exposure assessment and hence can be used to refine the First Tier risk assessment (see Risk Assessment Schemes). If as a result, risk quotient(s) are breached, then it is recommended to carry out semi-field studies, alternatively risk mitigation may be considered (see Chapter 3). These studies can be used to determine both the effect of the pesticide as well as determine the residues in pollen and nectar in the colony under exclusive foraging as well as the flowers. The residue information can be used to estimate the ratio between residues in flowers with those in the colony which will provide an adjustment factor for compound degradation and metabolism of the active substance. This adjustment factor will be used to validate field studies if/when undertaken.

Design of semi-field study

Choice of crop

The choice of crop that can be used for this study is up to the Applicant. It may be possible to carry out this study with the proposed crop outlined on the label, alternatively it may be possible to use a representative crop, e.g. *Phacelia tanacetifolia* or oilseed rape and extrapolate the findings to a range of crops. The key issue in selecting a suitable crop is to ensure that it is attractive to honey bees and that the residues, and hence the exposure to honey bees, is at least as high as predicted in the exposure section.

Number of colonies and plots

Each plot should have one colony. The number of test and control plots must be high enough to account for the normal inter-colony and inter-plot variability and allow for statistical analyses (Principle f).

Please note that further work is required by the Applicant to determine the number of plots required.

Size of plots

In order to ensure appropriate exposure (Principle a), the treated and control fields should each be >60m^2 and preferably >80m^2 in area.

Colony size and health

The use of small colonies is required in the semi-field methodology compared to field tests due to limited forage area. Colonies should be of similar size and the strength should be adapted to the forage area but as large as possible. It is recommended to use colonies of at least 6000 adult bees and 3 to 4 brood combs (at least 15000 brood cells), containing a high amount of capped brood. The study should start, if possible, early in the season. Major modifications of the colonies shortly before application should be avoided. At least 4 replicates per treatment are recommended.

At the beginning of the experiment, all colonies (treatment and controls) must be in the same state (population size, health status). In order to reinforce the level of exposure of honey bees to the contaminated nectar and pollen, most of the frames containing food stocks should be removed from the colony before the beginning of the experiment to a level that just prevents starvation but allows sufficient stores for survival. It is acknowledged that this operation is difficult as it could cause a weakening of the colonies. It should only be conducted by experienced beekeepers.

All colonies should be of equal strength initially and then allocated to treatment (control, exposed) at random (principle e). Applicants should ensure that genetic variation is properly controlled. Ideally, the experimental colonies should initially comprise sister queens and identical numbers of adults taken from a common stock. In practice, variation from this is allowable, but wherever possible uniform initial colony composition should be achieved among the colonies allocated between the control and treated fields at each site.

The colonies should be healthy at the beginning of the experiment, e.g. free of clinical signs of significant brood diseases such as American Foul Brood (AFB) and European Foul Brood (EFB). As most of the European colonies, even strong ones, contain infectious agents, it is not possible to use colonies that are completely free of them. Regarding the mite *Varroa destructor*, present in almost all European colonies, the level of infestation of the control and test colonies should be as low as possible. During and after the experiment, the health of the colonies should be evaluated for the whole

range of bee diseases (including *Nosema, acarine* and the main viruses, e.g. through molecular screening).

Number of sites and location of plots

The sites should be representative of the region(s) for which authorization is sought. As regards location of the control and treated plots within a single site, it is recommended that they should be as similar as possible in terms of size and surrounding landscape.

At each site, the location of the control and treated plots should be decided at random (principle e).

Duration of study

It is recommended that the study assesses effects on all 3 stages of brood. There are significant advantages to interpretation if the effects of pesticides on eggs, young larvae and old larvae are assessed. It is proposed that the development of at least 100 eggs, 100 young larvae and 100 old larvae per colony should be used, preferably by the use digital imaging instead of acetate sheets. The contents of all cells including deformities in pupae should be assessed as well as weight of pupae before and after treatment to determine any adverse effects on development, e.g. delayed development.

Determination of exposure

Residue analyses

Residue analyses must be performed on the nectar and pollen in the treated semi-field. These analyses should have two goals: the first one, to check that the bees from the experimental hives have been exposed to the pesticide, and the second one to check that the control bees have not been exposed to the pesticide of the treated field or by another one, also present in the environment. If there are residues detected in the controls then the study is not valid. In addition, residues in nectar and pollen in the colonies should be determined. All the residue analyses should be realized with the lowest possible LOD and LOQ.

METHOD FOR APPLICATIONS FOR A PESTICIDE APPLIED VIA A SOLID

A semi-field study with a pesticide applied as a solid may be triggered for two reasons:

- The potential risk from deposition of dust on to adjacent crops/weeds, and directly on foraging bees when they are flying over or near the sowed field, or
- The presence of the active substance in pollen and nectar of the treated crop, weeds, or adjacent crops.

The design of these semi-field studies will be fundamentally the same as outlined above, but will differ in the following respects:

Exposure via dust

If a risk from dust is predicted, then it is proposed that a semi-field study as outlined above for sprays is conducted, however it is essential that the exposure is in line with that determined in residue data from previously conducted studies (see Appendix J).

Exposure via the presented of the active substance in the pollen and nectar

If a risk is predicted via this route, it may be possible to address this as outlined above ensuring that the concentrations in pollen and nectar are in line with those determined in semi-field studies (see

above) and the Chapter 3. It will be important to ensure that the exposure profile in terms of duration is considered; for example in plants grown from treated seed residues may occur for the duration of flowering, hence bees will be exposed for many days and possibly weeks. In these circumstances, it may be appropriate to us the crop of concern, ensuring that the residues in pollen and nectar are at least as high as those predicted in the Chapter 3.

METHODOLOGY FOR THE HOMING STUDY

The aim of this study is to determine whether an active substance causes an adverse effect on the ability of forager honey bees to return to the colony. It is proposed that the following approach should be taken:

1. The study should be a dose-response study with up to 3 doses. It is recommended to carry out a dose-response study rather than a single dose as this will be of more use should the use rates change and therefore the doses should be based on the potential exposure of honey bees foraging the crop. There should be a control and a positive control (e.g. high dose that causes a clear detrimental effect).
2. A total of 100 bees per dose group should be used. These should be young foragers that have not been exposed to the a.s. before.
3. In order to ensure that the bees are adequately dosed, they should be exposed to treated sucrose in the same manner as in the LD50 oral study.
4. All bees should be individually marked so that it can be determined if and when they return to the colony. It is proposed that either RFID tags are used or colour number tags are used.
5. Once exposed the bees should be taken to a distance of 1 km from their colony and released.
6. The returning bees should be recorded.
7. Statistical analysis should test whether the proportion of dosed bees that return successfully differs from control levels. If there is no significant difference between treatments, then no further work is required and it can be concluded that the a.s. does not adversely affect the homing ability of foraging honey bees. If there is an effect that is treatment and dose related, then the importance of this effect needs to be determined. This can be evaluated by using the method of Henry et al. (2012), which depends on the model presented in Khoury et al. (2010).

The above is a proposal to determine the potential effect of the a.s. on the homing ability of foragers. Comments on this proposal are welcomed, as are alternative approaches.

As presented above a semi-field study is required whenever a field study is required. This is so that the exposure in the field study can be verified. If effects are determined in the primary assessment endpoints of a semi-field study then a field study is required. In addition, a homing study is required.

If, however a semi-field study is conducted and no effects are determined on the primary assessment endpoints, no field study is required. A homing study is still required.

Views are requested on whether this approach is appropriate or whether, due to the potential short-comings of semi-field studies field studies should always be requested.

P. TEST PROTOCOLS FOR BUMBLEBEES (*BOMBUS TERRESTRIS*)

***Bombus terrestris* as key species in the Risk assessment for bumblebees**

The genus *Bombus* (family Apidae) comprises approximately 250 species and they are mainly distributed in the Northern Hemisphere with many more species and subgenera in Eurasia than in North America (Michener, 2007). *Bombus terrestris* is proposed as test species in the risk assessment scheme for bumblebees because:

1) This species is commercially reared for the pollination of agricultural and horticultural crops in Europe;
2) Several toxicological studies are available in literature on this species and some protocols are already suitable for inclusion in the risk assessment (see Opinion 2012 for full list of references).

At the moment official test protocols are not available for bumblebees. In this section the methods from literature to test compounds on *Bombus* spp. are proposed in outline (see EFSA 2012a for the full list of references) but they have to be fully developed and validated by ring-testing.

Laboratory tests

Acute oral toxicity test (Adults)

The acute oral toxicity test is designed to establish the oral LD50 (median lethal dose) value, i.e. the dose, expressed in µg of active ingredient per bee, inducing 50% mortality following oral exposure of measured amounts of active ingredients or commercial pesticide formulations.

In the oral toxicity test for *Apis mellifera* (EPPO 170 and OECD 213) a common feeder is provided to a group of workers assuming that, through trophallaxis, all individuals will receive similar doses of test solution. However, bumblebees do not show trophallaxis behavior and thus individual feeding is required.

Test procedure: For the laboratory toxicity test it is recommended to collect worker bees of average size and ages. Thirty bees individually caged per dose should be used and kept in dark conditions at 25±2°C during the test. Bees should be starved for about 2-3 hr before dosing.

For each test product, five concentrations are selected so as to range from 10 to greater than 100% mortality with no more than 2-fold dilutions between doses. A control of bees fed with only sugar solution is included in each test. The reference compound, 40% dimethoate or 20% parathion, is used as toxic standard. After a single exposure to the test solution (see mode of treatment), bumblebees can be housed together by dose feeding sucrose *ad libitum*.

Mode of treatment: Bees should be individually fed 10 µL of test solution using an individual feeder and a 2 hr dosing period.

Data assessment and reporting: After dosing, mortality and sugar solution consumption should be checked daily (and corrected for evaporation). The LD50 values (µg/bee) at 24, 48 and 72 h from exposure with 95% confidence limits have to be determined using Probit analysis. The test is valid if the mortality in control is <=10%.

Acute contact toxicity test (Adults)

The OECD 214 protocol for contact toxicity test in *A. mellifera* can be easily applied to bumblebees or other species of bees. The endpoint of this test is the contact LD50 (µg/bee) following topical exposure.

Test procedure: As the acute oral toxicity test.

Mode of treatment: Bees are anaesthetised (by carbon dioxide, for example) for as short a time as possible until they stopped moving. One µL of test solution is then pipetted onto the ventral part of thorax between the 2nd and 3rd pairs of legs.

The test solution is prepared by dissolving each compound in acetone. A negative control with acetone and a positive one with either dimethoate or parathion are also recommended.

Data assessment and reporting: as the acute oral toxicity test.

Chronic oral toxicity test (Adults)

The chronic oral toxicity test is designed to establish the oral LC50 (median lethal concentration) value expressed in mg of active ingredient per kilogram of food ingested.

Because no official guideline is available the following protocol is based on the studies available in literature. In particular the use of bumblebee microcolonies in laboratory conditions is recommended (see Mommaerts et al. 2010) in order to cover a wide range of endpoints.

Test procedure: the study is performed with worker bumblebees under standardized laboratory conditions of 28–30 °C and 60–65% RH (Relative Humidity) and continuous darkness. The insects should be fed *ad libitum* with sugar solution and commercial pollen as energy and protein source, respectively. Newly emerged workers should be collected from the bumblebee colony and five workers should be placed in an artificial nest box (i.e., 15 cm x 15 cm x 10 cm). In each nest box a worker will normally become dominant and begin to lay the eggs within a week, playing the role of a queen (only male progeny because the false queen is not inseminated). The four other workers help the false queen for brood care, which mainly consisted in feeding larvae, building and heating cells.

Mode of treatment: The duration of the exposureis chosen to reflect the environmentally relevant period of exposure, which depends on the blooming period of the crop. In the experiment, the adult workers should be exposed orally to the test compound via syrup feeders over a period up to 11 weeks, or bees can be fed for a period of 30 days after which they are then provided for 30 days with untreated food. The experiment requires a range of different concentrations and in the control nests, workers were exposed with untreated sugar solution. For each concentration, at least four artificial nests, each containing five worker bees, should be used. Each experiment should be repeated twice.

This protocol can be improved using the new bioassay of Mommaerts et al. (2010) in order to assess the impact of sublethal concentrations on the bumblebee foraging behavior under laboratory conditions. In brief, the experimental setup of this behavior test consists of two artificial boxes connected with a tube of about 20 cm and use of queenless microcolonies of 5 workers. One box is used as nest where the worker bees rear the brood, the other box is used for the food (sugar and pollen). Before exposure (for 2 days), the worker bees are allowed a training to forage for untreated food; afterwards this is replaced by treated food.

Data assessment and reporting: In the artificial nest boxes, worker survival should be evaluated daily for the first 3 days post treatment and then on a weekly basis for a period of up to 11 weeks. The adverse sublethal effects on reproduction should be monitored on a weekly basis for 11 weeks by scoring the numbers of offspring (total number of eggs, larval brood and adults) and/or drones produced per nest. These data are used to calculate the LC50 and the NOEC50 (expressed in mg/kg).

Oral toxicity test (larvae)

In this section a protocol to study the effects of pesticides (with specific reference to IGR) to larvae of bumblebees in laboratory conditions based on data available in literature (see in particular Gretenkord and Drescher 1996). In this protocol the toxicity of pesticide on brood is tested when the substance is

ingested by workers for 24 hours. At the moment there is no protocols to test the pesticide directly to brood.

Test procedure: Eggs should be removed from a queenright *Bombus terrestris* colonies and incubated in the laboratory (at 32°C and 55-60% HR) until hatching. For each test concentration, 10 young larvae have to be placed in small rearing boxes at 28°C and 50±5% HR. In each box, three nurse workers should be added with sucrose syrup and pollen. On the 7th day, the first larvae begin to pupate. After pupation, workers should be removed and the brood have to be reared until adult emergences.

Mode of treatment: The test substance have to be dissolved in the food and fed to the test groups for 24 hours. The exposure of the larvae to the test substance is carried out with larvae 1, 4 or 6 days old, each for 24 hours. For each larval age and each test substance three replications are necessary.

Data assessment and reporting: The amounts of pollen consumed by the larvae and the numbers of larvae developing into an adult have to be determined. To determine the amount of food consumed by the larvae, the amount consumed by a test group of larvae and a test group of 3 workers without larvae are compared. With these data the average consumption of each larva can be estimated.

Semi-field tests

Semi-field tests are higher-tier studies conducted in field cages or greenhouse cages or glasshouse compartments and they may be triggered as a result of possible concerns during laboratory studies in the Tier 1. By far the majority of higher-tier studies in bumble bees have been conducted in the glasshouse due to the widespread use of bumblebees for pollination. At the moment there are no formalised guidelines but a number of methods have been published (see EFSA 2012a for the full list of references or review in van der Steen, 2001). In this section the protocol from Tasei *et al*. (1993) is proposed but the method will need further development because the main problem with the use of crops in small compartments is that there is not enough pollen and nectar available in the cages for a colony of normal size and adding pollen and sugar syrup can dilute the possible effects.

Test procedure: Small bumble bee colonies are placed in glasshouse compartments (3 m x 2 m) containing flowering plants (2 m^2). *Phacelia tanacetifolia* plants should be used as crop.

Mode of treatment: The crop is spray with the pesticide at the recommended concentration.

Data assessment and reporting: Assessment endpoints can be similar to those used in semi-field trials of honey bees and may include adult and larval mortality, colony strength, amount of brood and foraging activity.

Field tests

Several approaches have been used to assess the effects of applications of pesticides on bumblebee colonies in the field (see Opinion 2012a for full list of references). In this section we proposed a protocol described by Schaefer and Mühlen (1996). However, significant further work is required to develop guidelines, including the minimum field size, number of colonies per treatment, methodology for dead bee assessments and foraging assessments and agreement of appropriate approaches for determining colony development. The recent paper of Whitehorn et al. (2012) can be used as alternative field test or as complementary test in the Higher Tier.

Test procedure: Six small bumble bee colonies (less than 50 workers) should be placed in a treated field (2400 m^2) with flowering *Phacelia tanacetifolia*. A further six are placed in a control field.

Mode of treatment: The crop should be spray with the pesticide at the recommended concentration three days after colony introduction. The control field should be treated only with water.

Data assessment and reporting: Assessments of effects should include colony vitality (numbers of brood, workers, and honey pots, and weights of queens, workers and whole colonies with hives), workers foraging activity (forager density on 5x1 m^2 spots and the flight activity for 10 minutes every day at the hive entrance), marking all introduced workers to assess homing rate and growth rate of the colony, and defensive response to an aggressive stimulus. Pollen and nectar sampling for residue sampling and assessment of forage should be undertaken by collecting foragers returning to the colonies.

Q. TEST PROTOCOLS SOLITARY BEES (*OSMIA CORNUTA* AND *OSMIA BICORNIS=O. RUFA*)

***Osmia cornuta* and *Osmia bicornis* (=*O. rufa*) as key species in the Risk assessment for solitary bees**

Two mason bees of the genus *Osmia* (*O. cornuta* and *O. bicornis*) are proposed as test species in the risk assessment scheme for solitary bees. *Osmia cornuta* and *O. bicornis* are very closely related species from Paleartic region, and share many life history and behavioral traits. *O. cornuta* is distributed in central and southern Europe, Turkey and parts of North Africa and the Middle East (Peters, 1977). *O. bicornis* can be found also in northern Europe (fig. 1). These two species can be suitable as key species because:

1) species of the genus *Osmia* are already used in ecotoxicological studies and some protocols are available in literature (see Opinion 2012 for the full list of references).
2) these species are quite easy to rear and it is possible to obtain large populations (Bosch et al. 2008; Krunic and Stanisavljevic 2006);
3) compared with other species of solitary bees, the biology of these species is well known (Bosch et al. 2008);
4) they are economically important species and management methods have been developed to use various *Osmia* species as commercial pollinators used in crop pollination in Asia, North America and Europe (Bosch and Kemp 2002);
5) the genus *Osmia* comprises more than 400 species in the world and their show several behavior and life cycle traits representative of many species of solitary bees nesting above the ground.

They show also some limitations:

1) the soil exposure contamination could be underestimated in *Osmia* if compared with the ground-nesting bees. In fact, *Osmia* spp. nest in pre-established cavities in which females build series of cells separated by mud partition, however, compared to the ground-nesting species, the genus *Osmia* are less exposed to pesticide applied into the soil;
2) *Osmia cornuta* and *O. bicornis* populations fly early in the year for about 2-3 months and are univoltine. This means the tests can be carried out only during spring.

Others two species were used in toxicological studies (*Nomia melanderi* and *Megachile rotundata*) in US because they are widely used as alfalfa crop pollinator in North America but not in Europe.
At the moment official test protocols are not available for solitary bees. In this section the methods from literature to test compounds on *Osmia* spp. are proposed (see EFSA 2012a for the full list of references) but they have to be ring-tested and validated. In order to obtain standardized results, it is recommended that *Osmia* populations used in the tests are reared under optimal temperature conditions according to their geographical origin (Bosch et al. 2008; Sgolastra et al. 2012).

Laboratory tests

Acute oral toxicity test (Adults)

The acute oral toxicity test is designed to establish the oral LD50 (median lethal dose) value, i.e. the dose, expressed in µg of active ingredient per gram of bee, inducing 50% mortality following oral exposure of measured amounts of active ingredients or commercial pesticide formulations. After emergence, each bee should be weighed in order to calculate the LD50 expressed in µg/g of bees.
In the oral toxicity test for *Apis mellifera* (EPPO 170 and OECD 213) a common feeder is provided to a group of workers assuming that, through trophallaxis, all individuals will receive similar doses of test solution. However, the current oral toxicity tests cannot be applicable to non-*Apis* bees because most other bee species don't show trophallaxis behavior and thus a individual feeding is required.

Test procedure: During spring, *Osmia cornuta* (or *Osmia bicornis*) females should be used to run the test approximately 24 h after emergence from their cocoons. Females should be starved overnight and than exposed to a compound the next morning.
For each test product, five concentrations are selected so as to range from 10 to greater than 100% mortality with no more than 2 fold dilutions between doses. A control with bees feed with only sugar solution is included in each test. The reference compound, dimethoate, is used as toxic standard. After single exposure to test solution (see mode of treatment), three set of 10 bees for dose are transferred to a holding cage, provided with an artificial feeder. The artificial feeder can consist of a 5 mL-LDPE sample vial, containing a sucrose solution, with a soaked cigarette filter inserted through the lid of the vial.
During the test bees are kept in an incubator at: t = 22 °C, R.H. = 60–80%, L:D = 12:12 h.
Mode of treatment: Osmia females should individually fed 10 µL of test solution using an individual feeder with the "flower method" proposed by Ladurner et al. (2003). In the "flower method" the test solution is pipetted into a plastic ampoule, inserted into the calyx of a flower (i.e. cherry, *Prunus avium* L.). Flowers and bees are individually housed in holding cages and kept in an incubator at 22 °C under artificial light for 1 h.
Data assessment and reporting: the LD50 values (expressed in µg/g of bee) at 24, 48 and 72 h from exposure with 95% confidence limits have to be determined using Probit analysis. LD50 after 7 days from exposure should be calculate if the mortality is still increasing. Mortality data are corrected for control mortality using Abbott's formula.

Acute contact toxicity test (Adults)
Methods used to study contact toxicity in *A. mellifera* can be easily applied to other species of solitary bees including *Osmia cornuta* and *O. bicornis*. The endpoint of this test is the contact LD50 (µg/g of bees) following topical exposure.
Test procedure: see the acute oral toxicity test.
Mode of treatment: Osmia females are cooled at 4 °C (for a maximum of 30 minutes) until they stopped moving. One µL of test solution is then applied to the dorsal surface of the thorax. The test solution is prepared by dissolving each compound in acetone and purified distilled water (50% v/v) to obtain desired concentrations.
Data assessment and reporting: see the acute oral toxicity test.

Chronic oral toxicity test (Adults)
The chronic oral toxicity test is designed to establish the oral LC50 (median lethal concentration) value expressed in mg of active ingredient per kilogram of food ingested.
As for the acute oral toxicity test a common feeder cannot be applicable to non-*Apis* bees thus, an individual feeding is required. A new artificial feeding method to provide test solutions to adult solitary bees *ad libitum* was developed by Konrad et al. (2009).
Test procedure: During spring, newly emerged females of *Osmia cornuta* (or *O. bicornis*) should be used to run the test. For each test product, five concentrations are selected so as to range from 10 to greater than 100% mortality with no more than 2 fold dilutions between doses. A control with bees feed with only sugar solution is included in each test. Thirty bees per concentration should be used and individually caged with the artificial feeder (see mode of treatment). During the test, bees are kept in an incubator at: t = 22 °C, R.H. = 60–80%, L:D = 12:12 h.
Mode of treatment: Osmia females should individually fed the test solution for 10 days using the individual feeder proposed by Konrad et al. (2009). The feeders are prepared by cutting off the Luer tips (leaving a drinking hole of approximately 2 mm in diameter) of a 5 ml-syringes and then affixing rings of yellow and blue adhesive tape around the drinking hole as colour cues. Syringes are filled with 1 ml test solution and two fresh flower petals of oilseed rape are pinned next to the drinking hole. Only bees that successfully drink from the test solution are used for the test.
Every day the feeders should be removed and replaced with fresh feed so that bees has continuous access to the treated feed throughout the study. The amount of sugar solution consumed by a bee

between two syringe replacements are determined by weighing the syringe before and after exposure. Weight loss due to evaporation is measured with control.
Data assessment and reporting: bee mortality and behaviour is recorded daily in order to calculate the LC50 and the NOEC values (expressed in mg/kg) after 10 days of chronic exposure to pesticide.

Oral toxicity test (larvae)
Unlike honey bee larvae that feed primarily on secretions (brood food or royal jelly) from nurse bees, the eggs of most non-*Apis* species are laid directly on a loaf of pollen mixed with nectar, on which the larvae feed. That provision may contain much higher levels of pesticide contamination than the glandular secretions of nurse bees on which honey bee larvae feed. In literature some tests are available for *Megachile rotundata* and *Osmia* spp. in laboratory conditions (see EFSA, 2012a for full list of references) however they need to further improvements. A critical point is to obtain an homogeneous distribution of the test product in the mass provisions.
Test procedure: Provision masses with eggs are obtained from nests of *Osmia cornuta* or *Osmia bicornis* released in glasshouse or in an organic field with flowering oilseed rapes or other attractive crops for *Osmia* spp. (i.e. phacelia). Artificial nests can consist of wood blocks with drilled holes filled with paper straws. During nesting period, nests should be checked daily and newly-plugged paper straws (completed nests) are pulled out of the wood block and taken to the laboratory. Nests are then dissected and provisions with eggs are weighed and individually placed in clay wells or in 48-well culture plates. Eggs were sexed based on provision size and cell position within the nest (females are produced deeper in the nest and are assigned larger provisions). After the pesticide application (see mode of treatment), the clay wells or the culture plates with provisions and eggs are transferred in an incubator at constant temperature condition until adulthood (late summer). The optimal temperature condition during development and the period of adult eclosion depends on the species and the origin of the population used in the test (Bosch et al. 2008; Sgolastra et al. 2012; Figure Q1). In the autumn, after ~ 30 days from adult eclosion, the bees are cooled for wintering (15 days at 14 °C + 150 days at 3-4 °C). After wintering, bees inside the cocoons are removed from the wells and individually caged with water availability but no food. Cocoons are checked daily for emergence of adult bees and their survival will be recorded.
Mode of treatment: Test product should be distributed within the mass provision as evenly as possible without removing the attached egg. The test product can be dissolved in water reaching the desired concentration and 50 µL of this solution per gram of provision is delivered into a longitudinal fissure or in an hole previously formed in the provision mass. Five different concentrations should be tested in order to calculate the LC50.
Data assessment and reporting: The fresh pollen provisions with the attached eggs are weighed before treatment. Larval development and mortality are observed daily until cocoon spinning. Bee mortality is observed and recorded also after emergence. The LC50 is calculated from percentage of bee mortalities (total number of bees dead during the development and not emerged from the cocoon after incubation). Other endpoints can be: the NOAEC (considered the highest concentration which do not induce mortality significantly higher than that observed in control), the longevity, the larval development duration (from egg to the completion of cocoon spinning). Usually, eggs are dated assuming a cell production rate of 1 cells/day.

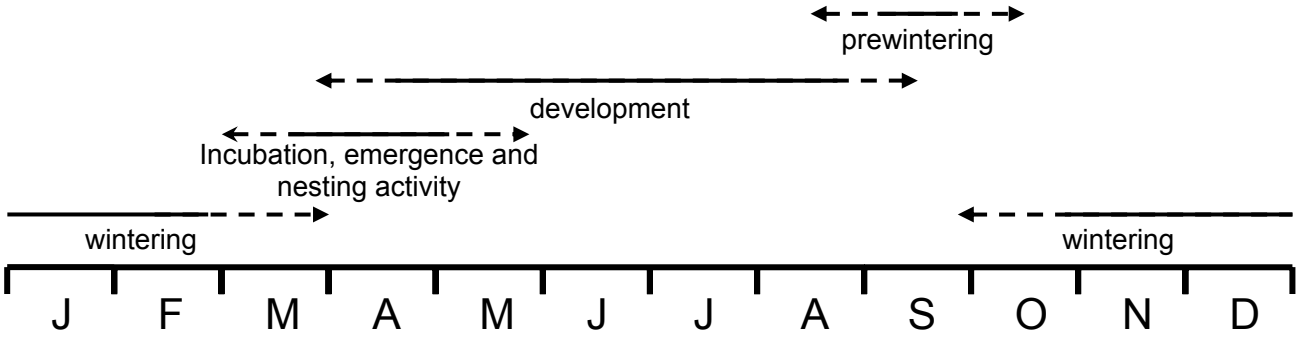

Figure Q1: Life cycle and phenology of a univoltine *Osmia* species. The phenological variability in *Osmia* populations from different geographic area is indicated by dashed lines.

Semi-field tests

Semi-field tests are higher-tier studies and they may be triggered as a result of possible concerns during laboratory studies in the Tier 1. Moreover, semi-field and field tests are more appropriate to test sub-lethal effects (nesting behaviour) of pesticide to solitary bees.

There are no standardized guidelines but a number of methods have been published to test pesticides on solitary bees in cage, tunnel or glasshouse conditions (e.g. Ladurner et al. 2008 but see EFSA, 2012a for the full list of references).

Test procedure: Nesting females of *Osmia cornuta* or *O. bicornis* are forced to forage on a attractive flowering crop in field cages. Common pollen-nectar sources for *O. cornuta* and *O. bicornis* are *Phacelia tanacetifolia* Benth and the oilseed rape (*Brassica napus* L.). With the onset of bloom, cages of ~40 m^2 each are confined within the field with anti-aphid screen cages (mesh size ≤ 3mm) and a nesting shelter should be placed in the center of each cage. Nesting shelters can consist of several wood blocks with drilled holes filled with paper straws. To facilitate observations, nesting cavities can numbered with white grease pencils.

During full bloom, new emerged females of *O. cornuta* or *O. bicornis* are released with an adequate number of males in the cages. From 10 to 15 individually marked females and 15-20 males should be released in each cage. After starting of nesting activities (once at least five females per cage has established) the active ingredient is applied in the crop.

Mode of treatment: Test product should be applied in separate cages at the highest recommended field rate when bees are actively foraging on the crop. However, this may be modified if appropriate for the objective of the study (e.g. when testing systemic compounds applied pre-flowering or for assessing mitigation measures). One cage should be treated only with water (control) while an other one should be treated with a toxic standard. Each cage should be randomly assigned to a treatment. More cages per treatment can be used as replicates.

Data assessment and reporting: Observations on nesting activity should be performed before and after treatment in each cage. The number of nesting females and other parameters should be recorded on day 0 (day of treatment for evening applications; day before treatment for morning application), and on days 1, 2 and 4. In case of systemic pesticides, the assessment period can be extended. For each nesting female, the following parameters are recorded on each of assessment days:

- In-nest time: the time spent inside the nest depositing pollen and nectar load in the morning during 1 hr of observation;
- Foraging time: the time spent outside the nest foraging for pollen and nectar in the morning during 1 hr of observation;
- Bee mortality: nesting cavities are inspected with a flashlight every night and the number of females inside is counted (night counts), in fact *Osmia* spp. females spend the night in their nesting cavity;
- Cell production rate: during the nigh counts, paper straws containing females are removed with forceps and nest progression is marked and dated on each straw.

Four days after treatment the cages can be opened in order to allow the free foraging activity of bees. At the end of the nesting activity, the marked nests are brought to the laboratory and dissected to record larval mortality. Temperature and relative humidity inside the cages should be recorded throughout the study. The endpoints (bee mortality rate, cell production rate, foraging and in-nest times, progeny survival) are compared between treatments with appropriate statistical analysis.

Field tests

Field studies are required when concern has not been adequately addressed at lower tiers. They can be suitable to study the sublethal effects in solitary bees under the worst case scenario in natural conditions. At the moment field studies are not available in literature for *Osmia* spp. (see EFSA, 2012a for reference). In this section it is proposed a protocol adapted from a study on *Megachile rotundata* (Torchio, 1983).

Test procedure: Nesting females of *Osmia cornuta* or *O. bicornis* are released in nesting shelters placed in the centre of test fields of flowering crops. Nesting shelters can consist of several wood blocks with drilled holes filled with paper straws. To facilitate observations, nesting cavities can numbered with white grease pencils. Test should be performed in spring during the natural period of *Osmia* nesting activity in according with the local climatic conditions. During blooming (with ~15% of open flowers), at least 400 nesting females with a relative number of males (ratio 1♀:2♂) should be released per hectare of field. Compared with honey bees, solitary bees show much smaller foraging area (range: 200-400 m) thus, a smaller size of field is necessary and the distance of 1 Km between nesting shelters should be sufficient for preventing cross-foraging between test and control fields. Alternatively, a large field divided into two nearly equal parts can be used. Each of these "half-field" (plot) is subsequently used as treatment or control field. In any case, at the end of the nesting period, accidental cross-foraging can be verified by residue analysis of the mass provisions. After starting of nesting activities and in coincidence with the full blooming, the active ingredient is applied in the crop.

Mode of treatment: Test product should be applied in the crop at the highest recommended field rate during daytime (when bees are actively foraging on the crop) or in the evening (if appropriate for the objective of the study). Control field/plot should be treated only with water and more fields/plots per treatment can be used as replicates. During spray applications, the nesting shelters should be protected from spray drift.

Data assessment and reporting: Observations on nesting activity should be performed before and after treatment in each field/plot. The number of nesting females and other parameters should be recorded on day -2, -1, 0 (day of treatment for evening applications; day before treatment for morning application), and on days 1, 2, 3, 4, 7. In case of systemic pesticides, the assessment period can be extended till the end of the blooming period. On each of the assessment days, the following parameters are recorded:

- Active nests: nesting cavities are inspected with a flashlight every night and the number of females inside is counted (night counts), in fact *Osmia* spp. females spend the night in their nesting cavity;
- Cell production rate: during the nigh counts, paper straws containing females are removed with forceps and nest progression is marked and dated on each straw.

For substances for which effects on growth or development cannot be excluded, it is possible to survey the progeny development and survival transferring the nests in laboratory. Progeny should be reared under standardized temperature conditions till next spring and the percentage of bee survival recorded (see laboratory test for larvae). The endpoints (number of active bees, cell production rate and progeny survival) are compared between treatments with appropriate statistical analysis.

R. TEST CROPS TO BE USED

Spray applications

The EPPO 170 (4) describes that for testing of effects on honey bees following spray applications that in the first instance, rape, mustard, *Phacelia* or another crop highly attractive to bees should be used as test plants, e.g. in the case of a standard semi-field or field trial based on acute toxicity.

The EFSA working group recommends *Phacelia* to be used in semi-field and field tests because of the following reasons:

1. It is a worst case crop for spray applications as the highest exposure can be achieved due to
   - maximum contamination of nectar and pollen in flowers is expected, as nectaries and anthers are directly exposed to the spray
   - Very high attractivity for bees
   - Very high density of foragers in semi-field and field trials per m²

2. It is a crop which has features making it particularly suitable for semi-field and field tests because:
   - Pollen is visually easy to distinguish from all other pollen sources (by purplish colour)
   - Flowering period can be adapted to time with low alternative forage in the surrounding to maximize exposure
   - Several plantings in season possible resulting in flowering at different times allows testing e.g. at different times of year according to GAP or assessment of repeated applications
   - ability to extrapolate the risk assessment carried out on *Phacelia* to a range of other crops

In the EPPO 170 (4) guideline it is stated that in other cases, identification of a surrogate (worst-case) test crop may be more difficult, e.g. for systemic compounds, where the test crop should be one for intended use.

This would also be recommended by the working group; for seed treatments the target crop e.g. Winter oilseed rape should be used. If the test is conducted with a crop which is not the target crop, residue analysis of nectar and pollen are required to determine the level of exposure to residues in these matrices.

S. CALCULATION OF THE ORAL EXPOSURE WITH WORKING EXAMPLES

Knowing the residue levels that may occur in nectar and pollen (PECnectar and PECpollen) and the consumption of these items by the bees and bee larvae, their exposure can be calculated using the formulas Eqn S1 or Eqn S2, below.

$$ORI = \frac{(PECpollen \times Cp) + (PECnectar \times Cn)}{1000} \quad \text{(Eqn S1)}$$

$$ORC = \frac{(PECpollen \times Cp) + (PECnectar \times Cn)}{Cp + Cn} \quad \text{(Eqn S2)}$$

Where: PECpollen is residue level in pollen (mg/kg)
PECnectar is residue level in nectar (mg/kg)
Cp is consumption of pollen in mg (mg/bee/day for adults or mg/larva)
Cn is consumption of nectar in mg (mg/bee/day for adults or mg/larva)
ORI is the overall residue intake expressed in µg/bee/day
ORC is the overall residue concentration in the diet expressed in mg/kg

The overall residue intake will be necessary to be calculated to compare with the LC_{50} value obtained from the chronic toxicity test on adult bees (calculation of ETR_{adult}). The overall residue concentration will be compared to the NOEC/NOAEC from the larval test (calculation of ETR_{larvae}).
It should be taken into account that for each assessment, several PEC values need to be generated such as PEC for the target crop, for weeds (except for seed treatments); for field margins, for adjacent crop and for succeeding crops (unless if the compound is not-persistent). However in the risk assessment, the highest PECs should always be used. For details regarding the calculation of PEC values, chapter 3 of the GD needs to be consulted.
As a screening step, the default residue values can be used as indicated in Table S1.

Table S1: Default conservative RUD or PEC values to be used in a screening assessment

Scenario	Residue level to be considered	Comment
For all PECs, except PEC for the target crop if the application is seed treatment	RUDnectar – 21 mg/kg RUDpollen – 150 mg/kg*	To derive PECs, these values needs to be multiplied with the application rate expressed in kg/ha before used in the risk assessment. Additional adjustment factors may be applied pending on the exposure flowchart that is followed (see chapter 3).
Seed dressing application for the target crop	PECnectar – 1 mg/kg PECpollen – 1 mg/kg	Considered as absolute values independently from the application rate.

*: the highest RUD values from Table 1 of Appendix I (rounded up from 20.7 and 149.8 mg/kg) are recommended to be used as default for screening, considering that the available data set for default RUDs is relatively small

Data for consumption of nectar and pollen by adult bees and larvae are indicated in Tables S2 and S3. The consumption data originates from EFSA, 2012a, except where a footnote clarifies the origin. Only the most exposed type/cast of bees are considered here (e.g. drone honey bees eat less diet than foragers or nurse bees, therefore a scenario for drones is not necessary). Since in most of the cases the energy demand of the bees or larva is available (sugar consumption) rather than the nectar

consumption, the sugar content of the nectar needs to be considered. The sugar contents of nectar, which maybe foraged by the bees, were agreed by the group of experts based on information from the scientific literature (Nicolson, 2008; Maccagnani et al., 2003; Monzon et al., 2004). It was noted by the working group that only very little is known about the distribution and frequency of the sugar content carried by bees and it was identified that further research are needed in this field. It was also noted that for example the nectar consumption of a forager honey bee varies largely on several factors, therefore the variation of the overall exposure of the colonies should be considerable.

Table S2: Data to be considered for nectar and pollen consumption by adult individuals

	consumption of sugar (mg/bee/day)	**sugar content of nectar (%)**	**consumption of pollen (mg/bee/day)**
Honey bee	forager: 32-128 nurse: 34-50	15-65	forager: - nurse: 6.5-12
Bumble bee	worker: 73-149	15-60	26.6-30.3
Solitary bee	female osmia 18-77[1]	10-60	10.2[2]

[1]: this value was erroneously reported as nectar consumption in EFSA, 2012a
[2]: estimated from bumble bee queen pollen consumption (Pridal et. al., 1996) considering the difference in bodyweight

Table S3: Data to be considered for of nectar and pollen consumption by a larva

	consumption of sugar (mg/larva)	**sugar content of nectar (%)**	**consumption of pollen (mg/larva/)**
Honey bee	59.4/5 days	15-65	1.5-2/5 days
Bumble bee	23.8/day	15-60	22-23/day
Solitary bee	54 mg nectar/30 days[1]	-	488 mg/30 days

[1]: this value refers to nectar instead of sugar (the sugar content of the nectar used in the study from where the data originate is assumed to be around 10 %)
Note: The data for honey bee larva refer to worker larva. The difference in the ratio of pollen and nectar consumption of drone larvae to worker larvae is negligible, therefore no separate scenario for drone larvae was considered necessary.

For the screening step, as a simply worst case approach, the 90th percentile of the ranges of consumption of nectar and pollen was calculated. In case of nectar, first the worst case sugar consumption was combined with the worst case sugar content and the best case sugar consumption with the best case sugar content to get the consumption ranges for adults. For example for honey bee forager the consumption of 128 mg sugar combined with 15% sugar content resulted in the maximum nectar consumption of 853 mg. The minimum consumption was calculated similarly (32 mg sugar consumption combined with 65% sugar content) and resulted in 49 mg (the 90th % of the range 49-853 was further considered). For larvae, the 90th% sugar content of nectar was combined with the relevant consumption data (consumption always a single value for larvae). It is noted that when more than one variable is considered (for most of the scenarios this was the case), the overall exposure level will be higher than 90th%. In case of solitary bee larva there was no variable. In this case simply the reported values were used. The values for the consumption to be used for the screening step are reported in Table S4.

Table S4: Nectar and pollen consumption (conservative estimates) to be used for the screening steps

	consumption of nectar by adults (mg/bee/day)	consumption of pollen by adults (mg/bee/day)	consumption of nectar by larvae (mg/larva)	consumption of pollen by larvae (mg/larva)

Honey bee	forager: 773 nurse: 305	forager: 0 nurse: 11.5	297	1.95
Bumble bee	906	29.9	159	22.9
Solitary bee	696	10.2	54	488

It is acknowledged that this approach is conservative and assumes higher exposure level than the target 90th%. The overall 90th% exposure can be calculated considering the variation of nectar and pollen concentrations combined with the variation of the consumptions of the feed items. Since the variation in nectar and pollen concentrations varies from pesticide to pesticide it is not possible to establish default percentiles for the consumption data, which can always be used (note that using the values in Table 4 will always result a higher exposure level than overall 90th%). Therefore, when the nectar and pollen concentrations of a pesticide molecule under evaluation is available, it is recommended to undertake a statistical exercise to identify the percentiles of the ranges of nectar and pollen consumptions and the ranges of sugar content of nectar to be combined with the variation of the residues to calculate the overall 90th% oral exposure.

Shortcut values and shortcut calculations

The consumption data reported in table S4 can be combined (using equations Eqn S1 or Eqn S2) with the default worst case RUD values (for the first screening steps) or with the calculated PEC values (which also based on the default RUDs in the initial steps). Table S5 contains the shortcut values considering the default RUD values and Table S6 contains the simplified equations to be used with the PEC values. It has to be noted that in case of seed treatment, for PEC calculations for the target crop the default of 1 mg/kg shall be used for both pollen and nectar (and not the values from Table S5). For further details see Table S1, above.

Table S5: Shortcut values based on default RUD values and conservative feed consumption of different bees and bee larvae

	the overall residue intake (µg/bee/day) to be used in calculation of ETR_{adult}	overall residue concentration (mg/kg) to be used in calculation of ETR_{larvae}
Honey bee	16.2	21.8
Bumble bee	23.5	37.2
Solitary bee	16.1	137.1

Notes: These values needs to be multiplied with the application rate expressed in kg/ha. Additional adjustment factors may be applied pending on the exposure flowchart that is followed (for details see chapter 3)
For seed teratment for the target crop use PECpollen and PECnectar of 1 mg/kg

Table S6: Simplified calculations taking into consideration conservative feed consumption of different bees and bee larvae

	the overall residue intake (µg/bee/day) to be used in calculation of ETR_{adult}	overall residue concentration (mg/kg) to be used in calculation of ETR_{larvae}
Honey bee	forager: 0.773 x PECnectar nurse: 0.305 x PECnectar + 0.0115 x PECpollen	0.9935 x PECnectar + 0.0065 x PECpollen
Bumble bee	0.906 x PECnectar + 0.0299 x PECpollen	0.8741 x PECnectar + 0.1259 x PECpollen

Solitary bee	0.696 x PECnectar + 0.0102 x PECpollen	0.0996 x PECnectar + 0.9004 x PECpollen

Hypothetical working example (oral exposure):

Pesticide X is used as spray in winter cereals in late growing stages, which encompass the time of flowering (e.g. May). The highest recommended application rate is 400 g a.s./ha.

- The toxicological profile is the following (keys: HB - honeybee, BB - bumble bee, SB - solitary bee):

  Oral LD_{50} for HB: 0.3 µg a.s./bee
  Oral LD_{50} for BB: 0.5 µg a.s./bee
  Oral LD_{50} for SB: 0.6 µg a.s./bee
  LC_{50} (HB): 0.03 µg a.s./bee
  $NOEC_{brood}$ (HB): 2.0 mg a.s./kg

- The calculations of Hazard Quotient (HQ=application rate/toxicity endpoint) using the oral LD_{50} values resulted in HQs of 1333, 1000 and 800 for HB, BB and SB, respectively. All HQs are above the relevant triggers (33, 5.5, 2), indicating high potential for acute risk.
- As suggested by the relevant flowchart in chapter 3, Pesticide X should not be used when honey dew occurs (unless special assessment is made to address this issue).
- Independently of these results, the chronic risk to adults and the risk to larval development needed to be addressed.
- The exposure chapter offers a screening step, which assumes that the bees will be exposed to the default worst case concentrations (default RUDs x application rate) that occur in flowering weeds in the treated field. The shortcut values (Table S5 above) includes the default RUD values and conservative estimations for consumption of nectar and pollen. Considering an application rate of 400 g/ha, the following conservative intake/overall concentrations and ETR values will be obtained:

	ORI	**ETR_{adult}**	**ORC**	**ETR_{larva}**
HB	16.2x0.4 = 6.5	6.5/0.03 = 216	21.8x0.4 = 8.7	8.7/2 = 4.4
BB	23.5x0.4 = 9.4	9.4/0.03 = 314	37.2x0.4 = 14.9	14.9/2 = 7.4
SB	16.1x0.4 = 6.5	6.5/0.03 = 215	137.1x0.4 = 54.9	54.9/2=27.4

- All ETR values are above the relevant triggers (0.03, 0.0024 or 0.0027 and 0.1 or 0.01), therefore further steps need to be considered.
- Since cereals are not considered to be attractive to pollinators, logically the exposure to target crop will not be the one, which drives the risk assessment and the concentrations in other crops within the foraging area should be lower than the concentrations for the target crop. Therefore, PEC calculations for nectar and pollen were undertaken using the recommendations of chapter 3. The first set of calculations still used the default RUD values and resulted in the following PEC values:

	PECpollen (mg/kg)	**PECnectar (mg/kg)**
target crop (cereal)	0	0
weeds	18	2.5
field margin	0.54	0.076
adjacent crop	0.18	0.025
following crop	0.002	0.002

- The updated risk assessment using the PECs calculated for weeds resulted in the following values:

	ETR_{adult}	**ETR_{larva}**
HB	forager: 1.9/0.03 = 64 nurse: 1.0/0.03 = 32	2.6/2 = 1.3
BB	2.8/0.03 = 93	4.5/2 = 2.2
SB	1.9/0.03 = 64	16.5/2=8.2

- Still, all ETR values are above the relevant triggers (0.03, 0.0024 or 0.0027 and 0.1 or 0.01), therefore further steps still need to be considered.
- To further refine the exposure estimates, field residue trials were undertaken in relevant crops, which represent relevant weeds that occur in the field at the time of application, therefore extrapolation is reliable. The measured concentrations were two and three order of magnitude lower than the PECs estimated using the default RUDs.
- The PEC values derived from the field measurement were combined with the consumption data available for the bees (Table 2 and 3) and the overall 90th% exposure level were calculated. These resulted in the following values:

	ORI	**ORC**
HB	forager: 0.0028 nurse: 0.0014	0.0033
BB	0.004	0.0057
SB	0.0028	0.021

- The repeated risk assessment resulted in the following ETR values:

	ETR_{adult}	**ETR_{larva}**
HB	forager: **0.0947** nurse: **0.0468**	0.0017
BB	**0.1354**	0.0029
SB	**0.0929**	**0.0105**

Bold values indicate ETR values when the relevant trigger is breached

- The results of these refinement steps indicate that further efforts needs to be undertaken to justify low risk to pollinators. It is also indicated that these steps should focus particularly on adult bees; in case of honeybees, both foragers and in hive bees are potentially under risk by the use of pesticide X. Regarding larvae only the scenario for solitary bees indicated high risk and the ETR value was only slightly above the trigger of 0.01.

T. LITERATURE REVIEW ON DAILY MORTALITY RATE

FORAGER HONEYBEES

Visscher and Dukas (1997) investigated the lifetime foraging duration and survivorship of individual honey bees (*Apis mellifera* L.) foraging in a natural setting.
In the experiment, bees were allowed to emerge in an incubator. Bees were individually marked with numbered tags and introduced into a 2-frame observation hive containing about 3000 bees. Totally, 3 introductions of 40 bees each 3 days apart were made. Two weeks after introducing the first bees into the hive, the few marked bees that had already begun foraging were removed, and the observations started. The nearest bee colonies were about 100 m away in the opposite direction from the flight line from their colony, and there were many nearby distinctive landmarks, so that drifting of foraging bees from their colony was minimized. A 50 cm transparent tunnel provided the bees access to the outdoors. A portion at the centre of the tunnel could be gated at each side and removed. In this removable cage, each marked bee was individually trapped each time it either departed on or returned from a foraging trip. The bee was weighted on a balance which reported the bee's weight with precision of + 0.1 mg, directly to a personal computer, which averaged a total of at least 5 readings. The computer recorded the time of day, and information about the bee's identification number and its direction was added, either exiting or returning to the hive. From these records, trip time was later calculated, net weight of nectar uptake, and net rate of nectar uptake (mg/min) for each foraging trip by each bee. The analysis includes 33 bees for which a complete lifetime record was available from the first foraging trip until the bee did not return; all 33 of these bees foraged exclusively for nectar.
The lifespan of foraging bees had a mean (+ 1 SE) of 7.7 days ± 0.75 days, median of 7 days, and range of 2 to 17 days. Then the daily mortality is about 13%.

Schippers et al., (2006) assessed honeybee foraging performance.
The research was carried out in southern Ontario, Canada from early June to early July 2004. The average (± s.e.m.) daily high temperature was 23.2±0.65°C. Forage during this period was abundant. The empty honeycomb placed in the observation hive at the start of the experiment was 100% full 29 days later. Assuming a full frame mass of 4.5 kg, this corresponds to an average daily increase in frame mass of 155 g. Newly eclosed bees (*Apis mellifera* L.) were marked with individually numbered tags and introduced into a two-frame observation hive containing approximately 2000 bees. Four introductions of 80 bees 3 days apart were made in order to have bees commencing foraging over several days. Two weeks after introducing the first bee cohort, a few bees that had already initiated foraging were removed and data recording began. All bees departing and entering the hive travelled through a transparent Plexiglas tunnel. These bees were collected at four different life stages: hive bees (11–15 days old), young foragers (2 days of foraging experience), mature foragers (4–11 days of foraging experience) and old foragers (12 days of foraging experience).
The average foraging life span of the 27 bees (out of 38) that died before the end of the experiment was 9.7±0.9 days, and the median foraging span was 8 days. This means a daily mortality rate of 10.3%

Rueppel at al., (2007) assessed the importance of extrinsic risk on worker mortality, how foraging is quantitatively related to mortality, how variation in life history between two selected strains correlates with mortality and how chronological age affects mortality.
Focal cohorts of honey bees (*Apis mellifera* L.) in colonies of a natural age composition were studied. Honey bee queens in the source colonies were induced to lay eggs in empty combs. These combs were brought into a humidity and temperature controlled incubator (33 °C/60% Rel. Humid.) 1 day prior to emergence of the focal cohort bees. Within 12 h of emergence, worker bees were marked with individually numbered colour-tags and introduced into an unrelated host colony. The host colonies were maintained in 4-frame observation hives in a dark, temperature-controlled room with immediate access to the outside (either flight cage or natural habitat).

During the experiments, resource and brood levels were maintained equal between the respective experimental groups by exchanging selected frames and additional feeding if necessary. The entrance of each hive was observed for incoming, tagged bees during the peak of foraging activity.
In the first experiment, the life-histories of workers that were free-flying was compared to those workers that were confined to foraging in a flight cage in which food (30% sucrose solution and ground, dried pollen) was offered from 10:00 am to 12:00 am daily.
Two simultaneous replicates of the following paired design were used. Two equal colony halves were established (ca. 4000 workers each) from a source colony, stocked with a queen, and introduced into a 4-frame observation hive. The two observation hives were connected at the back through a mesh-wire screen to permit food exchange between colony halves. For one hive the hive entrance opened into the natural foraging environment, for the other hive it led into a semi-circular flight cage (11 m long, 6.5 m wide, 3.3 m high, 60% shade cloth) with one sucrose and one pollen feeder located 5 m from the hive entrance.
At the beginning of the experiment 960 newly emerged, individually tagged workers were introduced into each colony half. Daily foraging observations and nightly survival censuses began the following day. Bees that died during the first 5 days were excluded from the analyses because the handling and marking can artificially increase mortality. Foraging activity of both colony halves was observed for 30 min each during the feeding period. All incoming bees were recorded to obtain an estimate of total foraging activity along with specific foraging data on the tagged bees to verify the experimental treatment.

Table T1: Results of the 1st experiment

	Free-flying		**Caged (2h)**	
	Col1	**Col3**	**Col2**	**Col4**
Foragers (n)	288	335	183	175
Forager lifespan (days)	26.3 (25.6-27.0)	25.6 (24.8-26.3)	30.7 (29.6-31.9)	32.9 (31.7-34.1)
Mortality rate (1/lifespan*100)	3.80%	3.91%	3.26%	3.04%
Flight span (days)	3.3(2.9-3.8)	4.9(4.4-5.4)	5.3(4.4-6.1)	4.7(3.9-5.5)
Daily mortality rate (1/flight span*100)	30.3%	20.4%	18.9%	21.3%

In the second experiment, the quantitative effect of foraging into flight cages was assessed. Worker mortality was compared between cohorts that had access to pollen and nectar sources in the flight cages either ad-libitum or for only 1 h per day. Each cohort was introduced into a separate host colony, controlled for levels of brood and food. In the ad-libitum treatment, three pollen and three nectar feeders were available throughout the day. The other group of bees only had access to one pollen and one nectar feeder from 10:00 am to 11:00 am. During feeding, foraging activity was not significantly lower in the limited colony than in the unlimited colony but it was significantly reduced when no food was available .
A focal cohort of 480 workers was introduced into both colonies. In contrast to the first experiment, these were initially installed in small hive boxes and only transferred to the 4- frame observation hives at the onset of the observations (5 days after the introduction of the focal bees). Overall foraging activity was assessed during 6 min entrance scans, but individual foraging data was collected by directly observing the feeders (between 20 and 40 min daily).
Individual survival was additionally monitored by nightly censuses, as in the first experiment.

Table T2: Results of the 2nd experiment

	Caged (24 h food)	Caged (1h food)
Foragers (n)	113	60
Forager lifespan (days)	20.4 (19.6-21.2)	21.0 (20.1-21.9)
Mortality rate (1/lifespan*100)	4.90%	4.76%
Flight span (days)	7.3 (6.2-8.4)	11.3 (9.2-13.5)
Daily mortality rate (1/flight span*100)	13.7%%	8.85%

The third experiment compared the mortality between the workers from the bidirectionally selected high and low pollen-hoarding strains. One host colony received 350 high and 530 low pollen-hoarding bees, the second host colony received 250 of each as focal cohorts. As in the second experiment, the colonies were transferred to observation hives 5 days after the introduction of the focal cohorts, just before the beginning of the observations. Both colonies foraged into the natural environment but their resource and brood levels were maintain at comparable levels.

Table T3: Results of 3rd experiment

	Low pollen		High pollen	
	North	**South**	**North**	**South**
Foragers (n)	131	246	165	168
Forager lifespan (days)	26.7 (25.9-27.1)	26.5 (25.9-27.1)	23.4 (22.6-24.1)	23.2 (22.3-24.1)
Mortality rate (1/lifespan*100)	3.74%	3.77%	4.27%	4.31%
Flight span (days)	3.6 (3.0-4.1)	3.6 (3.0-4.1)	3.3 (2.8-3.7)	6.1 (5.3-6.7)
Daily mortality rate (1/flight span*100)	27.8%	27.8%	30.3%	16.4%

Dukas (2008) tested the effects of senescence on honey bees foraging in natural settings and documented the predicted pattern of exponential increase immortality rate with forager age. Those data indicated that, in spite of high rates of external mortality, senescence was an important factor determining the performance of insects such as honey bees in the wild.
The main experiment involved a two-frame observation hive containing about 2500 bees. A second similar observation hive was used primarily for another study, but the marked bees in that hive were also monitored and are included in the data set. Dukas made 3 introductions of newly eclosed honey bees with individually numbered plastic tags each about 10 days apart. The first hive received 250 marked bees at each introduction and the second hive received 100, 50 and 100 bees in the first, second and third introductions respectively. The successive introductions resulted in bees commencing foraging over a long period of time. This made monitoring of the bees easier and also decoupled effects of age and day effects owing to variation in hive conditions, weather and other external factors such as predator activity and competitors. Overall, bees initiated foraging at an average age of 12.8± 0.28 days, and foragers from the two hives had nearly identical mean life spans (6.6±0.3 and 6.8±0.2)
The observation hives were placed inside a research trailer and connected to the outdoors through transparent Plexiglas tunnels.
Out of the total of 852 marked bees observed throughout the study, 611 bees were recorded as foragers. Only these 611 bees were included in the analysis.

The results indicated an exponential increase in mortality rate with age in forager honey bees under natural settings. This was in spite of the relatively high value (~13.4%) of the age-independent mortality rate. It was likely that both the age-independent and age dependent mortality rates were caused primarily by predation, with the age-dependent factor increasing exponentially owing to physiological and mechanical deterioration.

Rueppel et al., (2009) set up an experiment to compare individual worker life-histories and lifespan between two differently-sized colonies as social environment. Large cohorts of individually marked worker honey bees were used and monitored their foraging activity in addition to survival because the transition from in-hive duties to foraging is a major determinant of honey bee worker lifespan.
Two pairs (experimental trials) of one small and one large hive were made up from respectively one and two pounds (one pound approximates 4500 individuals) of worker bees. The bees were shaken from a mixture of European source hives and then randomly divided into the experimental treatment groups. These groups were then installed in five-frame nucleus hives with queens that had mated naturally. One week later, twelve frames of brood comb with ready-to emerge worker brood were collected from the same European source hives kept in the experimental apiary. Bees emerged overnight in a temperature (34 °C) and humidity (50%) controlled incubator. Bees were individually marked by gluing numbered plastic tags on their dorsal thorax and 796 were introduced into each observation hive. Just prior to that, 400 and 800 untagged new workers were introduced to the small and large hive, respectively, to facilitate the introduction process for the tagged, focal individuals. One day later, colonies were transferred into glass-walled observation hives that each contained one frame of honey, one fully drawn, empty frame, and two frames of foundation. One day after this transfer, daily survival and foraging observations began.
Worker survival was monitored daily after sunset by systematically recording all marked individuals present in the colony. Since worker bees return daily to their hive as long as they are alive, death was inferred for one day after the last recording of a bee.
All bees returning from foraging trips were recorded daily for 2 h during the peak of foraging activity to determine the age of foraging initiation. Workers returning with pollen on their legs were classified as pollen foragers, all others were classified non-pollen foragers. From the foraging records, the number of foraging days was calculated and the pollen foraging bias as the proportion of foraging observations for each worker that included pollen collection.

Table T4: Worker life span and flight span

	Worker Lifespan	**Flight span**	**Daily mortality rate (1/flight span*100)**
Large Hive 1	22.8 ±9.4 (22.1-23.5), n=671	7.5±6.6	13.3
Large hive 2	22.3 ±7.6 21.7-22.9), n=609	6.5±5.3	15.4
Small hive 1	26.6 ±8.9 (26-27.3), n=680	6.7±6.0	14.9
Small hive 2	26.4±9.7 (25.6-27.1), n=709	8.8±6.9	11.4

Khoury et al., (2011) developed a quantitative model of honey bee colony population dynamics. As input parameters the values for life span reported by Rueppel et al., (2009) were used.

WORKER ADULT HONEYBEES

Sakagami & Fukuda (1968) gave tables for workers honeybees throughout their all developmental stages. Their results showed an average longevity for June adult bees of 28.345 days (mortality rate 3.53%); an average longevity for July adult bees of 32.424 days (mortality rate 3.08%); an average longevity for wintering adult bees of 154.095 days (mortality rate 0.65%) and an average longevity for postwintering adult bees of 23.431 days (mortality rate 4.27%).

Schmid-Hempel and Wolf (1988) randomly selected workers of a single colony and forced them to restrict their foraging activities to different degrees while leaving in the natural context of their hive to maintain homogeneity among the tested workers with regards to colony, external conditions and heritable components. The relationship between life-span and work loads given under field conditions was studied.

One comb containing sealed cells ready for eclosion, together with nurse bees, was removed from the hive and put in an incubator at 35°C. From this comb, freshly hatched bees were collected several times a day, individually marked and reintroduced to the colony. This procedure was repeated until 280 bees had been marked.

The emerging bees were randomly assigned to one of the five treatment groups which differed in the amount of the individuals were allowed to forage outside the hive. An observer was placed at the entrance of the hive for 8h each day during the main foraging activity period. Within the 8h treatment period, the individuals could forage for 0, 2, 4, 6 and 8 hours (H0, H2, H4, H6, H8). Individuals of the H8 were always allowed to forage and thus served as control where the individuals of H0 could never leave the hive.

Tab T5: Life span for forager bees in the 5 treatments.

	H0	**H2**	**H4**	**H6**	**H8[(a)]**
Sample size	49	59	57	46	49
Life span (days)	41.6	41.3	41.9	45.1	39
Mortality rate % [(1/life span)*100]	**2.40**	**2.42**	**2.39**	**2.22**	**2.56**

(a) H8 is the control

Schmickl and Crailsheim (2007) used the following values as mortality rate for their model:

For adult bees: Base mortality = 1%;
Nursing mortality = 0.5%;
Processing mortality = 0.5%;
Foraging mortality = 3.5%;

For immature stages: Eggs = 3%;
Larvae = 1%;
Pupae = 0.1%

They created a simple mathematical model for honeybee population model, using difference equations to model the population dynamics and the resource dynamics of a honeybee colony. They generated a simulated life-table based on the mortality rates they used in their model and compared the resulting survivorship with the one reported by Sakagami & Fukuda (1968).

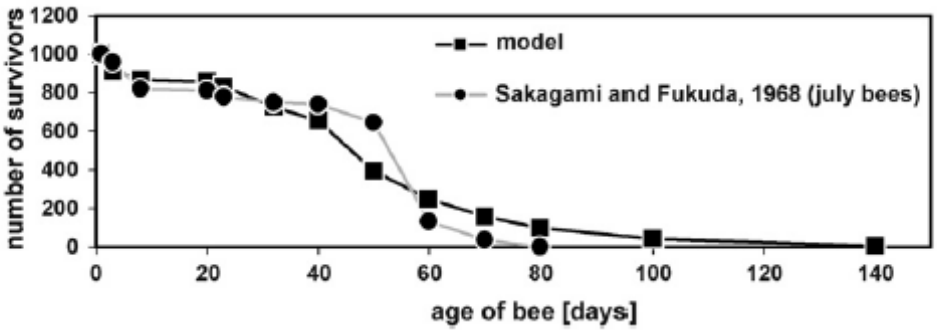

Figure T1: Comparison of life-table given by Sakagami & Fukuda and the model's simulated life table

BUMBLEBEES

Schmid-Hempel and Heeb (1991) reported an average mortality rate for *B. lucorum* worker bees in the control colonies of 31.1 % per week. This gives a daily mortality rate of 4.4%

Da Silva-Matos and Garofalo (2000) aimed at examining **adult worker longevity** in queenright (QR) and queenless (QL) colonies of *B. atratus* in order to verify if this bionomic character differs between the two types of colonies. Queenright colonies produced 1605 (QRC-1) and 639 (QRC-2) workers while in queenless colonies the number of workers produced was 798, in QLC-1, and 1119, in QCL-2. No distinction between house-bees and foragers was made in either colony because all workers, except the egg-laying ones, were observed to forage, although some of them began foraging early than others. The mean longevity for the workers from QLC was not significantly different from those of QRC . The daily mortality rate was QLC-1=4.50%; QLC-2=4.95%; QRC-1=4.11%; QRC-2=5.68%.

Remark: *B. atratus* is a neotropical species and it is uncertain if the mortality rates are representative for European species. Therefore the analysis of the daily mortality rates relied on the study of Schmid-Hempel and Heeb (1991).

6289 **Table T6:** Overview on daily honey bee forager mortality rates
6290

Study	Flight span	Daily mortality rate
Visscher and Dukas (1997)	7.7	12.99
Schippers et al (2006)	9.7	10.31
Rueppel (2007) (median values)	4.8	20.83
Dukas (2008)	7.5	13.33
Rueppel et al (2009) (median values)	7.1	14.1
Sakagami and Fukuda (1968)* average of June and July bees (life spans 8.345, 12.424)	10.4	9.63
Schmid-Hempel and Wolf* (1988) (only control group)	19	5.26
min	4.8	5.26
max	19	20.83
median	7.5	13
10th percentile	5.72	7.88

6291
6292 *The total adult life span was reported. It was assumed that adult bees will be 20 days in-hive before
6293 they start foraging. The forager flight span was calculated from the total life span minus 20 days.
6294
6295

U. TRIGGER VALUES

Use of HQ approach for solid formulations

EFSA (2012a) propose that it is possible to use the HQ approach, along with the associated trigger value as part of the seed treatment/granule, or solid formulation scheme. In particular EFSA (2012a) propose using it in the assessment of risk from dust drift.

The original concept behind the HQ approach and the associated trigger value was developed for spray applications. To read across to solid formulations, there needs to be an assessment of whether a solid formulation poses an equivalent (or lower) risk to sprays. In order to do this there should be a consideration of the toxicity of a spray formulation versus the toxicity of dust from a solid formulation, as well as a consideration of exposure

As regards toxicity, it is likely that in terms of toxicity, that when expressed in equivalent terms (i.e. µg a.s./bee), that a spray formulation is *potentially* more toxic than the active substance and that a solid formulation is probably of similar toxicity to the active substance.

Exposure from spray formulations will mainly consist of oral and contact. Exposure via the oral route may occur when the bees consume contaminated pollen or nectar, water, guttation fluid which has either been contaminated directly by spray deposit or via systemic action of the active substance. As regards contact exposure, this is possible if the bee is sprayed directly or comes in to contact with spray deposits. It should be noted that when a bee cleans itself, it may then consume what is deposited on it.

As for exposure from dust from solid formulations, it is considered that the routes will be similar as for sprays above. In addition, it is feasible that if dust is present in or on the flower then a bee may come in to contact with this when working flowers. This may then be taken up orally when the bee cleans or is cleaned by others in the hive; it is feasible that this route could be greater compared to the similar route for spray applications.

According to the above, the toxicity of the formulation of a solid formulation is likely to be less than that for a spray formulation, as regards exposure, this is likely to be similar, although there is a possibility that the may be greater exposure compared to the spray from deposition of the dust in flowers. Taking all this together it is feasible that using a HQ approach may be appropriate and hence would mean the same as for a spray treatment – see earlier.

The HQ is calculated with the in-field dose. Soil treatments and sowing of seeds are usually performed on bare soil, which means that bees are not expected to be exposed in the field. The off-field dose will always be (much) lower than the in-field dose (*refer to dust drift values elsewhere*). This means that the calculated HQ is much higher than the HQ relevant for the off-field. This may possibly cover the uncertainties regarding the extrapolation of the LD50 determined for liquid formulation to dust.

Risk quotients and First Tier trigger values

The Toxicity Exposure Ratio, or TER, is a risk quotient that is calculated for each particular combination of a non-target organism and a PPP. Conventionally, the quotient is calculated as the ratio of the intake of the PPP that is lethal to half the subjects exposed, or the LD_{50}, and the level of environmental exposure, denoted E. Here we generalize the principle to any response variable, lethal or sublethal. Therefore, the dose required to reduce performance on any variable, including survivorship, is denoted by D_{50}. Thus, the TER is given by:

$$\text{TER} = D_{50}/E \qquad \text{Eqn U1}$$

Higher Tier testing is invoked when the TER is less than the trigger criterion, T, i.e.

$$D_{50}/E < T \qquad \text{Eqn U2}$$

Algebraic rearrangement of Eqn U2 shows that Higher Tier testing is invoked when the environmental exposure exceeds $100/T$ % of the D_{50}:

$$E > D_{50}/T \qquad \text{Eqn U3}$$

For lethal effects, the trigger criterion typically has been set at ten, so that Higher Tier testing is invoked when the environmental exposure exceeds 10% of the LD_{50}:

$$E > D_{50}/10 \qquad \text{Eqn U4}$$

It is necessary to establish the maximum level of potential threat that can be expected from a PPP that has been eliminated from further consideration by First Tier testing. Specifically, we must establish the effect of a PPP that has just exceeded the trigger value by having a level of environmental exposure of $E = D_{50}/T$. The degree of detrimental effect due to a dose of D_{50}/T depends on the dose-response relationship, which is typically a sigmoidal function (Figure U1).

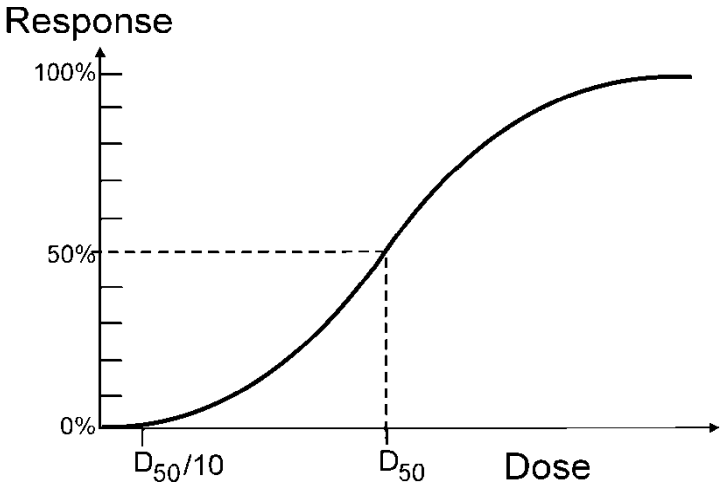

Figure U1: A typical dose-response relationship where 'Dose' (x-axis) indicates the environmental exposure of an individual organism and 'Response' (y-axis) indicates the percentage of individuals that exhibit the response being measured. D_{50} denotes the dose at which 50% of individuals respond and for the case where the trigger criterion $T = 10$, $D_{50}/10$ denotes one tenth of this exposure.

Provided that the dose-response relationship is sigmoidal and that its gradient accelerates at the lowest doses, the maximum response to a particular dose is given by a linear relationship, *response* = *dose* × 50/ D_{50} (Figure 2).

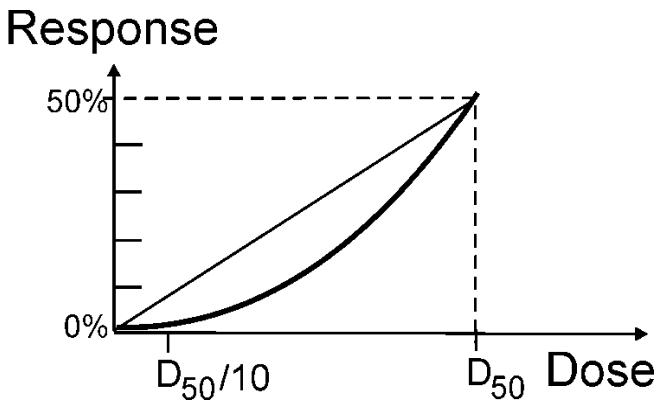

Figure U2: The lower left quadrant of the dose-response relationship from Fig. 1. If the dose-response relationship is sigmoidal, its gradient must accelerate in this quadrant, which implies that the maximum response to $D_{50}/10$ is given by a linear relationship, *response* = *dose* × 50/ D_{50}. The slope of this relationship is obtained because starting from the origin there is a rise of 50% in response across a run of D_{50} and the slope of a linear relationship is given by rise over run.

Given that *response* = *dose* × 50/ D_{50}, the maximum response to an exposure, or dose, of D_{50}/T is obtained by $D_{50}/T \times 50/D_{50}$, or (50 / *T*)%. For the case where the trigger criterion $T = 10$, we obtain a maximum response of (50/10)%, or 5%. Consequently, we consider that the use of a trigger criterion of $T = 10$ provides a reasonable safeguard for most protection goals.

Notes

To defend this conclusion, the following must be further justified by evidence: that dose-response relationships for PPPs are linear or sigmoidal. Gathering this evidence is a target for further research.

Note that the dose-response relationships presented here are generic and not necessarily based on mortality. It is an open question as to whether an exposure of $D_{50}/10$ based on mortality testing will safeguard sublethal responses to a level below 5%. Other endpoints may be more sensitive than mortality and so resolving this question requires further research.

There is always statistical uncertainty associated with working from dose-response relationships fitted to experimental data. Our guidelines will need to make reference to necessary levels of statistical power etc. in this context.

Determining a trigger value for an acute oral exposure

Overview:- By assuming that the dose-response relationship is linear in the low-dose range, it is possible to identify the maximum exposure whose impact (imposed mortality) meets a specified protection goal. By definition, it is possible to link this maximum exposure, or uptake, to the HQ.

Principles:- Let *A* denote the field application rate of a compound (kg a.i. ha^{-1}) and let *RUD* denote the residue unit dose of the bee's diet (mg a.i per kg diet at A = 1 kg a.i. ha^{-1}). Let *c* denote the daily consumption rate (kg diet day^{-1}) and let *d* denote the duration of the exposure in days. If *U* denotes the uptake of a compound by an individual bee (mg a.i), then

$$U = A \times RUD \times c \times d \qquad \text{Eqn U1}$$

Let LD_{50} (units of mg) denote the 48 h consumption of a.i. that causes mortality in 50% of exposed bees. Dividing both sides of Eqn U1 by LD_{50} yields:

$$U / LD_{50} = (A \times RUD \times c \times d) / LD_{50} \quad \text{Eqn U2}$$

Since by definition the hazard quotient is given by $HQ = A / LD_{50}$, we replace this quotient in the right hand side of Eqn U2 and rearrange terms to obtain:

and hence:

$$HQ = U / (RUD \times c \times d \times LD_{50}) \quad \text{Eqn U3}$$

Assuming that the dose-response relationship is linear through the origin (i.e. zero dose-dependent mortality in the control dose) in the dosage range from zero to LD_{50} (see justification above), the maximum dietary exposure (mg a.i. kg^{-1}) that meets a protection goal of mortality less than *M*% is given by $U = M \times LD_{50}/50$, which is explained as follows.

Let *X* denote the exposure that causes the maximum mortality permitted under the Specific Protection Goals. Assume that the dose-response relationship is a straight line defined by *mortality* = *exposure**50/LD_{50}. (This assumption is conservative because it produces higher mortality at low doses than an accelerating sigmoidal curve). Note that this dose-response relationship passes through the origin (zero dose-dependent mortality above background at zero dose) and that *mortality* = 50% at *exposure* = LD_{50} as required.

The point (*U*, *M*) lies on the dose-response relationship with coordinates *mortality* = *M, exposure*= *U*, so we can find *U* given *M*. When *mortality* = *M* and *exposure* = *U*, we use *mortality* = *exposure**50/LD_{50} to obtain:

$$M = U*50/LD_{50} \quad \text{Eqn U4}$$

and rearrangement yields the required

$$U = M \times LD_{50}/ 50 \quad \text{Eqn U5}$$

We now use this result as follows. Substituting the expression for U given by Eqn U5 into Eqn U3 yields:

$$HQ = (M \times LD_{50}/ 50) / (RUD \times c \times d \times LD_{50}) \quad \text{Eqn U6}$$

and algebraic simplication produces:

$$HQ = M / (50 \times RUD \times c \times d) \quad \text{Eqn U7}$$

Worked example.

Assume $RUD = 12.5 \times 10^{-3}$ mg a.i. mg^{-1} (which is 12.5 ppm), $c = 128 \times 10^{-3}$ mg d^{-1}, and $d = 2$.

If the protection goal specifies $M \leq 5.3\%$ then solving Eqn U7 yields

$HQ = 5.3/(50 \times 12.5 \times 10^{-3} \times 128 \times 10^{-3} \times 2) = 5.3/0.16 = 33$

The HQ trigger values are calculated as follows based on daily mortality rates based on life span/mortality data of foragers retrieved from literature (see Annex T on mortality rates):

	Lowest observed mortality	10th percentile	Median
Daily background mortality	5.3	7.8	13
HQ trigger	33	49	81

The HQ trigger values for bumble bees and solitary bees were recalculated based on daily mortality rates of 4.4% (bumble bees) and 5% (Osmia) resulting in values of 27.5 and 31.5. An additional assessment factor of 5 is suggested to account for higher susceptibility of forager losses in bumble bees and uncertainties related to differences in species sensitivity distribution in solitary bees.

Determining a trigger value for an acute contact exposure

This scenario covers direct overspray of bees sitting on a plant or on the ground in field. In the Opinion of the PPR panel (EFSA, 2012a) it is proposed to assume "as a conservative assumption that honey bees in the field during or shortly after spray applications are exposed to a mass corresponding to the mass sprayed to 1 cm^2 of the field". (Note that 1 $cm^2 = 10^{-8}$ ha.)

As above the exposure/dose a bee receives is denoted as U and can be calculated as follows:

$$U = A \times 10^{-8} \qquad \text{Eqn U8}$$

Since the application rate is given in kg a.s./ha it needs to be multiplied by 10^6 to express it in mg a.s./ cm^2.

$$U = 10^{-2} \times A \qquad \text{Eqn U9}$$

Dividing both sides of the Eqn U9 by LD50 (contact) yields:

$$U / LD_{50} = 10^{-2} \times A / LD_{50} \qquad \text{Eqn U10}$$

The hazard quotient is given by $HQ = A / LD_{50}$. We replace the quotient on the right hand side of Eqn U10:

$$U / LD_{50} = 10^{-2} \times HQ \qquad \text{Eqn U10}$$

The rearranged equation is:

$$100U / LD_{50} = HQ \qquad \text{Eqn U11}$$

As above the point (*U,M*) in the dose-response curve can be used to find the dose at a certain mortality.

When *mortality* = *M* and *exposure* = *U*, we use *mortality* = *exposure**50/LD_{50} to obtain:

$$M = U*50/LD_{50} \qquad \text{Eqn U4}$$

and rearrangement yields the required

$$U = M \times LD_{50}/ 50 \qquad \text{Eqn U5}$$

We now use this result as follows. Substituting the expression for U given by Eqn U5 into Eqn U11 yields:

$$HQ = 100\ (M \times LD_{50} / 50) /\ LD_{50} \qquad \text{Eqn U12}$$

and algebraic simplication produces:

$$HQ = 2M \qquad \text{Eqn U13}$$

Workedl example.

If the protection goal specifies $M \leq 5.3\%$ then solving Eqn U13 yields

$HQ = 5.3 \times 2 = 10.6$

The HQ trigger values are calculated as follows based on daily mortality rates based on life span/mortality data of forager honey bees retrieved from literature (see Annex T):

	Lowest observed mortality	10th percentile	Median
Daily background mortality	5.3	7.8	13
HQ trigger	10.6	15.6	26

The HQ trigger values for bumble bees and solitary bees were recalculated based on daily mortality rates of 4.4% (bumble bees) and 5% (Osmia) resulting in values of 8.8 and 10. An additional assessment factor of 5 is suggested to account for higher susceptibility of forager losses in bumble bees and uncertainties related to differences in species sensitivity distribution in solitary bees.

Determining a trigger value for an oral 10 day exposure.

Overview:- This procedure finds the maximum dietary exposure of a compound that causes a level of mortality over 10 days that would impose no more than a negligible impact on a honeybee colony, as required by the Specific Protection Goals. The required proportional elevation in mortality is determined from the Khoury model (Khoury et al. 2011) and assuming the standard parameterisation of Henry *et al.* (2012. Science 336: 348-50), which is conservative in assuming that the colony has a relatively low capacity to replenish lost foragers (Cresswell & Thompson 2012. Science, *in press*) and then this is applied to a more conservative estimate of the background rate of mortality under field conditions. The exposure required to cause this elevation is determined from a laboratory dose-response relationship.

1. Find the daily mortality rate in the Khoury model that causes a 7% decrease in colony size over 10 days (see the magnitude of a 'negligible effect' in the Specific Protection Goals). Denote this rate by $m_{7,10}$

2. Find ratio of $m_{7,10}$ to the 'background' rate of daily mortality assumed in the Khoury model* (i.e. 0.154). The maximum relative increase in daily mortality rate that meets the Specific Protection Goal is $I = m_{7,10}/0.154$

3. Assume that the environmentally relevant background rate of daily mortality under field conditions is m_E. Therefore, the maximum rate of mortality that meets the Specific Protection Goals for the

relevant environment is $I \times m_E$. The maximum increment above background level is therefore $max.increment = (I - 1) \times m_E$

4. For the compound in question, consider the dose-response relationship between oral dietary exposure dosage (mg a.i. kg^{-1}) and mortality rate and determine the compound's LC_{50}, where LC_{50} denotes the exposure dosage necessary to produce 50% mortality after 10 days.

Assuming that the dose-response relationship is linear through the origin (i.e. zero dose-dependent mortality in the control dose) in the dosage range zero to LC_{50} (see justification in Appendix A), the maximum dietary exposure (mg a.i. kg^{-1}) that meets the protection goal is given by $max.increment \times LC_{50}/50$, which is explained as follows.

Let X denote the exposure that causes the maximum mortality permitted under the Specific Protection Goals. Assume that the dose-response relationship is a straight line defined by $mortality = exposure*50/LC_{50}$. (This assumption is conservative because it produces higher mortality at low doses than an accelerating sigmoidal curve). Note that this dose-response relationship passes through the origin (zero dose-dependent mortality above background at zero dose) and that $mortality = 50\%$ at $exposure = LC_{50}$ as required.

The point ($max.increment, X$) lies on the dose-response relationship with coordinates $mortality = max.increment, exposure = X$, so we can find X given $max.increment$. When $mortality = max.increment$ and $exposure = X$, we use $mortality = exposure*50/LC_{50}$ to obtain:

$$max.increment = X*50/LC_{50}$$

and rearrangement yields

$$X = max.increment \times LC_{50}/ 50.$$

5. Let T denote the trigger value for the TER and by definition $T = LC_{50} / exposure$ so substituting $exposure = X = (max.increment \times LC_{50}/ 50)$ yields

$$T = LC_{50} / (max.increment \times LC_{50}/ 50)$$

and algebraic simplification yields $T = 50/ max.increment.$

Worked example (labelled by steps above).

1. The solution to the Khoury model that yields 7% reduction in colony size after 10 days is $m_{7,10} = 0.195$.

2. Therefore $I = 0.195/0.154 = 1.27$

3. If $m_E = 5.3\%$, $max.increment = 0.27 \times 5.3 = 1.43$

5. Trigger value = $50/1.43 = T = 34$

The TER trigger values are calculated as follows based on daily mortality rates based on life span/mortality data of foragers retrieved from literature (see Annex T):

	Lowest observed mortality	**10th percentile**	**Median**
Daily background mortality	5.3	7.8	13
I	1.27	1.27	1.27
Max. increment	0.27 x 5.3 = 1.43	0.27 x 7.8 = 2.1	0.27 x 13= 3.5
TER Trigger	34	23	14
ETR Trigger	0.03	0.04	0.07

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6634 The ETR trigger values for bumble bees and solitary bees were recalculated based on daily mortality
6635 rates of 4.4% (bumble bees) and 5% (Osmia) resulting in values of 0.024 and 0.027, respectively.
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6639 **GLOSSARY [AND/OR] ABBREVIATIONS**

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a.i.	active ingridient
a.s.	active substance
BBCH	Growth stage; uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species
CA	Concentration Addition
EA	Exposure Assessment
EC50	Concentration required killing half the members of a tested population after a specified test duration
ECx	Concentration with x% level of effect compared to the control
EPPO	European and Mediterranean Plant Protection Organization
ERC	Ecotoxicologically Relevant type of Concentration
ETR	Exposure toxicity ratio
EU	European Union
FOCUS	FOrum for Co-ordination of pesticide fate models and their Use
Guttation	Appearance of drops of xylem sap on the tips or edges of leaves of some vascular Plants
GD	Guidance Document
HQ	Hazard quotient i.e. the quotient of the application rate and the acute oral or contact toxicity
ICPBR	International Commission Plant Bee Relationship
IGR	Insect growth regulator, group of compounds that affect the ability of insects to grow and mature normally
Lab	Laboratory
LC50	Dose required killing half the members of a tested population after a specified test duration
LOD	Level of Detection
LOQ	Level of Quantification
NOAEC	No Observed Adverse Effect Concentration

NOAEL	No Observed Adverse Effect Level
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
OECD	Organization for Economic Co-operation and Development
PEC	Predicted Exposure Concentration
PPP	Plant Protection Product
PUF	Plant Uptake Factor
RAC	Regulatory Acceptable Concentration
RUD	Residue Unit Dose
SCFoCAH	Standing Committee on Food Chain and Animal Health
SPG	Specific Protection Goal
TU	Toxic Unit