ECHA
EUROPEAN CHEMICALS AGENCY

Guidance on the assessment of risks to bees from the use of biocides

February 2024

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Version	Changes	
Version 1.0	First edition	February 2024

Guidance on the assessment of risks to bees from the use of biocides
Reference: ECHA-24-G-03-EN
ISBN: 978-92-9468-352-6
Cat. Number: ED-02-24-128-EN-N
DOI: 10.2823/99631
Publ.date: February 2024
Language: EN

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Abstract

The European Commission asked ECHA to develop guidance for assessing the risks to arthropod pollinators (including bees) from the use of biocides, considering EFSA's Guidance on the risk assessment of plant protection products on bees. This Guidance Document describes how to perform a risk assessment for bees, in accordance with Article 19(1)(b)(iv) of the Biocidal Products Regulation (BPR). It proposes a tiered approach scheme for biocidal active substances for the exposure estimation in different scenarios, hazard characterisation and a risk assessment methodology covering both dietary and contact exposure. This document also provides recommendations for higher tier assessment, metabolite risk assessment and biocidal product risk assessment (mixtures). For arthropod pollinators other than bees, an overview of the literature and a database search on the ecology and sensitivity of non-bee pollinators are provided together with recommendations for further research and considerations for future development of guidance.

Keywords

Bees, pollinators, biocides, Guidance Document, *Apis mellifera*, *Bombus spp*., solitary bees, risk assessment, lower tier studies, Specific Protection Goals, toxicity, exposure

Summary

In December 2019, ECHA received a mandate from the European Commission to develop guidance for assessing the risk to pollinators (including bees) from the exposure to biocides. According to the mandate, ECHA should take into account the revised EFSA Guidance document on the risk assessment of plant protection products on bees, which was published in May 2023.

The mandate also stated that the ECHA Guidance on pollinators should specify the information required to enable a conclusion by the evaluating authority on whether the biocidal product complies with the criteria under BPR Article 19(1)(b)(iv) concerning bees and other arthropod pollinators. However, due to the current lack of data on non-bee pollinators, this guidance document only covers the risk assessment to bees from the use of biocides.

ECHA has developed this guidance document together with a group of experts from different Member States, as well as with the support from stakeholder organizations.

This guidance document provides a methodology to assess the risk to honey bees that are exposed to biocides. This is done by following a tiered approach for the exposure and the effect assessment. In the risk assessment of honey bees, the *magnitude* dimension of the Specific Protection Goals (SPGs) is applied as a threshold for acceptable effects. In regard to bumble bees and solitary bees, a risk assessment methodology is provided but the magnitude dimension of the SPG is not defined due to current lack of knowledge.

This guidance considers two main routes of exposure to bees, via intake of contaminated pollen and nectar through the diet, and via contact, when the bees come to physical contact with the biocidal product.

In the risk assessment, a tiered approach is applied both for exposure and effect assessments, i.e., an exposure-Tier and an effect-Tier have been defined. In the exposure tiers, residue intake or residue deposition need to be quantified by calculating the Predicted Exposure Quantity (PEQ) to address the dietary and the contact exposure of the bees from the use of a biocide through

the different routes of exposure. In the effect tiers, the imposed exposure is called 'Dose' in the laboratory tests or 'Estimated Exposure Dose' in the higher tier tests.

The routes of exposure to bees for both exposure and effect assessments are approached considering both acute and chronic effects, and adults and larvae as different life-stages. Thereby, four risk cases have been defined: acute-contact; acute-dietary; chronic-dietary; larvae-dietary. For each of these risk cases, a PEQ_j is derived in the exposure estimation with suffix j indicating the specific risk case.

The guidance proposes a risk assessment approach to assess the risk to bees from product type (PT) 18 (insecticides, acaricides and products to control other arthropods) emission scenarios. The possible sources of exposure covered in the guidance are (1) application of manure/sludge from animal housing, (2) spraying on walls and foundation of buildings, (3) irrigation of private gardens with treated water, and (4) large scale spraying of trees, bushes or natural water bodies. Bee exposure through direct consumption of bait products is not considered in this guidance. Also, for spot applications by spraying, no quantitative risk assessment is required.The focus of the guidance is on PT18 uses. However, a risk assessment may be required for a biocidal product under a PT other than PT18 when the potential exposure to bees is considered significant enough to warrant further consideration and if the product contains an active substance with an insecticidal mode of action.

In the effects assessment, the lower tier assessment will define dose response curves (DRC), which are parameters to describe the steepness of the dose-response relationship obtained from the standard laboratory test.

As part of the effect assessment, this guidance document includes two additional aspects for honey bees: considering and assessing whether the concerned compound presents increasing toxic effects due to long-term exposure to low doses – Time Reinforced Toxicity (TRT) and potential concerns due to sublethal effects.

The guidance also provides advice for higher tier effect assessment as a potential way for refinement, in case unacceptable effects are observed in the lower tier assessment. Furthermore, a risk assessment scheme for metabolites and biocidal products (mixtures), and considerations of risk mitigation measures, and instructions for use are included in the document.

This guidance document for the risk assessment of bees from the use of biocides is developed by taking into account the existing guidance available by EFSA for the risk assessment of plant protection products. For further information on the detailed aspect of the risk assessment methodology as well as the scientific background information, the reader is referred to the EFSA guidance (Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)) and its supplementary documents.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Biocidal products are substances or mixtures that are used to control unwanted organisms harmful to human or animal health or to the environment. These products include for instance insecticides, insect repellents, disinfectants, and preservatives. Via their action against harmful organisms such as pests (e.g., insects) and microorganisms (e.g., bacteria), biocides play an important role in controlling diseases, infections, and protection of materials. However, due to their intrinsic properties, biocidal products can pose risks to humans, animals, and the environment.

In December 2019, the European Commission ("COM") mandated ECHA to develop a guidance for assessing the risks to arthropod pollinators (including bees) from biocides exposure to ensure a high and harmonised level of protection of the environment, taking into account the *Revised guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)* by EFSA ("**EFSA Bee guidance**"). The revised EFSA Bee guidance was published in May 2023 (EFSA 2023). In addition, ECHA was requested to specify the information required to enable a conclusion by the evaluating authority on whether products comply with the criteria under Article 19(1)(b)(iv) of the Biocidal Products Regulation (BPR) concerning bees and other arthropod pollinators.

According to the mandate, the following elements were to be considered by ECHA when addressing this question:

- *In order to develop a specific guidance to assess the risk to arthropod pollinators (including bees) from the use of biocides, ECHA shall use any information already available, and in particular the past and current work of EFSA in this field.*

- *To ensure that all available information can be considered in the opinion a targeted consultation of stakeholders should occur. For this consultation, if ECHA considers it appropriate, an overview of biocidal active substances and biocidal products to which arthropod pollinators could be exposed and may trigger directly or indirectly the occurrence of adverse effects in them could be prepared.*

- *The current references to the assessment of risk to arthropod pollinators included in the ECHA Guidance on the Biocidal Products Regulation shall also be considered, along with the work in this field already carried out by the competent authorities and scientific bodies from the EU Member States.*

Throughout the development process of this ECHA Guidance on the assessment of risks to bees from the use of biocides ("**ECHA Bee guidance"**), ECHA carried out several actions to consider the elements included in the mandate:

- Establishment of Expert Group ("**ECHA EG**"): a scientific expert group composed of experts from Member States with specific scientific competence in risk assessment to bees, other arthropod pollinators and bee biology with the support from experts from the European Food and Safety Authority (EFSA) was set up by ECHA.

- Consideration of EFSA Bee guidance: in reference to the work being done at EFSA, ECHA and EFSA were in constant communication in relation to risk assessment to bees. The EFSA Bee guidance published in May 2023 has been taken as reference in the development of this ECHA guidance document for assessing the risks to bees from the

use of biocides.

- Consultation of stakeholders: a dedicated Guidance Consultation Expert Group (GCEG) was established by ECHA for the consultation of stakeholders. The group was defined with a specific role, composition and responsibility. Written consultation and dedicated meetings were organised to consult BPC Environment Working Group, Biocidal Products Committee and the representatives of Members States Competent Authorities for the implementation of Regulation (EU) No 528/2012.

- Scoping document: in the initial stage of the guidance development, the ECHA EG chose to start with a scoping document before proceeding to the actual drafting phase of the ECHA Bee guidance. The scoping step was critically important in the guidance development for biocides, as there was no specific guidance available to assess risk for bees or other arthropod pollinators. Results of the work have been reported in a standalone document (ECHA 2020).

Risks to arthropod pollinators other than bees (**non-bee pollinators, NBP**): Within the ECHA EG, several experts focussed on NBPs with the goal of ensuring that a risk assessment methodology will be available in the future to protect these organisms. Firstly, a literature review related to the ecology and the sensitivity to insecticides of Diptera, Lepidoptera, non-bee Hymenoptera, and Coleoptera was done. Furthermore, a collection of toxicity endpoints of NBPs exposed to active substances was conducted. The results of the work have been reported in a standalone document (ECHA 2022a).

In reference to the mandate, the present guidance document is intended to assist applicants and competent authorities to carry out **assessment of risks to bees** from the use of biocide active substances and biocidal products.

The following areas are covered in this guidance document:

- Introduction (Chapter Introduction)
- Scope of the Guidance Document (Chapter 2)
- Overview of the risk assessment (Chapter 3)
- Problem formulation (relevant exposure scenarios) (Chapter 4)
- Exposure assessment methodology (Chapter 5)
- Effect assessment in lower tiers (Chapter 6)
- Lower tier risk assessment (Chapter 7)
- Time reinforced toxicity and sub-lethal effects (Chapter 8 and 9)
- Higher tier risk assessment (Chapter 10)
- Metabolite assessment (Chapter 11)
- Mixtures (Biocidal products) (Chapter 12)
- Risk mitigation measures and instructions for use (Chapter 13)
- Recommendations (Chapter 15)

In addition, the approach for the development of guidance for arthropod pollinators other than

bees (NBPs) is explained (Section 1.6).

1.2. Legal framework

Regulation (EU) No 528/2012 of the European Parliament and of the Council (Biocidal Products Regulation, the BPR) lays down rules and procedures for approval of active substances in biocidal products and for the authorisation of biocidal products.

The process of evaluation of active substance applications is given in Article 8 (BPR) and the common principles for the evaluation of dossiers for biocidal products (including the representative biocidal product in the context of active substance approval) is given in Annex VI (BPR). The evaluating or receiving competent authority (CA) uses the data submitted in support of an application for active substance approval, or authorisation of a biocidal product, to make a risk assessment based on the proposed use of the (representative) biocidal product.

Article 19(1)(b)(iv) of the BPR establishes that a biocidal product, when used as authorised, shall not generate unacceptable effects on the environment, having particular regard to the impact of the biocidal product on non-target organisms, which, among many other organisms, include also bees and other arthropod pollinators under the terrestrial compartment. The risk assessment is therefore a principal part of the evaluation process.

Study data and other information must enable the conduct of a proper risk assessment in order to allow a decision on the suitability of the substance to be approved or the product to be authorised. The BPR sets out rules on information requirements that are specified for active substances in Annex II, and for the respective biocidal products in Annex III. The common core data set (CDS) forms the basis of the requirements and is information that always has to be submitted. The additional data set (ADS) includes supplementary information that may be required depending on the characteristics of the active substance and/or the product-type and on the expected exposure of humans, animals, and the environment. Data requirements in relation to risk assessment of bees are explained in Section 6.1.2.

While the COM mandate to ECHA includes a requirement to consider the EFSA Bee guidance, it is important to notice that there are differences in the assessment of plant protection products (PPPs) and biocidal products (BP). For instance, the type of application of biocides is fundamentally different to the type of application of PPPs which leads to potentially different routes and levels of exposure of arthropod pollinators to active substances which has an impact on the focus of the ECHA Bee guidance. In addition, at the time of the ECHA Bee guidance development, there was a difference in the amount of available data for arthropod pollinators in pesticide and biocide dossiers, especially from experiments with applications comparable to biocides exposure patterns. Furthermore, while in the EFSA Bee guidance the scope is limited to the species *Apis mellifera*, the family *Bombus spp*. and the various groups of solitary bees, the ECHA Bee guidance, in line with the COM mandate, in addition considers the first steps needed for the development of guidance on assessment of risks to other arthropod pollinators.

In regard to the 'unacceptable effects on the environment', the aim of the BPR is to provide sufficient protection to the environment from exposure to biocidal active substances and biocidal products at a general level. That is normally, for aquatic, terrestrial, and sewage treatment plant (STP) compartment, performed by comparing the predicted environmental concentration (PEC) in the relevant environmental compartment with the predicted no effect concentration (PNEC) below which no adverse effects in the environmental compartment are expected to occur (PEC/PNEC ratio). In the context of PPPs, EFSA PPR Panel (2010) and EFSA Scientific Committee (2016) have proposed a methodology to define specific protection goals (SPGs) based on ecosystem services and biodiversity with the underlying principle that the general protection goal of Plant Protection Products Regulation (PPPR) may be achieved via the protection of providers of ecosystem services. This PPP approach served as the basis for the assessment approach for bees outlined in this guidance for biocides.

1.3. Specific Protection Goals

The environmental protection goals outlined in the BPR encompass biodiversity and the ecosystem. These broad goals are translated into specific (operational) protection goals (SPGs), or threshold of acceptable effects on colony/population size, in order to be directly applicable for bee risk assessment in line with the methodology defined in the EFSA Bee guidance. An overview of the SPGs for honey bees, bumble bees, and solitary bees from the EFSA Bee guidance is presented in Table 1.

Table 1: Overview of the SPGs for honey bees, bumble bees, solitary bees (EFSA Bee guidance).

Dimensions	Honey bees	Bumble bees	Solitary bees
Ecological entities	Colony	Colony	Population
Attribute	Colony strength[1]	Colony strength[1]	Population abundance
Magnitude[2]	≤ 10%	Undefined	Undefined
Temporal scale	Any time	Undefined	Undefined
Spatial scale	Edge of field	Edge of field	Edge of field

At the 93rd meeting of representatives of Members States Competent Authorities for the implementation of Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products[3], the Commission indicated, underlining the differences between biocides and plant protection products, that these goals should be considered as far as possible in the development of ECHA Bee guidance.

The following definitions of the dimensions are presented in the EFSA Supplementary Document:

> *The **ecological entity** dimension refers to the level of biological organisation for the identified service providing units, i.e., populations that deliver a given ecosystem service.*
>
> *The **attribute** dimension allows to identify the most ecologically relevant elements that must be protected relative to the ecological entities.*
>
> *The **magnitude** dimension refers to the level of tolerated effects for the attribute to be measured relative to the defined ecological entities. Note that for the EFSA guidance, risk managers agreed on a magnitude dimension for honey bees (A. mellifera) for the entire EU corresponding to a value of 10% as the maximum permitted level of colony size reduction following pesticide exposure. For bumble bees and solitary bees, based on the consolidated information provided in EFSA et al. (2022a), an evidence-based decision for a threshold of acceptable effects could not be finalised by risk managers due to the lack of data. The majority decision was for an 'undefined threshold' that was given as an option in EFSA et al. (2022a).*
>
> *The **temporal scale** dimension defines the duration of tolerated effects.*
>
> *The **spatial scale** dimension 'edge of field' refers to the location of the colonies/populations, i.e., directly adjacent to the treated field, from where the bees forage*

[1] Colony strength is defined operationally as colony size reduction.
[2] The magnitude was the only dimension reviewed and agreed by EFSA risk managers for the EFSA Bee guidance. For bumble bees and solitary bees, a threshold will be defined when more data will become available.
[3] Minutes of the 93rd meeting of representatives of Members States Competent Authorities for the implementation of Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products, available at https://health.ec.europa.eu/events/93rd-meeting-expert-group-implementation-biocidal-products-regulation_en

in the treated field or immediate off-field areas.

For biocides, the spatial scale dimension refers to the location of the colonies/populations directly next to the treated area.

1.4. Pathways of biocides exposure for bees

Biocidal products are used to protect humans, animals, materials, or articles against harmful organisms like pests or bacteria, by the action of the active substances contained in the biocidal product. Biocides are widely used and there is concern that emissions of biocides in the environment may result in the exposure of bees. The way a biocide can become a source of exposure for bees is determined by the emission of a biocide to the environment. Bees may come into direct contact with biocides (e.g., droplets of spray), as well as be exposed via contaminated matrices (e.g., by contact with contaminated surfaces or the consumption of nectar or pollen).

An overview of the pathways of exposure for bees to biocides which are evaluated in this guidance is shown in Figure 1 (adapted from EFSA Bee guidance).

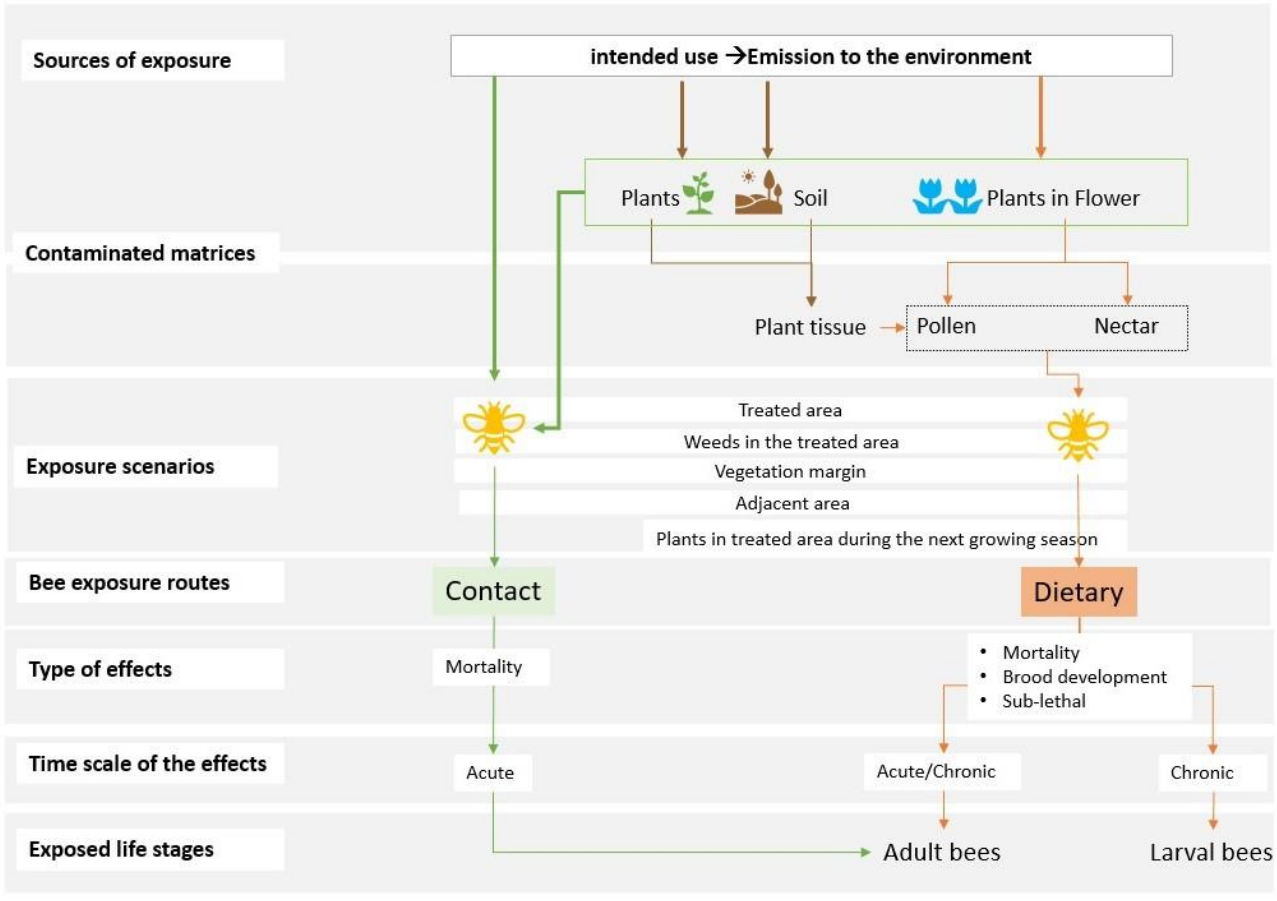


Figure 1: Bee exposure pathways evaluated in the context of biocide risk assessment (adapted from EFSA Bee guidance)

The following series of processes of the way environmental matrices may be contaminated is adapted from the EFSA Bee guidance: Environmental matrices may be contaminated directly (e.g., by spray liquid or dust deposits to the pollen/nectar) or via a series of processes:

For example,
1) a biocide is sprayed onto the plant surface (e.g., leaves) → the biocide enters the plant and is distributed through the plant tissue → reaches the reproductive organ(s) → excreted into e.g. pollen and nectar;
2) a proportion of the biocide is sprayed onto the soil → a proportion of the biocide is taken up by the roots of the plant → distributes within the plant → reaches the reproductive organ(s) → diffused to e.g. pollen and nectar.

Bees may be exposed to contaminated matrices either in areas where biocides are used or in areas that have been unintentionally contaminated. To aid in the development of the exposure assessment, various exposure scenarios for the assessment of risk to bees due to the use of biocides have been defined. These biocide exposure scenarios have been developed on the basis of the exposure scenarios presented in the EFSA Bee guidance. The transposition of the scenarios and the correspondence of terms under biocides and PPPs is presented in Table 2.

Table 2: Exposure scenarios covered in the scope of the EFSA Bee guidance (PPP) and ECHA Bee guidance (Biocides).

EFSA Bee guidance (PPP)	ECHA Bee guidance (Biocides)
Treated crop	Treated area
Weeds in the field	Weeds in the treated area
Plants in the field margin	Vegetation margin
Adjacent crop	Adjacent area
Succeeding crop	Plants in treated area during the next growing season

It is assumed that bees may forage on a plant if it is attractive to bees. In the treated area scenario, it is therefore assumed that the plant is flowering. Similarly, weeds growing in the same area are assumed to be flowering at the time of application of the biocidal product.

Furthermore, the ECHA Bee guidance considers that, for spray applications, spray drift during spray application reaches areas beyond the edge of the treated area and therefore bees could be exposed by foraging on plants growing in a vegetation margin or adjacent area. Spray drift deposition is assumed to decrease with the distance from the treated area. For both the vegetation margin and the adjacent area scenario it is assumed that there are flowering plants at the time of application, and that all foragers from a hive forage on the attractive plant as a worst-case.

The ECHA Bee guidance presumes that bees may forage also on plants in the area during the next growing season. This scenario takes into account that soil residues of substances may lead to root uptake in the next growing season or the following year and that these residues are subsequently transported via the plants to nectar and pollen.

The relevance of the exposure in those scenarios as well as the level of exposure varies pending on the source of exposure (see Chapters 4 and 5).

In line with the EFSA Bee guidance, there are two main ways (i.e., bee exposure routes) through which biocides or their residues can reach the bees (different life stages) in the above defined scenarios and potentially cause adverse effects:

- By contact: it occurs when bees enter in physical contact with the biocides or with contaminated matrices, but that does not involve ingestion.
- By (or via) the diet: it occurs when bees orally consume contaminated material and therefore, they ingest residues of biocides with their diet.

In addition, the following assumptions from the EFSA Bee guidance have been taken over for the ECHA Bee guidance:

- Insufficient information is available to consider the exposure through inhalation.

- It is acknowledged that for most species of bumble bees and solitary bees nesting in the soil, repeated exposure by contact with contaminated soil/mud/leaves may be relevant. However insufficient information is still currently available to address these exposures.

- EFSA has evaluated the relevance of exposure via consumption of contaminated water and concluded that data were not sufficient to achieve a reliable estimation of quantified water consumption or frequency and magnitude of water collection. In consideration of this, exposure from contaminated water is not included in the ECHA Bee guidance document.

- In the EFSA Bee guidance, dietary exposure is considered as ingestion of contaminated nectar and pollen for both adult bees and larvae. Other contaminated matrices (e.g., honey dew, extrafloral nectar, resin, wax etc.) that could lead to oral residue intake are not explicitly covered in this guidance due to lack of sufficient data to propose a quantitative risk assessment approach.

Overall, areas which are not covered in the ECHA Bee guidance may be addressed in future revisions of the guidance document and the ECHA EG recognises the need to generate further research and data. ECHA Bee guidance Chapter 15 outlines areas where further research is needed.

1.5. Bee ecology

In this guidance, pollinating arthropods are divided in two main groups: "bees" and "non-bee pollinators" (NBPs). The group "bees" (taxonomic order: Hymenoptera, taxonomic family: Apidae) covers honey bees (*Apis mellifera*), bumble bees (*Bombus sp.*), as well as solitary bees (e.g., *Osmia spp., Megachile sp., Andrena sp.*). For more information on bee ecology, please refer to the Supplementary Document of the EFSA Bee guidance (Section 1.1) which describes general information on bee life history as well as specific aspects of honey bees, bumble bees and solitary bees.

The other group, the NBPs, is much more diverse and consists of different taxonomic orders: *Diptera* (mainly dominated by flies), *Lepidoptera* (moths and butterflies), *Hymenoptera* (bees, wasps, and ants), and *Coleoptera* (various beetle families). More information can be found in the ECHA publication *European arthropods and their role in pollination: scientific report of their biodiversity, ecology and sensitivity to biocides* (ECHA 2022a).

1.6. Non-bee pollinators

According to the Commission's mandate (see Section 1.1), guidance for the risk assessment from the use of biocides was requested not only for the species *Apis mellifera*, the family *Bombus spp*. and the various groups of solitary bees (bee pollinators), but also for other arthropod pollinators.

The first version of the ECHA Bee guidance will consider other non-bee pollinators (NBPs) to a limited extent only. The reason behind this decision is the lack of information in the literature regarding inter-species sensitivity to biocides and lack of standardised test guidelines for NBPs. This is currently preventing the development of a scientifically based methodology to assess the risk arising from the use of biocides to these non-bee taxa. Nevertheless, as a part of the work under the COM mandate and guidance development, the ECHA EG performed a literature and a database search (ECHA 2022a) to assess the available data to investigate the sensitivity of *Diptera*, *Lepidoptera*, non-bee *Hymenoptera*, and *Coleoptera* to insecticides and to compare it to that of honey bees. The aim of this analysis was to find out whether the honey bee could be

used as a surrogate species when assessing risks to NBPs.

The ECHA EG's scientific report explains which arthropod species may be regarded as relevant pollinators, then further describes the main characteristics of relevant NBP orders, their ecological profiles, and roles as pollinators. The report also outlines the variations in life stages (e.g., foliage- or soil-dwelling) and feeding habits (e.g., herbivorous or feeding on pollen/nectar). As the data available were few and the distribution of data were uneven across the active substances and NBP taxa considered, a comparison of sensitivity data between bee and non-bee species was only possible for some representatives of the orders *Lepidoptera* and *Coleoptera*, and three *Dipteran* species. Thus, the report indicates that at the time of its publication it was not possible to conclude on sensitivity differences between bee and NBPs, as information is scarce for all relevant taxa. In summary, the report identifies the following data gaps:

- Lack of validated standard test guidelines for NBPs.
- Lack of information on the basic biology, ecology and e.g., feeding behaviour to allow conclusion on species vulnerability and further the selection of representative species (surrogates) or alternatively assessment factors for NBPs.
- Lack of information on the most relevant route of exposure, and at which life stage NBPs are most exposed to biocides.
- Lack of commercially available NBP species.

In the report, ECHA EG has highlighted that also NBPs significantly contribute to pollination. NBPs can be exposed to biocidal products during application (contact), via soil (contact), and/or by uptake of contaminated matrix (oral). Therefore, NBPs should be considered in the risk assessment for biocidal products.

The full details on the sensitivity analysis, results and conclusions are explained in the ECHA 2022 publication.

Regarding the risk assessment for NBPs, the ECHA EG concluded that future development of guidance is needed. Furthermore, the ECHA EG has agreed to consider the NBPs under the terrestrial compartment of the environmental risk assessment (according to BPR Annex VI)[4]. However, as at the time of publication of the first version of the present guidance there are significant data gaps, it is not possible to define a method for quantitatively assessing the risks to NBPs arising from the use of biocides. Therefore, risk assessment of NBPs is not covered in this guidance.

2. Scope of the Guidance Document

This document is intended to provide guidance to applicants and risk assessors for the risk assessment of bees in the context of the evaluation of biocidal products and their active substances under Regulation (EC) No 528/2012 for authorisation process at EU or Member State level and the approval at EU level, respectively.

The ECHA Bee guidance covers the risk assessment for chemical biocidal substances, applied as or reaching the environment through outdoor spraying, application of manure/sludge from animal housings, and irrigation. The ECHA Bee guidance covers mainly these sources of exposure, for which exposure estimation approaches are available and consolidated, although the principles of proposed risk assessment schemes may be relevant for other sources of exposure as well. The ECHA Bee guidance does not cover the risk assessment for micro-organism

[4] Currently there is no method available for biocides on how to perform the risk assessment for non-target arthropods, thus an update of BPR Guidance Volume IV: Environment Part A: Information Requirements and Volume IV Environment - Assessment and Evaluation (Parts B + C) is foreseen once new data on NBPs become available. The development of assessment methods for NBPs is currently under discussion.

active substances. According to the EFSA Bee guidance, micro-organisms are not covered since specific considerations are needed. Furthermore, for indoor uses, no exposure to bees living in the surrounding areas is expected and therefore a risk assessment is normally not necessary. However, it is noted in the EFSA Bee guidance that for applications made indoors and where seedlings are subsequently transported to the field, exposure to bees may occur. By analogy to this, for biocides that are applied in animal housing and where treated or contaminated manure/sludge is subsequently transported to the field, exposure to bees may occur.

When a biocide is used in a way that is likely to result in significant exposure of bees that is not covered by the ECHA Bee guidance, it is considered that the applicant has the responsibility to provide a proper characterisation of the exposure in line with the principles of this guidance.

2.1. Focus on PT 18 uses

2.1.1. Emission scenarios with potential exposure to bees

The strategy used to identify emissions of biocides in the environment, with potential exposure to bees significant enough to be further considered for exposure assessment, is outlined below.

The ECHA EG screened and evaluated all available Emission Scenario Documents (ESDs) and Technical Agreements for Biocides (TAB) for the 22 biocide product types (PTs). The intention was to rule out applications of biocides where exposure to bees is not likely. The potential for exposure to bees was considered from releases, i.e., emissions to the first receiving compartments, and emissions to subsequent compartments separately. Manure is, for example, not considered as an "environmental compartment" as such, and for scenarios where manure is applied on soil, soil is considered as a first receiving environmental compartment.

The following criteria were considered relevant in order to identify biocides emission scenarios which might potentially contribute to the exposure of bees following release:

- outdoor use/release.
- release pathway/application type considered relevant (e.g., spray drift).
- release scale of a certain magnitude (e.g., spray or manure applications).
- insecticidal mode of action[5].

Additional considerations were made in some cases with regard to the release being temporary, or whether bees are likely to be present. Expert judgements were made to some scenarios where exposure to bees was not considered probable, due to e.g., the compartment not being relevant for bees, or area not attractive or not of interest to bees due to lack of food sources. On the basis of the work of the ECHA EG and input from Accredited Stakeholder Organisations (ASO) and Member State consultations, it was concluded that emissions from active substances used as insecticides, acaricides and products to control other arthropods (PT 18) have potential exposure to bees significant enough to warrant exposure assessment. The ECHA Bee guidance therefore proposes a risk assessment approach to assess the risk to bees for the PT 18 Emission Scenarios presented in Table 3. Note, however, that this list is not exhaustive and does not exclude future scenarios which might become relevant.

[5] In the scope of the ECHA Bee guidance, mode of action is meant to refer to key events at various levels of biological organization, starting with cellular interaction and leading to functional and/or anatomical changes. Biocide active substances with insecticidal mode of action are usually approved under PT18 (Insecticides, acaricides and products to control other arthropods). However, active substances approved under PTs other than PT18 may also have a mode of action relevant for insects or other arthropods for instance when (Q)SAR model prediction or other available data indicate a substance having structural or functional similarities to a substance (or group of substances) with insecticidal mode of action.

Table 3: PT 18 ESD Scenarios with potential exposure to bees meriting exposure assessment for biocides

PT 18 ESD Scenario	Potential exposure to bees
Insecticide application in animal housings and at manure storage systems*	YES
Outdoor applications (spray application)**	YES
Irrigation scenario (TAB ENV 205)	YES
Outdoor large scale spraying (TAB ENV 248)	YES

*Emission Scenario Document for Insecticides for Stables and Manure Storage Systems (2006)
** Emission Scenario Document for Insecticides, Acaricides and products to control other arthropods for household and professional uses (2008). In the ECHA Bee guidance, the scenario is covered under name "Spraying on walls and foundation of buildings" (see Section 5.3).
TAB ENV = Technical Agreements for Biocides Environment

Note that while the focus is on PT 18 uses, there may be instances where a biocidal product in another PT containing an active substance with an insecticidal mode of action warrants an assessment. In such a case, where the potential exposure is considered significant enough to warrant further consideration for exposure assessment and an exposure scenario is available, such assessment should be performed.

2.1.2. Bait, spot and nest spray applications: no risk assessment

PT 18 products applied as bait or spot applications could be broadly defined as products in the form of gel, blocks, liquids, granule, or powder, containing an insecticidal active substance and - in most but not all cases - sugars as a food attractant.

2.1.2.1 Oral direct consumption of baits

Based on available information, exposure of bees through direct oral consumption of PT 18 bait products is not considered in this guidance.

The ECHA EG initially considered that bees may forage directly on sugar-containing bait products. However, the literature search and ASO consultations showed that there is no data that confirms this assumption. During an ASO consultation conducted in 2022, several peer-reviewed publications were submitted which indicate that the attractiveness of bait products is low for insect pollinators in general and for bees in particular. Firstly, the publications showed that baits in solid form are not attractive to bees because the bees prefer to access sugar in liquid form. This is because bees have mouthparts that are used to lap nectar, to ingest it by dipping their tongue into and then extracting it from the nectar (Kim et al., 2011). Lapping is thus not possible for solid baits and limited for high viscosity baits. In this regard, Nicolson et al. (2013) also showed that honey bees prefer to forage on less viscous nectar. Secondly, studies have shown that as long as food alternatives such as honey, nectar or pollen were available, bees prefer to move to these sources instead of sugar-containing baits (Toledo-Hernández et al., 2021; Mangan et al., 2009).

Nonetheless, the attractiveness of sugar-containing products in the absence of other food sources cannot be completely excluded. Single bees might find and feed on these baits. For several reasons, it is however considered unlikely that local and small scale bait and spot applications will have a major effect on bees leading to colony collapse at a larger scale. Firstly, single bees feeding on baits are likely to take up a lethal dose very quickly and will not be able to transport the product back to the hive or nest. Secondly, the baits are used around residential buildings. If no natural food sources like flowers are present in a garden, only few bees are expected to be present in such a garden, as bees would not localize the garden as a "food patch". Bees use combinations of visual, olfactory and tactile cues to localize flowers. Color cues and floral scents are long distance signals. Once a rewarding food source is identified, this information

is passed on in order to recruit an adequate number of foragers at this site. Therefore, baits would not be recognised as food sources from longer distances because they are lacking color and scent cues. Lastly, as described above, highly viscous gel baits or solid baits are energetically not favourable for use as a food source for bees.

2.1.2.2 Small-scale bait and spot applications

Exposure of bees due to small-scale bait or spot applications outdoors (around the house) was considered very limited by the ECHA EG based on the currently available information, and therefore, no risk assessment for these uses is provided in this guidance.

As shown in Figure 1, exposure of bees could occur, for all applications including small-scale applications, (1) when bees get in physical contact with the product (e.g., with a spray cloud) during or shortly after the treatment, (2) due to contamination of nectar and pollen after direct deposition and (3) when the active substance deposited on soil is taken up by plant roots and consumed by bees via nectar and pollen. For small scale bait and spot applications, both the area where bees could get into direct contact with the product and the area of contaminated soil are small. It is therefore unlikely that such uses will have significant effects on colony size of bees. Therefore, no quantitative risk assessment is required.

Specifically, for the outdoor treatment of wasp or hornet nests (scenario covered in the ESD PT 18, 2008), no quantitative risk assessment is required. The size of the exposed soil area from deposition after the spray application (30 % of the biocide applied) onto the nests is only a circular surface of 50 cm diameter (resulting in a contaminated area of 0.2 m^2) just below the treated nest (ESD PT 18, 2008). No spray drift beyond this area is assumed in the ESD.

Furthermore, the same reasoning also applies to spot applications with baits or sprays (including application around terraces, scenarios covered in the ESD PT 18, 2008). The size of the exposed soil area from emissions during spot application is 0.25 m^2 (ESD PT 18, 2008) or 8.5 m^2 for applications on terraces (TAB ENV 154).

In this regard, the exposed soil areas from the small-scale bait and spot applications (less than ten m^2) in a private garden are considered small when compared to treated areas in the range of hectares for large scale biocidal uses (or plant protection product applications).

Example calculations for small-scale applications were conducted by the ECHA EG with a hypothetical toxic active substance. Those calculations confirmed that with a Tier 2 assessment (refinement of exposure with $PEC_{pw,2}$, see Section 5.3), such a biocidal use resulted in acceptable risk for bees.

2.2. Comparison between EFSA and ECHA Bee guidance documents including justifications of all uses and substances not considered in the ECHA Bee guidance

Although the EFSA Bee guidance exclusively refers to agricultural setting, there is a potential to transfer the logic of the PPP exposure scenarios to biocides uses. It is assumed that for the biocidal uses, which entail applications by spray, spreading of manure or sludge containing biocides on agricultural fields or grassland, as well as irrigation, the exposure scenarios in the EFSA Bee guidance may be taken over for biocides by applying certain adaptations. For some biocides uses, which are outside of a field context, some caution needs to be applied when applying the principles of the EFSA Bee guidance.

It is important to highlight the differences in the assessment of PPPs and biocidal products that result in the approach in the ECHA Bee guidance being different in some aspects to the EFSA Bee

guidance. In consideration of how to make best use of the available methodology of the EFSA Bee guidance, the following has been agreed regarding differences and similarities between the assessment framework for PPPs and biocides:

- The standard testing methods for assessing the risk to *Apis mellifera*, *Bombus* spp. and solitary bees can be generally used in the ECHA Bee guidance.

- Some exposure calculations for biocidal uses can follow representative exposure scenarios considered in the EFSA Bee guidance, with certain adaptations. Adaptations are needed as the application methods and use context as well as the availability of certain basic information for PPP and biocides are not the same (e.g., application to crops/fields versus application around houses, type of treated plants, lack of measured residue levels, etc.).

- The risk assessment principles presented in the EFSA Bee guidance at the first tier can in principle be followed for biocides uses with some adaptations.

- The higher tier assessment presented in the EFSA Bee guidance may not be directly suitable for the bee risk assessment for biocides since standard higher tier studies designed for PPPs may not represent real use conditions for biocides.

3. Overview of the risk assessment

3.1. Implementation of the SPG in the risk assessment including tiered approach

3.1.1. Exposure Assessment Goal and Effect Assessment Goal

In line with the EFSA Bee guidance, the implementation of the agreed SPGs in the risk assessment requires the combined evaluation of the exposure generated by the use of a biocide in the field (which can be predicted, simulated, or measured) and of the ecotoxicological effects (which are assessed as part of the hazard characterisation based on an imposed exposure in the laboratory or higher tier effect experiments).

The same principles can be applied to the risk assessment of bees from the use of biocides. To define what exposure and which ecotoxicological effects should be used to implement the SPGs, the concepts of Exposure Assessment Goal (ExAG) and Effect Assessment Goal (EfAG) have been transposed from the EFSA guidance:

> *The ExAGs relate to e.g. definition of the environmental exposure, type and duration (see supplementary document for more details) and EfAGs relate to e.g. definition of relevant model species, type of toxicity endpoints.*
>
> *The definition of the ExAG allows to answer questions such as:*
> - *where, in which matrix and for what time frame the exposure should be estimated; or*
> - *what level of conservativeness the exposure estimate should aim for, i.e. what percentage of the exposure situations in the field should be covered in the risk assessment?*
>
> *The definition of the EfAG allows to answer questions such as:*
> - *what should be the measured endpoints for the relevant species;*
> - *what extrapolation approaches should be used to cover other species, endpoints*

and untested exposure regimes; or
- *which percentile of a probabilistic effect assessment should be selected?*

According to the EFSA Bee guidance, bees will experience various levels of exposure due to temporal differences (e.g., the same hive/nest may experience different exposure level in spring or during summer) or due to spatial differences (e.g., different hives/nests placed at different locations in the area of use of the active substances). Therefore, it is necessary to define the Exposure Assessment Goal, which can be determined by selecting a percentile that will result in realistic worst-case exposure estimation from the distribution of the various levels of the exposures. Since a 90th percentile is commonly used in ecotoxicology risk assessment e.g., for the EU FOCUS surface water, the EFSA Bee guidance uses the 90th percentile and this has been taken over also in the ECHA Bee guidance document.

The EFSA Bee guidance furthermore notes the following:

> *The exposure and the effect (or hazard) tier assessments should address coherently the agreed SPGs in all the tiers and thus should be completely consistent with each other.*
>
> *Both the ecotoxicological endpoints and the exposure in the field can be defined through the concept of ecotoxicologically relevant exposure quantity (EREQ), which can be described as a type of quantity, that gives the best mechanistic link between exposure in the field and effects in an ecotoxicological experiment. Therefore, they should be expressed as the same type of exposure quantity (e.g. µg/bee per day) in order to enable a consistent linking between each effect and exposure assessment tier (see supplementary document for more details).*

The definitions of the key terms used in the risk assessment of bees are summarised in Table 4.

Table 4: Definition of key terms in the risk assessment of bees as defined in the EFSA Bee guidance.

Terminology	Explanation
EREQ Ecotoxicologically Relevant Exposure Quantity	Not a value, but a **type** of quantity, that gives the best mechanistic link between exposure and effects in an ecotoxicological experiment, and that is calculated/estimated both in the field[a] (PEQ) and the ecotoxicological tests (dose/EED)
PEQ Predicted Exposure Quantity	A value, i.e., the quantification of an EREQ for a specific compound in the field/area of use.
Dose	A value: administered exposure in laboratory ecotoxicological tests
EED Estimated Exposure Dose	A value: estimated in effect field studies

a) For biocides, the exposure is calculated in the area of use.

3.1.2. Tiered approach for biocides

The tiered approach for biocides largely follows the principles of tiered approach established by the EFSA Bee guidance; according to ExAGs and EfAGs, both exposure estimation and effect assessment can be performed following a tiered approach, moving from relatively simple, conservative assessments to more realistic assessments. The concept of tiered approaches is to start with a simple assessment such as a screening assessment, or Tier 1 and add reality and complexity by moving to Tier 2, or higher tier, if necessary to refine the risk i.e., when an unacceptable risk is not excluded at the lower tier. A fundamental aspect of the tiered approach in the EFSA Bee guidance is that every Tier of the exposure assessment should address the same

ExAG and every Tier of the effect assessment should address the defined EfAG.

Both the ecotoxicological endpoints and the exposure in the area of use or the area contaminated should be expressed as the same type of exposure quantity (e.g., µg/bee per day) to enable a consistent linking between each effect and exposure assessment tier. Since both the exposure and effect assessments are operationalised in the tiered approach, it is appropriate to define an exposure-tier and an effect-tier, separately (EFSA Bee guidance):

- Exposure-Tier: In the exposure tiers, residue intake or residue deposition need to be quantified by calculating the PEQ to address the dietary and the contact route of exposure of the bees following the use of a biocidal product.
- Effect-Tier: In the effect tiers the imposed exposure is called 'Dose' in the laboratory tests or 'Estimated Exposure Dose' in the higher tier tests.

For both exposure and effect-tier assessment, the routes of exposure for bees should be addressed by considering the different timescale of effects (acute and chronic) and the different life stages (adults and larvae). To this purpose, four risk cases have been defined (EFSA Bee guidance):

- Acute-contact risk.
- Acute-dietary risk.
- Chronic-dietary risk.
- Larvae-dietary risk.

The exposure estimation in the different tiers will provide PEQ for each of the above risk cases for considered exposure release routes/exposure scenarios and it is indicated as PEQ_j, where the suffix j indicates the four risk cases. In parallel, the effect assessment in the lower tier will provide dose-responses for the different timescales of the effect (acute and chronic) and different life stages (adult and larvae) and therefore address the four risk cases.

In the lower tiers of the exposure assessment, the exposure estimation is based on default parameters, while in higher tiers the exposure of the colony (or population) may be based on measured parameters (e.g., concentrations measured at the plant or brought into the hive/nest by bees). Similarly, in the lower tiers, the effect or hazard assessment is based upon ecotoxicological experiments with individual bees in laboratory studies, while the highest tier is formed by different type of studies e.g., semi-field, colony feeder and/or field tests.

For biocides, the first tier is generally conservative and based on laboratory toxicity data and exposure estimations based on default values. If the risk is found not acceptable at first tier, the applicant is offered the opportunity to submit additional information for conducting a refined risk assessment. This guidance follows in general the concepts from EFSA's tiered approach but includes simplifications and assumptions assuming different level of information and exposure patterns relevant for biocides, see Figure 2.

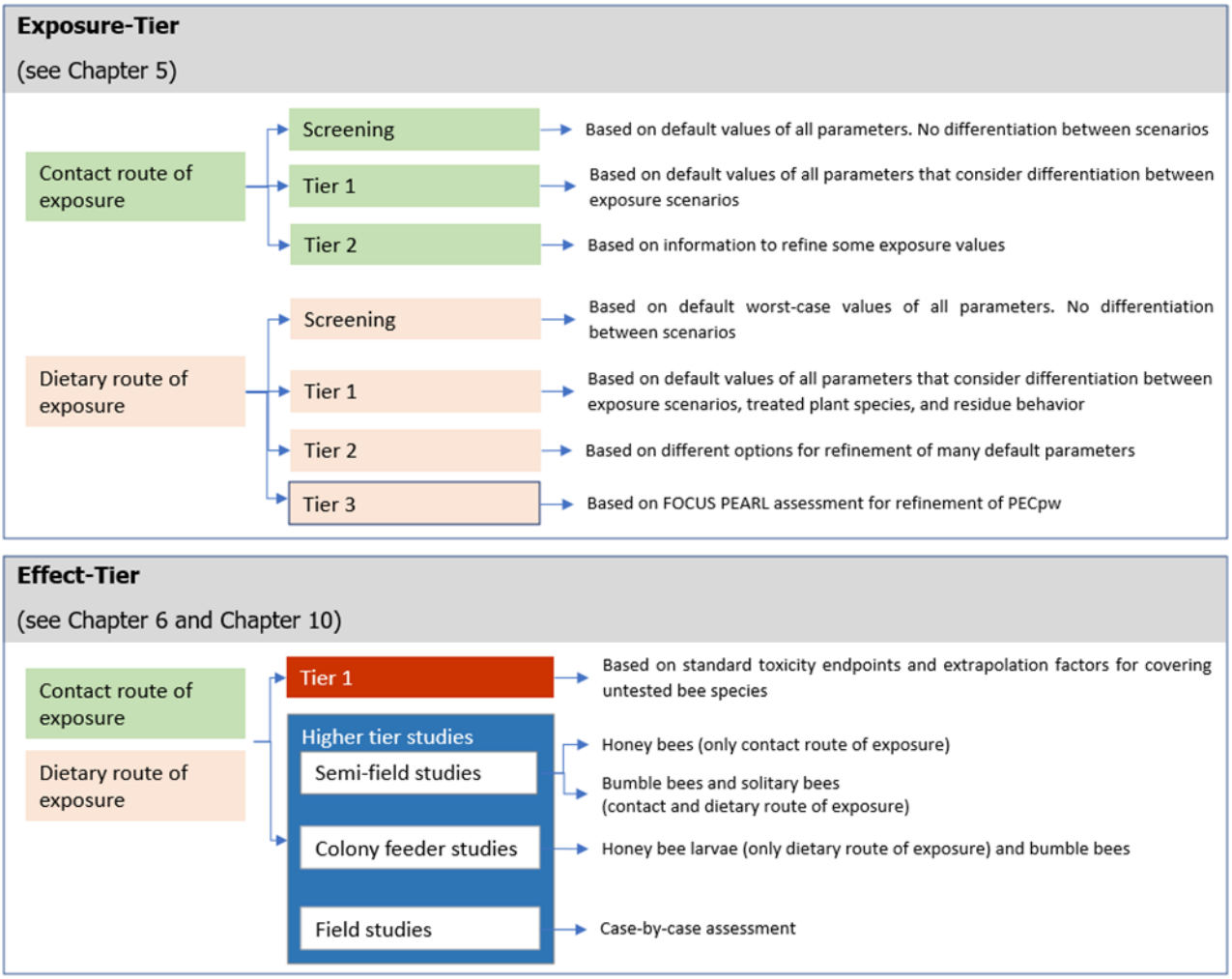


Figure 2: Tiered approach and explanations what each exposure or effect-tier implies for the risk assessment of an active substance (adapted from EFSA Bee guidance). According to the principle of the tiered approach, each exposure-tier can be linked to each effect-tier. FOCUS = FOrum for the Co-ordination of pesticide fate, PEARL = Pesticide Emission Assessment at Regional and Local scales, PECpw = Predicted Environmental Concentration in pore water. Note that the screening step only is relevant for the sources of exposure described in Sections 5.5-5.7.

3.2. Risk assessment scheme

In this section, the bee risk assessment scheme for biocides is described, which follows the principles of the risk assessment scheme for PPPs presented in EFSA Bee guidance. The flowchart as presented in Figure 3 gives an overview of the risk assessment referring to honey bees (for the lower effect tiers). Nevertheless, the scheme may be applied also to bumble bees and solitary bees. However, for those two bee categories, it is not possible to interpret the outcome of the lower tier risk assessment because the calculated risk cannot be compared to the corresponding SPG since no SPG could be defined for bumble bees and solitary bees (see above).

In case the risk remains unacceptable after exposure refinement and no appropriate risk mitigation measures can be applied, higher tier effect studies would be the last option to refine the risk (see Chapter 10). If an applicant intends to perform higher tier effect studies for biocides in order to show acceptable risk for bees, the applicant shall consult the evaluating competent authority during the preparation phase of the dossier and prior to conducting such tests. Together with the evaluating competent authority, appropriate test conditions and study design should be discussed and defined, as the standard higher tier effect studies designed for PPPs

might not be directly applicable for biocides.

In Chapters 2 and 4, it is described for which types of biocidal applications a quantitative risk assessment is needed. Therefore, when a quantitative risk assessment is required, exposure estimation and effect assessment should be performed to identify the worst-case PEQ_j and the relevant effect endpoints for each of the four risk cases (see Chapters 5 and 6, respectively).

In line with the EFSA Bee guidance, for the lower tier risk assessment, a combined approach which will integrate the four different risk cases (Section 3.1.2) is presented. The approach is described in Chapter 7; it allows to calculate the predicted individual level effect (PIE_j) for each worst-case PEQ_j, on the basis of the selected dose-response curve (see Chapter 6). The individual effects are extrapolated to the colony/population level effect (PCE_j) based on 1:1 relationship and then they are combined to predict the overall effect at colony/population level (PE_{SPG}), which is directly compared to the SPG which is the threshold of acceptable effects.

The lower tier risk assessment scheme starts with the screening step or Tier 1 exposure assessment and proceeds to the next exposure-tier when unacceptable risk is identified. When an exposure-Tier 2 assessment is needed, applicants should refine the exposure emission in Tier 1 as proposed in Chapter 5 of this guidance.

If an unacceptable risk cannot be concluded based solely on the exposure refinement (i.e., exposure-Tier 2 or 3), a higher effect-tier assessment may be required.

Alternatively, risk mitigation measures could be proposed by the applicant at exposure tier 1 or higher, but not for the screening step (see Chapter 13).

Furthermore, as part of the effect-tier assessment of biocidal products, three additional aspects should **in parallel** be addressed at lower tier:

- *Time-Reinforced Toxicity (TRT) assessment (Chapter 8):* the potential for the substance under evaluation for showing increasing toxic effects due to long-term exposure to low doses;
- *Sublethal effects (Chapter 9):* the potential concerns due to sublethal effects; and
- *Warning sentence (Chapter 13.3)*: the need to apply a warning sentence for biocidal products

TRT assessment is determined via extrapolation from the standard 10-day chronic honey bee toxicity study (OECD 245). Regarding sublethal effects, the Tier 1 allows to identify potential concerns which should be addressed with further testing after consulting the evaluating competent authority. The evaluation of TRT and sublethal effects is for the time being only performed for honey bees.

As part of the risk assessment scheme, also the risk from **metabolites** should be addressed. A risk assessment scheme for metabolites is presented in Chapter 11.

Also, an approach for the risk assessment of **mixtures** is provided in Chapter 12. The risk assessment of mixtures is performed in case the biocidal product contains more than one active substance. Note that the mixture assessment for bees deviates from the standard approach for aquatic and terrestrial compartments in biocide assessment.

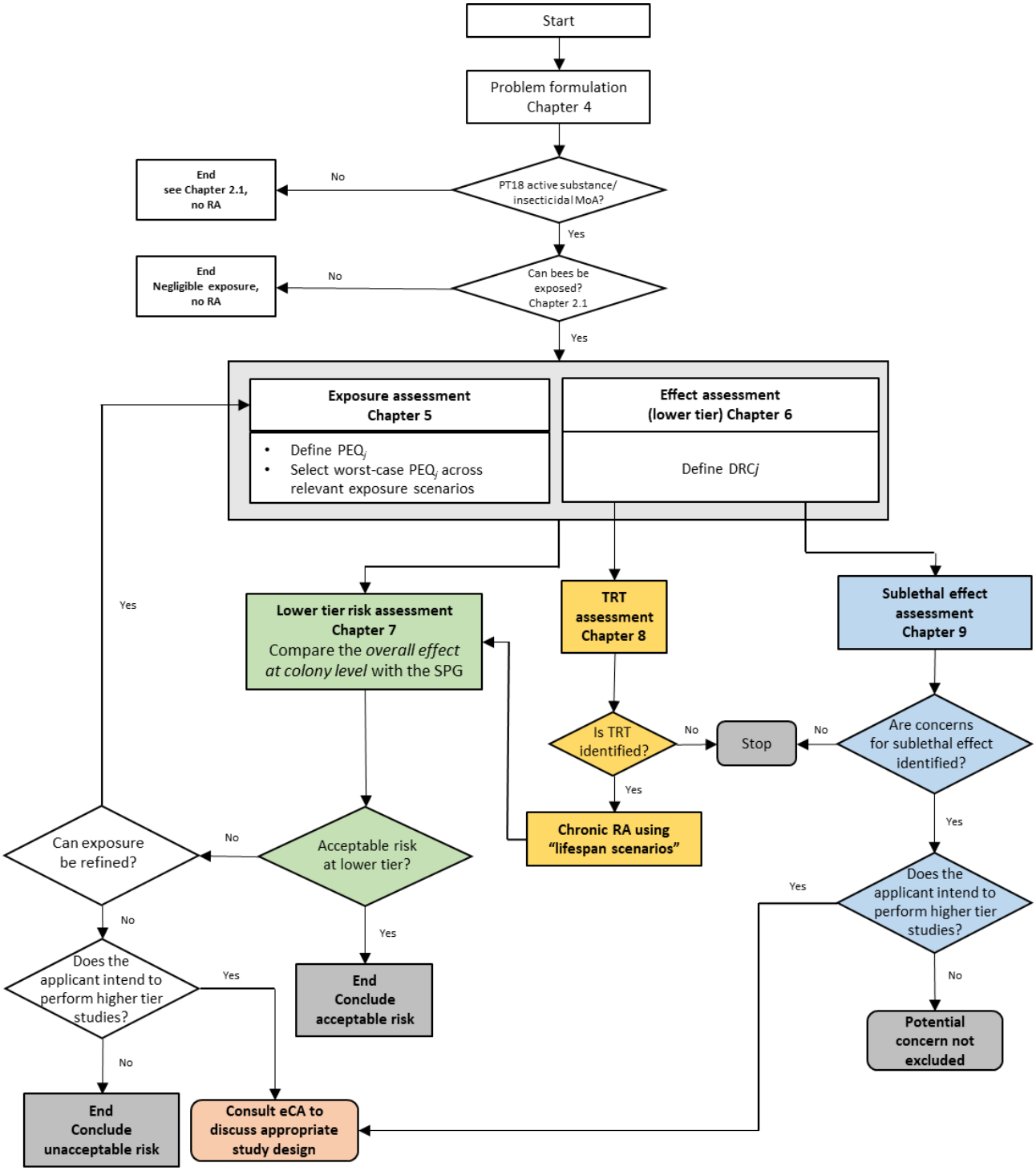


Figure 3: Overview of the biocide lower tier risk assessment scheme for honey bees (adapted from EFSA Bee guidance). MoA = Mode of action, RA = risk assessment, PEQ_j= Predicted exposure quantity for the four risk cases (indicated by the suffix *j*, i.e., acute-contact, acute-dietary, chronic-dietary and larvae-dietary), DRC_j =dose-response curve for the risk case *j*, TRT= time reinforced toxicity (only relevant for HB), SPG = specific protection goal, eCA = evaluating competent authority. Note that also a RA for metabolites (Chapter 11) and for mixtures (Chapter 12) need to be conducted, where relevant. For higher tier risk assessment, see Chapter 10.

4. Problem formulation

As outlined in the EFSA guidance, problem formulation is the first step of the risk assessment which allows applicants and risk assessors to identify the potential hazard and exposure pathways for a biocide and to formulate risk hypotheses and identify the proper risk assessment methodology. The problem formulation sets the boundaries for risk assessment such that it is 'fit for purpose'. For a risk to occur, it entails an exposure to a biocide which result in a direct harm to the bees that exceeds a specified SPG.

When evaluating a biocide, the first step of the risk assessment is to determine through a problem formulation, if and how emissions of the biocide could reach bees; and estimate the level of the exposure (see Chapter 5). Therefore, the starting point is a careful consideration of the source of exposure which includes an analysis of the method of application or form of release, the area where the biocide is applied/released, the number of applications, the application/release rate, and any particular conditions of use.

An overview of sources of exposure from the use of biocides covered by this guidance is reported in the Table 5 together with a consideration of the relevance of the routes of exposure (see Section 1.4) and exposure scenarios (see Section 4.1).

Table 5: Overview of the possible sources of exposure in relation to the contact and dietary routes of exposure in the exposure scenarios for biocides

Routes of exposure	Contact			Dietary			
Exposure scenarios / Source of exposure	Treated area	Weeds in the treated area	Vegetation margin	Treated area	Weeds in the treated area	Vegetation margin	Plants in treated area during the next growing season
Application of manure/sludge to agricultural soil or grassland	N	N	N	Y	(Y)	N	(Y)
Spraying of walls and foundation of houses	N	N	Y	N	N	Y	N
Irrigation of private gardens	Y	(Y)	N	Y	(Y)	N	(Y)
Outdoor large scale spraying*	Y	Y	Y	Y	Y	Y	Y

Y = YES, the scenario is relevant.
N = NO, the scenario is not relevant.
(Y) = scenarios considered covered by another worst-case scenario since either sugar content of the vegetation covered by the scenario is higher or equal to that of the worst-case scenario and/or exposure models result in the same results as for the worst-case scenario.
* Chapter 5 presents situations when 'Treated area'/'Weeds in the treated area' exposure scenarios are relevant for Outdoor large scale spraying.

4.1. Exposure scenarios

Bees may be exposed in the treated areas (i.e., 'treated area' and 'weeds in the treated area' scenarios) and/or in the surrounding areas (i.e., 'vegetation margin' and 'adjacent area'

scenarios). Furthermore, in some situations, bees may be exposed to residues in pollen and nectar that are taken up by the plants in the next growing season. In those scenarios bees may be exposed by contact and/or by dietary routes.
In relation to the contact exposure, it is considered that bees can be over-sprayed in the treated areas and/or could come in contact with spray drift in the surrounding areas at the time of the application.

In relation to the dietary exposure via consumption of contaminated pollen and nectar, the EFSA Bee guidance notes that the proportional contribution of the various exposure scenarios to the daily food intake by bees is unknown. Therefore, it is assumed that each scenario contributes to 100% of the contaminated food consumed by bees, as worst-case.

Further, according to the EFSA Bee guidance, among the most relevant scenarios, only those scenarios that will strongly dominate the exposure on the basis of the exposure estimation will be used for risk assessment, since the "dominant scenario" is considered to cover all the others. This means that worst-case PEQ_j will be selected across scenarios for risk assessment (see Chapter 7) and the worst-case PEQ_j should be identified at each tier of the risk assessment.

Sections 4.1.1 to 4.1.4 present the exposure scenarios for biocides that have been adapted from the EFSA Bee guidance.

4.1.1. Treated area scenario

The exposure of bees to biocides in treated areas entails that bees visit and interact with plants; therefore, it is necessary to ascertain whether the plants are attractive to bees. As pollen and nectar are the main sources of nutrition for bees, the attractiveness is based on the presence and availability of pollen and nectar. A list of plants/crops that are attractive to bees is presented in Appendix A of the EFSA Bee guidance. Note that when a plant is attractive to bees, contact exposure and dietary exposure cannot be excluded. When the treated area does not contain attractive plants, the exposure is assumed to be zero and therefore the treated area scenario is not relevant.

For biocides, the "treated area" may consist of trees or bushes subject to biocide treatment such as large scale spraying against mosquitoes or processionary moths. The trees could be treated in a row or as solitary trees. In case of treated trees, it is the crown of the tree (i.e., the branches, leaves, and reproductive structures extending from the trunk or main stems) that determine the borders of the treated area.

4.1.2. Weeds in the treated area scenario

When the 'treated area' scenario consists of treated plants/area that is considered not attractive/not relevant for bee exposure, bees may still be exposed in the treated areas while foraging on the flowering weeds present in those areas. Bees may be exposed both by contact exposure and dietary exposure when foraging on flowering weeds. For biocides, "weeds" may include also bushes, flowers, grass, or berries that grow within the treated area.

4.1.3. Vegetation margin and adjacent area scenarios

Areas surrounding the treated area can be defined as "vegetation margin" and "adjacent area". "Vegetation margin" may be park lawns, meadows, or countryside roads located at the edge of the treated area, forest, or tree crown. The vegetation margins are assumed to consist of mixed vegetation that is flowering at the time of application. "Adjacent area" may be meadows, countryside roads or fields. The adjacent areas are also assumed to be covered by various plants/mixed vegetation which are flowering at the time of application. The vegetation in these areas is exposed by spray drift (spray application). The exposure of bees in the adjacent area is considered to be lower than in the vegetation margin and therefore only vegetation margin scenario may be relevant (for e.g., large scale spraying).

The vegetation margin has to be considered a relevant exposure scenario for both the contact and the dietary routes of exposure, since this represents a relevant area of interest for bee habitats. For biocides applications, it is assumed, that the vegetation margin is always downwind.

4.1.4. Plants in treated area during the next growing season

In the 'Plants in treated area during the next growing season' scenario, bees are exposed to pollen and nectar contaminated with residues of the active substance that are already present in the soil following a previous treatment. Residues that persist in soil are taken up by the roots of plants and then translocated via the vascular system and the tissues of plants to nectar and pollen. This may happen for plants that are cultivated twice in a growing season or the following year. Residues may be found in the pollen and nectar of for example treated trees and bushes in the following year. It is assumed that these plants are attractive for both pollen and nectar and the dietary route of exposure is the only relevant route of exposure for bees in case of this exposure scenario.

5. Exposure assessment

Exposure of bees to biocides can occur, when biocidal products are intentionally applied outdoors or if matrices that were unintentionally contaminated with biocidal active substances (e.g., manure or sewage sludge) are released to the outdoor environment. Biocides might then reach the flowers of plants directly or can be taken up by the plants from soil and can accumulate in their pollen and nectar (see Figure 4). Hence, during consumption of pollen and nectar, bees take up the active substance orally. Furthermore, contact exposure of bees to biocides is possible when bees come in direct contact to the biocide (e.g., droplets of spray drift) or are exposed to contaminated matrices (e.g., spray deposition of biocides on plants).

Further exposure routes which are not considered in this document, but might become relevant once more knowledge has been acquired, are:

- the consumption of contaminated water (e.g., puddles formed during spraying, contaminated surface water, or guttation water),
- the consumption of other contaminated matrices (e.g., honey dew, extrafloral nectaries, resin, wax etc.),
- contact exposure of bee species which are breeding in contaminated soil or wood, or which use other contaminated materials (e.g., leaves, mud) to build their brood cells.

Therefore, in alignment with the EFSA Bee guidance only the main exposure pathways via the consumption of pollen and nectar (oral exposure) or via direct contact to the biocide or contact to contaminated plant surfaces are considered, if relevant, for the identified sources of exposure to biocides.

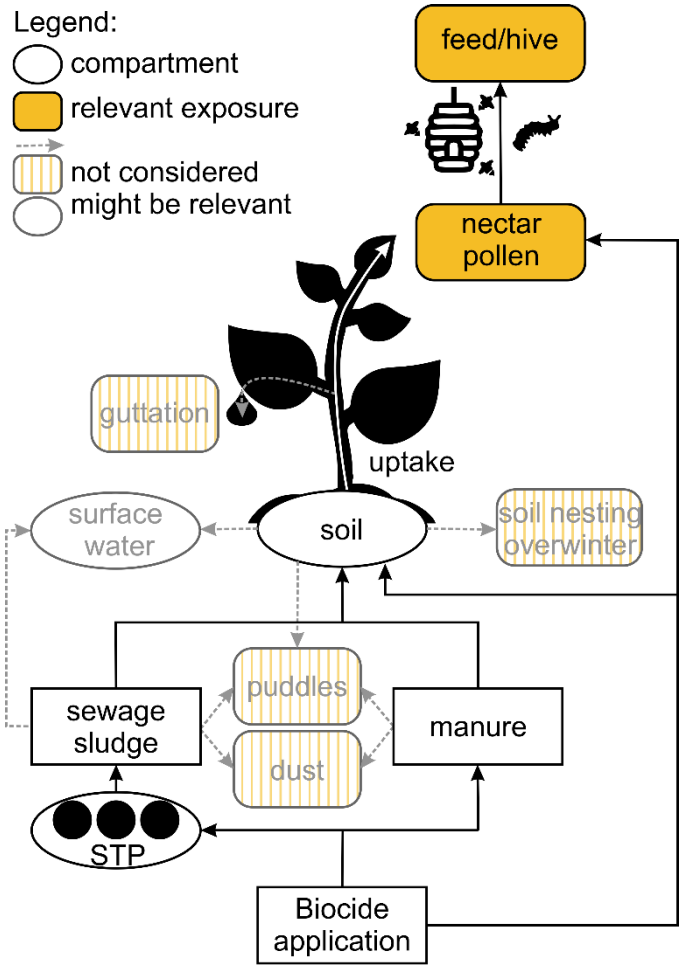


Figure 4: The main exposure pathways of bees to biocides via the consumption of pollen and nectar (oral exposure) are shown. Shaded are further exposure pathways that could become relevant once more knowledge has been acquired. (STP = sewage treatment plant)

Chapter 5 describes 1) the mathematical models to be used for the estimation of exposure of bees for the different exposure routes to be covered by the risk assessment in general; and 2) the relevant exposure pathways and applicability of the mathematical models for the exposure estimation by each relevant biocides source of exposure.

For the biocides sources of exposure presented in Sections 5.2-5.7, the input and output data and calculations are specified in the tables 12 to 36. The input and output data are divided into four groups:

S Set — Parameter must be present in the input data set for the calculation to be executed (no method has been implemented in the system to estimate this parameter; no default value is set, data either needs to be supplied by the applicant or should be available in the literature).

D Default — Parameter has a standard value (defaults can be changed by the user in justified cases).

O Output — Parameter is the output from another calculation (most output parameters can be overwritten by the user with alternative data).

P Pick list — Parameter value can be chosen from a "pick list" of values.

Pick list values and default parameters are to be adapted, when specific data is available, instead of a mandatory use of these values as defaults.

It should be noted that the input data to be used for the purposes of the assessment of risk to bees should be in line with the specific input data for the a.s./b.p. used in the environmental exposure assessment (soil/surface water).

5.1. The exposure assessment models

The exposure assessment models that are described in this chapter showcase the mathematical expressions of the exposure assessment. They are to be used to estimate the exposure quantity of an individual bee through the two main routes of exposure: contact and dietary exposure to biocides. These predictions are to be used in the lower tier risk assessments.

The two main routes of exposure (dietary and contact) lead to three relevant exposure assessment models for biocides: dietary above-soil model (during flowering), dietary through-soil model and contact model. The scope, applicability, and parameters of the dietary and contact models are described in more detail in Sections 5.1.1 and 5.1.2, respectively.

The calculation of exposure of bees to biocides results in Predicted Exposure Quantities (PEQs), which are to be included in the subsequent risk assessment (see Chapter 7).

5.1.1. Dietary models

The dietary models are to be used when adult bees or bee larvae are exposed to the biocidal product by directly consuming the contaminated pollen or nectar. They allow the estimation of the quantity of the biocide intake by an individual bee. Three dietary models have been described in the EFSA Bee guidance to assess the exposure of bees, of which the following two are considered relevant for the sources of exposure to biocides:

- The dietary model for during flowering contamination, which predicts the residue intake for during flowering applications i.e., when direct contamination of pollen and nectar is involved.

- The dietary model for through soil contamination, which predicts the residue intake when the contamination of pollen and nectar originates from the residue uptake process from soil.

The dietary models are briefly introduced below. For more detailed information on the dietary route of exposure models, see Section 5.1.2 of the EFSA Bee guidance.

The dietary model for the during flowering contamination is to be used (for all risk cases) when the open flowers of the treated/contaminated plants might directly be contaminated by the biocide. The model has been set up in the following way:

$$PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times \left(SV_{po,du} + SV_{ne,du}\right)$$ **Equation 1[6]**

The shortcut value (SV) parameters are derived with the following expressions:

$$SV_{po,du} = \frac{1}{1000} \times LF_{po} \times PCUD_{po,du} \times CMP_{po}$$ **Equation 2[7]**

$$SV_{ne,du} = \frac{1}{1000} \times LF_{ne} \times PCUD_{ne,du} \times \frac{CMP_{su}}{SN}$$ **Equation 3[7]**

The dietary model for through soil contamination is to be used (for all risk cases) when the plants can only be contaminated via the soil. The model has been set up in the following way:

$$PEQ_{di} = SV_{po,soil} + SV_{ne,soil}$$ **Equation 4**

The SV parameters are derived using the following expressions:

$$SV_{po,soil} = \frac{1}{1000} \times LF_{po} \times PEC_{pw} \times CMP_{po}$$ **Equation 5[7]**

$$SV_{ne,soil} = \frac{1}{1000} \times LF_{ne} \times PEC_{pw} \times \frac{CMP_{su}}{SN}$$ **Equation 6[7]**

The third dietary model presented in the EFSA Bee guidance is the dietary model for pre-flowering contamination, which is intended for situations when the contamination of pollen and nectar dominantly originates from contamination of the above-soil parts of the treated/contaminated plants before their flowering stage. Since for biocides applications it is unlikely to know the timing of the application in relation to flowering of the treated/contaminated plants and/or the treated/contaminated plants are mixed and therefore there may always be flowering plants in the affected areas, the dietary model for pre-flowering contamination is not considered relevant for biocides and therefore also not further described in the ECHA Bee guidance. The dietary model for the during flowering contamination as the only model in this ECHA Bee guidance to address the dietary exposure of bees due to above soil contamination is referred to as a dietary above-soil model.

The parameters of the above models are described below. This parameter description is supposed to give a general description of each parameter, which applies to all scenarios where these parameters are present. For more specific information regarding the definition of the

[6] Units of the parameters in this equation are as follows: [µg/bee or µg/bee/day or µg/larva/developmental period] = [g/ha]/1000 * [-] * [µg/bee or µg/bee/day or µg/larva/developmental period]. While SVs are developed based on an application rate of 1 kg a.s./ha, the units of the AR in the equation are cancelled out.

[7] Units of the parameters in these equations are as follows: [µg/bee or µg/bee/day or µg/larva/developmental period] = 1/1000 * [-] * [mg a.s./kg nectar or pollen] * [mg nectar or pollen/bee or mg nectar or pollen/bee/day or mg nectar or pollen/larva/developmental period]. Since to convert mg a.s. to µg a.s. requires a factor of 1000 but to convert mg nectar or pollen to kg nectar or pollen requires a factor 1/1000000 a multiplier 1/1000 appears in the equations. Note that for SVsoil equations, a.s. concentration in the soil pore water has been assumed to represent the a.s. concentration in the consumed nectar and pollen.

parameters for the different sources of exposure, see Chapter 5.2 onwards.

Parameter description:

Input parameters

AR Application rate (g/ha).
This parameter is established and named in line with the EFSA Bee guidance. AR refers to the mass of a biocidal active substance applied or released to an area with flowering plants assuming 100% deposition.
Depending on the source of exposure, this definition can deviate from the definition of AR in the biocides exposure assessment, where AR e.g., refers to a mass of a biocide per treated surface. In these cases, the emission of the biocide from the treated surface to the surrounding area with flowering plants in mass per area assuming 100% deposition is considered as AR in this guidance. In other cases, the biocide is directly applied to flowering plants and this AR can be used as such in the calculations for bee risk assessment.
If the application rate is given in a different unit, it should be recalculated to the application rate in g/ha.

CMP Consumption of sugar (CMP_{su}) or pollen (CMP_{po}) (mg/bee or mg/bee/day or mg/larva/developmental period).
Consumption rates refer to the active period of bees during the flowering period. CMP values are specific for bee categories.

Table 6: Food consumption rates (CMP) of bees during the active period (EFSA Bee guidance)

Category for the risk assessment	Representative species and bee role category	Daily sugar consumption (mg/bee/day) or over developmental period (mg/larva/developmental period)		Daily pollen consumption (mg/bee/day) or over development period (mg/larva/developmental period)
Honey bee	*Apis mellifera* forager	acute: chronic:	78.9* 74.7*	0
	A. mellifera nurse	34		11.6
	A. mellifera larva	81.5		1.99*
Bumble bee	generic model bumble bee species	acute: chronic:	79.8* 75.6*	11.7
	Bombus terrestris larva	194.6		60.23
Solitary bee	generic model solitary bee species	acute: chronic:	4.27* 4.05*	0.6
	Osmia bicornis larva	91		91.3*
	O. cornuta larva	165		91.3*

* Represents the 90th percentile value of the range and was used for shortcut value calculations.

EF_{di} Exposure factor for dietary exposure (-).
EF_{di} represents the proportion of the applied chemical that deposits on the plants due to spray drift or dust formation including a safety factor. It is dependent on the spray drift/dust drift (if relevant) and the growth stage of the crop/plants and the resulting interception. Default values for EF_{di} are tabulated in EFSA Bee

guidance Appendix B.

LF — Landscape dilution factor for pollen (LF_{po}) and nectar (LF_{ne}) (-).
This factor accounts for potential dilution in the residue levels entering the hive. An LF of 1 would mean that 100% of the food entering the hive/nest originates from contaminated pollen/nectar.
It is considered that the landscape factor for the acute exposure assessments for pollen and nectar should be 1 (100% of the collected pollen origin from the contaminated crop). A LF <1 is recommended for the chronic dietary exposure estimation in pollen for honey bee adults and larvae for the scenarios 'Treated area' and 'Plants in treated area during the next growing season' when a single plant species is assumed to be growing in the given area. The LF is not a single value, but a range of values that feeds into the Monte Carlo method for calculation of SVs. LF is already included in the SVs tabulated in ECHA Bee guidance Appendix B.

PCUD — Predicted Concentration per Unit Dose in pollen ($PCUD_{po,du}$) and nectar ($PCUD_{ne,du}$) from during flowering application (mg/kg).
PCUD is where relevant included in the SVs tabulated in ECHA Bee guidance Appendix B. For further information on how PCUDs are calculated, see EFSA Bee guidance Section 5.1.2.3.

PEC_{pw} — Predicted Environmental Concentration in pore water (mg/kg ≙ mg/L).
PEC_{pw} parameter for the Tier 1 exposure estimation is a single default value, which is 1 mg/kg (≙ mg/L).

SN — Sugar content in nectar expressed as mass/mass (e.g., kg/kg).

The sugar content of the nectar is crop/plant-dependent, lower sugar contents result in higher nectar consumption by bees. EFSA defines SN values for several crops. For biocides, the crop/plant species is often unknown, therefore, the default SN of 10% for solitary bees and 15% for honey bees and bumble bees applies. In habitats with mixed vegetation, a sugar content of 30% is to be considered for the risk assessment.

In rare cases where the crop/plant species is known, it can be checked if the species is listed in EFSA Bee guidance, Table 17. Otherwise, the following values from Table 7 should be used.

Table 7: Sugar content in nectar (SN) (EFSA Bee guidance)

Vegetation type	Sugar content in nectar (%)		
	Honey bee	Bumble bee	Solitary bee
Unknown crop/ grassland or Treated specific species of vegetation (not listed in EFSA Bee guidance Table 17)	15	15	10
Mixed vegetation	30	30	30

SV — Shortcut value (µg/bee or µg/bee/day or µg/larva/developmental period).

SVs represent the 90th percentile of the distribution of the residue intakes by bees, based on an application rate of 1 kg a.s./ha. The units are mentioned for brevity as µg/bee in some places in the text, but they are in fact µg/bee per day for chronic exposure and µg/bee per developmental period for larvae. SVs have been calculated with a Monte Carlo method and are tabulated by EFSA (see EFSA Bee guidance Appendix B). The values are classified according to the type of food

(nectar or pollen), role of the bee (forager acute/chronic, nurse acute/chronic or larvae), period of flowering (before or during application), and exposure pathway (spraying (downward or sideward/upward, via soil). Although SVs are very PPP specific, ECHA EG decided that certain SVs can be applied for biocides as a worst-case approach, considering that the AR for biocides is usually << 1 kg a.s./ha. Relevant SVs for biocides are shown in Appendix B.

Table 8: Overview shortcut values (SV)

Parameter	Definition
$SV_{po,du}$	Shortcut value for pollen for during flowering situations
$SV_{ne,du}$	Shortcut value for nectar for during flowering situations
$SV_{po,soil}$	Shortcut value for pollen for situations for contamination from soil
$SV_{ne,soil}$	Shortcut value for nectar for situations for contamination from soil

Output parameters

PEQ_{di} Predicted Exposure Quantity due to dietary exposure (µg/bee or µg/bee/day or µg/larva/developmental period).
PEQ_{di} refers to the intake of biocide mass per bee. For the chronic adult assessments, this quantity has to be expressed per day, but for the larvae it has to be expressed as the sum of the intake over the entire developmental period.

The calculated PEQs are to be included in the subsequent risk assessment (see Chapter 7).
For detailed information, refer to EFSA Bee guidance Chapter 5.

5.1.2. Contact model

The contact model is to be used when there is physical contact between the surface of the bee and the biocide (EFSA Bee guidance). This route of exposure may take place during or shortly after the spray application of the biocidal product. Thus, it is mainly relevant for foraging honey bees, foraging worker bumble bees and adult solitary bees. For more information on the contact route of exposure models, see Section 5.1.1 of the EFSA Bee guidance.

The model to be used in the lower tier exposure assessment is the following:

$$PEQ_{co} = AR \times EF_{co} \times BSF$$ **Equation 7**

The parameters of this model are described below.

Parameter description:

Input parameters

AR Application rate (g/ha) (see Section 5.1.1).

BSF Body surface factor (dm²/bee).
BSF is applied to take into consideration the size differences between bee species when performing the exposure assessment.

Table 9: Body surface factor (BSF) (EFSA Bee guidance)

Category for the risk assessment	Representative species	BSF (dm²/bee)
Honey bee	*Apis mellifera*	0.0114
Bumble bee	5th percentile (by body surface) bumble bee species	0.0146
Solitary bee	5th percentile (by body surface) solitary bee species	0.00184

EF_{co} Exposure factor for contact exposure (-).
EF_{co} represents the proportion of the applied chemical that deposits on plants due to spray drift or dust. It is dependent on the spray drift/dust drift (if relevant) and the growth stage of the crop/plants and resulting interception. Default values for EF_{co} are tabulated in the EFSA Bee guidance Appendix B.

Output parameters

PEQ_{co} Predicted Exposure Quantity for contact exposure (µg/bee).
The calculated PEQs are to be included in the subsequent risk assessment (see Chapter 7). For detailed information, refer to EFSA Bee guidance Chapter 5.

5.1.3. Screening step

The exposure assessment for the dietary route of exposure is a complex process, involving several steps and numerous parameters (EFSA Bee guidance). Therefore, EFSA has formed a simplified method for the derivation of PEQ_{di} values. This screening PEQ_{di} can be used in the combined risk assessment (see Chapter 7). Applying this screening is an option, but not mandatory and can only be used as far as the cumulative application rate (AR x n) is not higher than 4.5 kg/ha. In the ECHA bee guidance, the screening step is suggested only for the sources of exposure presented in Sections 5.5-5.7. The method for PEQ_{di} derivations for the screening step is a simplified version of the models described in Sections 5.1.1 and 5.1.2, resulting in conservative exposure estimations compared to Tier 1. In the simplified model for the during flowering contamination model, application rate (AR; g/ha), and the number of applications (to soil) (n) has to be combined with a constant B in the following way:

$$PEQ_{di} = \frac{AR}{1000} \times n \times B \qquad \textbf{Equation 8}^{8}$$

where B is a constant that depends on the risk case and the application method as presented in Table 10. Where details on the orientation of the spray nozzles to the treated area are not available during the assessment, constant B for sideward/upward spray application is to be used as a worst-case.

[8] Units of the parameters in this equation are as follows: [µg/bee or µg/bee/day or µg/larva/developmental period] = [g/ha]/1000 * [-] * [µg/bee or µg/bee/day or µg/larva/developmental period]. While constant B is developed from SVs and CMP (see Equation 1) where SVs are based on an application rate of 1 kg a.s./ha, the units of the AR in this equation are cancelled out.

Table 10: The values of constant B (µg/bee or µg/bee/day or µg/larva/developmental period) for each risk case by application methods (EFSA Bee guidance)

Category for the risk assessment	Risk case	Constant B to be used for downward (DW) spray	Constant B to be used for sideward/upward (SUW) spray application
Honey bee	acute adult	6.4	9.0
	chronic adult	6.2	9.0
	larva	7.2	9.2
Bumble bee	acute adult	10	13.7
	chronic adult	9.6	13.3
	Bombus terrestris larva	33.7	48.5
Solitary bee	acute adult	0.70	0.94
	chronic adult	0.67	0.90
	Osmia bicornis larva	38.2	57.5
	Osmia cornuta larva	47.2	68.8

This method results in more conservative PEQdi values for all situations which would be calculated by the model for the above soil contamination.

As regards the through soil contamination, single default PEQ_{di} values are available (sum of the Tier 1 SVs), which are independent of the application rate as presented in Table 11.

Table 11: The Predicted Exposure Quantity values due to dietary exposure (PEQ_{di}) (µg/bee or µg/bee/day or µg/larva/developmental period) relevant for situations or scenarios where the through soil contamination model is to be applied (EFSA Bee guidance)

Category for the risk assessment	Risk cases	PEQdi
Honey bee	acute adult	0.530
	chronic adult	0.500
	larva	0.542
Bumble bee	acute adult	0.541
	chronic adult	0.511
	Bombus terrestris larva	1.357
Solitary bee	acute adult	0.044
	chronic adult	0.041
	Osmia bicornis larva	0.993
	Osmia cornuta larva	1.783

The next step is to compare the PEQ_{di} values calculated by applying Equation 8 with the PEQ_{di} values reported in Table 11. For each risk case, the highest of the two PEQ_{di} values has to be considered in the risk assessment for the screening step (EFSA Bee guidance).

As regards to the screening step for the contact exposure, the PEQ_{co} is calculated using the following simplified model:

$$PEQ_{co} = AR \times BSF$$ **Equation 9**

The units of the parameters are the same as described in Section 5.1.2.

5.2. Source of exposure – application of manure/sludge from animal housing

5.2.1. Description of source of exposure

This section concerns the assessment of exposure and risk to bees due to application of manure (including slurry) or sewage sludge on agricultural soil and grasslands. Following the consideration that the exposure of bees would not be negligible for this release route (Section 2.1), it is required to assess risk for bees. Exposure of bees to biocidal active substances due to the application of manure or sewage sludge is considered to be relevant for the following emission scenario for biocides:

- PT 18: Insecticides used in Stables and Manure Storage Systems – Emission to Manure
- PT 18: Insecticides used in Stables and Manure Storage Systems – Emission to sewage sludge: Regarding application of sewage sludge on soil, the risk assessment for bees needs to be performed for releases of biocides to municipal STP after treatment of animal housings for animal subcategories i8, i11-12 and i16-18 with biocides (ESD PT 18 for Insecticides for stables and manure storage systems 2006).

Following the application of the biocidal product in animal housings or manure storage systems, the biocidal active substance can, depending on the release pathway, reach manure or sewage sludge (see ESD PT 18 (2006)), which might be emitted to the environment either via the spreading of manure or through the application of sewage sludge on agricultural soil or grassland. Active substances and their metabolites can then be taken up by plants growing on the agricultural soil/grassland and translocated via the vascular system and the tissues of plants to pollen and nectar. From there they can be taken up and consumed by bees.

The area, where sewage sludge or manure is applied to, is unknown. Consequently, it is also unknown which crop (or grass) is grown there. However, it can be assumed that one type of crop or grass species is grown in the considered area.

Bees may be mainly exposed to biocides in manure/sludge via the consumption of contaminated pollen and nectar (oral exposure) after spreading of manure or sewage sludge on agricultural land or grassland.

The main exposure scenario that needs to be addressed for this source of exposure is the 'Treated area' scenario as a worst-case. This scenario covers the 'Weeds in the treated area' and 'Plants in treated area during the next growing season' scenarios. The exposure of bees relies on the dietary model for through soil contamination. The 'Vegetation margin' scenario is not relevant for this source of application as there is no spray drift.

5.2.2. Dietary exposure model

5.2.2.1. Tier 1

Tier 1 is based on default shortcut values for "through soil contamination" from the EFSA Bee guidance.

Specific input parameters for Tier 1

$SV_{po,soil}$ The shortcut value is based on a PEC_{pw} of 1 mg/kg (≙ mg/L), which can be considered as an extreme worst-case for biocides. SVs for pollen for through soil contamination apply, see Appendix B. SVs are based on SN for "unknown crop/grassland".

$SV_{ne,soil}$ The shortcut value is based on a PEC_{pw} of 1 mg/kg (≙ mg/L), which can be considered as an extreme worst-case for biocides. SVs for nectar for through soil contamination apply, see Appendix B. SVs are based on SN for "unknown crop/grassland".

Calculations for Tier 1

Table 12: Application of manure/sludge from animal housing - Tier 1 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg /bee or µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = SV_{po,soil} + SV_{ne,soil}$				**Equation 4**	

5.2.2.2. Tier 2

Basically, the same calculation as in Tier 1 is conducted but the shortcut values are multiplied with a product specific PEC_{pw}, which is a standard output value of the biocide environmental exposure assessment for manure or sewage sludge application and can be derived according to Guidance on BPR, Vol. IV, Part B+C (2017, p. 93, equation 70). In the SV for Tier 1, PEC_{pw} is already included in 1 mg/kg (≙ mg/L) (see Equation 5 and Equation 6), thus for Tier 2, $PEC_{pw,2}$ in Equation 10 must be considered unitless for the correct final unit (µg/bee or µg/bee/day or µg/larva/developmental period), but is given in mg/kg (≙ mg/L).

Specific input parameters for Tier 2

$PEC_{pw,2}$ Predicted environmental concentration in porewater after application of manure or sludge mg/kg (≙ mg/L). Prior to calculating a PEC_{pw}, a PEC_{soil} needs to be derived. PEC_{soil} is calculated as the time-weighted average (TWA) concentration in soil over 180 days after the last application on land after 10 consecutive years of manure or sludge application (TAB ENV 237).

For all the other parameters, see Section 5.2.2.1.

Calculations for Tier 2

Table 13: Application of manure/sludge from animal housing - Tier 2 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Predicted Environmental Concentration in pore water – Tier 2	$PEC_{pw,2}$		Unitless (but in fact mg/kg (≙ mg/L)	S	Biocides exposure assessment
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure – Tier 2	$PEQ_{di,2}$		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di,2} = (SV_{po,soil} + SV_{ne,soil}) \times PEC_{pw,2}$				**Equation 10**	

5.2.2.3. Tier 3

In Tier 3, the same calculation as in Tier 1 is conducted but the shortcut values are multiplied with a product-specific $PEC_{pw.}$ The PEC_{pw} in Tier 2, which is calculated from PEC_{soil} in the biocides exposure assessment and is considered as a conservative value, can be refined by the modelling tool FOCUS PEARL. For all other input parameters, a refinement is not considered feasible as the area of application of manure or sludge is unknown and no crop specific parameters can be derived. In the SV for Tier 1, PEC_{pw} is already included as 1 mg/kg (≙ mg/L) (see Equation 5 and Equation 6). Thus also for Tier 3, $PEC_{pw,3}$ in Equation 11 must be considered unitless for the correct final unit (µg/bee or µg/bee/day or µg/larva/developmental period) but it is still given in mg/kg (≙ mg/L).

Specific input parameters for Tier 3

$PEC_{pw,3}$ PEC_{pw} is refined by the use of FOCUS PEARL v. 5.5.5 software. The agreed general input parameters and settings for FOCUS PEARL v. 4.4.4 according to TAB ENV 23, 165 and 166 (ECHA 2022b) for modelling of groundwater concentrations are also valid for modelling of PEC_{pw}. The "runs" produced during modelling of groundwater concentrations can be processed further to generate an output on porewater concentrations. Similar to the EFSA Bee guidance, the refined PEC_{pw} should be derived over 20 cm soil depth, and 150 days after the last application on agricultural land and 120 days after the last application on grassland. A detailed description of settings of the FOCUS PEARL v. 5.5.5 for modelling of porewater concentrations can be found in Appendix C.

For all the other parameters, see Section 5.2.2.1.

Calculations for Tier 3

Table 14: Application of manure/sludge from animal housing - Tier 3 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Predicted Environmental Concentration in pore water – Tier 3	$PEC_{pw,3}$		Unitless (but in fact mg/kg (≙ mg/L)	S	Biocides exposure assessment
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure – Tier 3	$PEQ_{di,3}$		µg/bee or µg/bee/day or µg/larva/developmental period	O	
End Calculation					
$PEQ_{di,3} = (SV_{po,soil} + SV_{ne,soil}) \times PEC_{pw,3}$					**Equation 11**

5.3. Source of exposure – spraying on walls and foundation of buildings

5.3.1. Description of source of exposure

A quantitative risk assessment for bees needs to be performed for the products applied around residential and non-residential buildings, i.e., for spray applications onto walls for flying insects and/or foundation for crawling insects (corresponding to outdoor emission model "spray application: treatment around the building" on p. 136 of the ESD PT 18 for household and professional uses (2008)). For wall treatment, it is assumed that the whole surface of the walls of a building is sprayed, whereas for foundation treatment only the bottom wall area (0.5 m of height) is treated. Spray treatment of walls and/or foundation with biocidal products leads to the contamination of a soil strip that surrounds the treated building. The contamination occurs through spray deposition or through run-off and/or wash-off by rainfall. For the risk assessment for bees, the contaminated soil surrounding the building is assumed to be part of the garden (residential buildings) or surrounding vegetation (non-residential buildings) that is covered with flowers or bushes that are attractive to bees.

For both the treatment of walls and foundation, dietary (above and through soil contamination for the same contaminated soil area) and contact exposure of bees are relevant. Due to deposition from the spray application, nectar and pollen of flowering plants around the building are directly contaminated by biocides (above soil contamination). At the same time, due to run-off and wash-off by rainfall, biocides reach the soil. They are taken up by the plants via their roots (through soil contamination). Those plants might be already present during the biocidal

application or are yet to emerge. Bees can also get in direct contact with the biocides due to the spray drift or contact with contaminated plant matrices shortly after the biocidal treatment (contact exposure).

The 'Vegetation margin' scenario is considered the only relevant exposure scenario for the spray application on walls and/or foundation because bees are exposed in the area adjacent to the wall and/or foundation treated with biocides. The plants growing on the band of contaminated soil surrounding the treated building are considered mixed vegetation which is in flower at the time of application and attractive to bees. In contrast to the PPP assessment, where only above soil contamination is considered for the 'Vegetation margin' scenario (in the EFSA bee guidance, this scenario is called Field margin scenario), both above and through soil contamination need to be considered for this biocidal source of exposure (see above). Since the wall or the foundation is the object of the biocidal treatment but as such is not relevant for exposure of bees, the exposure scenarios 'Treated area', 'Weeds in the treated area' and 'Plants in the treated area during the next growing season' are not relevant for the risk assessment.

5.3.2. Dietary exposure model

5.3.2.1. Tier 1

Table 15 presents the input and the output parameters for the dietary model for above soil contamination.

Specific input parameters for Tier 1 – above soil contamination

Q_{prod}	Quantity of product applied to treated surface.
F_{ai}	Fraction of active substance in the product.
$AREA_{treated}$	Treated area for wall or foundation spraying.
$AREA_{soil}$	Area of soil around the house that is contaminated during and after spraying. The width of this soil band is 0.5 m according to ESD PT 18 (2008).
$SV_{po,du}$	Shortcut values for pollen, sideward/upward (SUW, wall) or downward (DW, foundation) spraying is applicable taking into account that mixed vegetation is growing on the band of soil around the house, see Appendix B.
$SV_{ne,du}$	Shortcut values for nectar, sideward/upward (wall) or downward (foundation) spraying is applicable taking into account that mixed vegetation is growing on the band of soil around the house, see Appendix B.
EF_{di}	Exposure factor for dietary exposure, value is set to 0.1 which equals the deposition fraction for wall treatment according to ESD PT 18 (2008).
F_{extrap}	Extrapolation factor.

Calculations for Tier 1 – above soil contamination

Table 15: Spraying on walls and foundation of buildings - Tier 1 calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value Res. Building	Value Non-res. Building	Unit	Origin	Source
Input						
Quantity of product applied (e.g., to wall, foundation)	Q_{prod}			g/m^2	S	
Fraction of a.s. in the product	F_{ai}			[-]	S	

Treated surface (wall, foundation)	$AREA_{treated}$	wall: 125 foundation: 25	wall: 400* foundation: 50*	m^2	D/S	ESD PT 18 (2008) TAB ENV 52, 140 and 159 (2022)
Area of soil that is contaminated	$AREA_{soil}$	26	51*	m^2	D/S	ESD PT 18 (2008) TAB ENV 159 (2022)
Shortcut value for pollen for above soil contamination – for SUW or DW spraying	$SV_{po,du}$			µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for above soil contamination – for SUW or DW spraying	$SV_{ne,du}$			µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Exposure factor for dietary exposure	EF_{di}	0.1	0.1	[-]	D	ESD PT 18 (2008)
Extrapolation factor	F_{extrap}	10000	10000	m^2/ha	D	
Output						
Application rate	AR			g/ha	O	
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}			µg/bee or µg/bee/day or µg/larva/developmental period	O	
$AR = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times F_{extrap}$ **Equation 12** $PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$ **Equation 1**						

* The parameters for non-residential buildings are derived as follows:

Surface (floor) area of non-residential building	= 609 m^2 (TAB ENV 140)
Perimeter	= 100 m (= $609^{0.5}$ x 4 sides) (TAB ENV 159)
Height of non-residential buildings	= 4 m (TAB ENV 52)
Length of one wall	= 100 m / 4 = 25 m
Treated area of wall	= 25 m x 4 m x 4 sides = 400 m^2
Treated area of foundation	= 25 m x 0.5 m x 4 sides = 50 m^2
Area of soil exposed	= (26 m x 26 m) – (25 m x 25 m) = 51 m^2

For the dietary model for through soil contamination, the only input parameters are the SV parameters for through soil contamination (Table 16). Thus, there is no differentiation between wall or foundation treatment.

Specific input parameters for Tier 1 – through soil contamination

$SV_{po,soil}$ Shortcut values for pollen for through soil contamination apply taking into account that mixed vegetation is growing on the band of soil around the house, see Appendix B. PEC_{pw} parameter for the Tier 1 SV estimation is 1 mg/kg (≙ mg/L).

$SV_{ne,soil}$ Shortcut values for nectar for through soil contamination apply taking into account that mixed vegetation is growing on the band of soil around the house, see Appendix B. PEC_{pw} parameter for the Tier 1 SV estimation is 1 mg/kg (≙ mg/L).

Calculations for Tier 1 – through soil contamination

Table 16: Spraying on walls and foundation of buildings - Tier 1 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value Res. Building	Value Non-res. Building	Unit	Origin	Source
Input						
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$			µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$			µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output						
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}			µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation						
$PEQ_{di} = SV_{po,soil} + SV_{ne,soil}$ **Equation 4**						

5.3.2.2. Tier 2

For the dietary model for above soil contamination in Tier 1, the application rate to the unintentionally contaminated soil area is extrapolated to cover a contaminated soil area of 1 ha. However, within this 1 ha there are also uncontaminated soil areas, as well as non-attractive parts such as terraces or impermeable pathways. Thus, in Tier 2 it is proposed to refine the dietary model for above soil contamination with a so-called contamination factor (F_{cont}). The calculation for the refinement is presented in Table 17.

Specific input parameters for Tier 2 – above soil contamination

$N_{buildings}$ Number of buildings within 1 ha is set to 16 for residential buildings according to TAB ENV 157. For non-residential buildings, $N_{buildings}$ was derived as follows:
For outdoor uses, 2500 houses and 300 non-residential buildings need to be assessed for the emission to STP (TAB ENV 140). Keeping the same ratio for the number of residential and non-residential buildings within 1 ha, we obtain $N_{buildings}$ 1.92 (rounded up to 2) for non-residential buildings per ha = 300 / 2500 x 16.

F_{cont} Contamination factor. The F_{cont} is a fraction between 0 and 1 and describes the area within 1 ha that is covered with contaminated vegetation attractive to bees. Therefore, it accounts for the dilution of the contaminated area within the total area of 1 ha.

For all the other parameters, see 5.3.2.1.

Calculations for Tier 2 – above soil contamination

Table 17: Spraying on walls and foundation of buildings - Tier 2 calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value Res. Building	Value Non-res. Building	Unit	Origin	Source
Input						
Quantity of product applied (e.g., to wall, foundation)	Q_{prod}			g/m^2	S	
Fraction of a.s. in the product	F_{ai}			[-]	S	
Treated surface (wall, foundation)	$AREA_{treated}$	wall: 125 foundation: 25	wall: 400 foundation: 50	m^2	D	ESD PT 18 (2008) TAB ENV 52, 140 and 159 (2022)
Area of soil that is contaminated	$AREA_{soil}$	26	51	m^2	D	ESD PT 18 (2008) TAB ENV 159 (2022)
Shortcut value for pollen for above soil contamination – for SUW or DW spraying	$SV_{po,du}$			µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for above soil contamination – for SUW or DW spraying	$SV_{ne,du}$			µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Exposure factor for dietary exposure	EF_{di}	0.1	0.1	[-]	D	ESD PT 18 (2008)
Number of buildings on a hectare	$N_{buildings}$	16	2	1/ha	D	TAB ENV 98 and 140 (2022)
Extrapolation factor	F_{extrap}	10000	10000	m^2/ha	D	

Output						
Contamination factor	F_{cont}			[-]	O	
Application rate – Tier 2	AR_2			g/ha	O	
Predicted Exposure Quantity due to dietary exposure – Tier 2	$PEQ_{di,2}$			µg/bee or µg/bee/day or µg/larva/developmental period	O	
Intermediate Calculation						
$F_{cont} = AREA_{soil} \times \frac{N_{buildings}}{F_{extrap}}$ **Equation 13**						
End Calculation						
$AR_2 = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times F_{extrap} \times F_{cont}$ **Equation 14**						
$PEQ_{di,2} = \frac{AR_2}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$ **Equation 15**						

For the dietary model through soil contamination in Tier 1, the equation for PEQ_{di} derivation is based on a PEC_{pw} of 1 mg/kg (≙ mg/L) (Equation 4). However, this is overly conservative for biocidal uses. Therefore, the PEQ_{di} can be refined using the actual calculated PEC_{pw} from the terrestrial exposure assessment. Table 18 presents the input and output parameters for Tier 2. In the SV for Tier 1, PEC_{pw} is already included as 1 mg/kg (≙ mg/L) (see Equation 5 and Equation 6). Thus for Tier 2, $PEC_{pw,2}$ in Equation 10 must be considered unitless for the correct final unit (µg/bee or µg/bee/day or µg/larva/developmental period) but it is still given in mg/kg (≙ mg/L).

Specific input parameters for Tier 2 – through soil contamination

$PEC_{pw,2}$ Predicted environmental concentration in porewater derived in the terrestrial exposure assessment according to Guidance on BPR, Vol. IV, Parts B+C (2017, p. 93, equation 70) in mg/kg (≙ mg/L). It is the result of the fraction of the product applied to the treated surface reaching the soil through deposition (10%), run-off (20%), and wash-off (50%) according to ESD PT 18 (2008).

For all the other parameters, see Section 5.3.2.1.

Calculations for Tier 2 – through soil contamination

Table 18: Spraying on walls and foundation of buildings - Tier 2 calculations for dietary model for through soil contamination

Parameters	**Nomenclature**	**Value Res. Building**	**Value Non-res. Building**	**Unit**	**Origin**	**Source**
Input						
Predicted concentration in porewater – Tier 2	$PEC_{pw,2}$			Unitless (but in fact mg/kg (≙ mg/L)	S	Biocides exposure assessment

Shortcut value for pollen for through soil contamination	$SV_{po,soil}$			µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$			µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output						
Predicted Exposure Quantity due to dietary exposure – Tier 2	$PEQ_{di,2}$			µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation						
$PEQ_{di,2} = (SV_{po,soil} + SV_{ne,soil}) \times PEC_{pw,2}$ **Equation 10**						

5.3.2.3. Tier 3

For the dietary model for above soil contamination, if unacceptable risks remain after Tier 2, any default parameters used in the exposure calculations could be refined if applicants provide experimental data to substantiate the deviation. Proposals for such refinements need to be consulted with the evaluating competent authorities.

For the dietary model for through soil contamination, however, the $PEC_{pw,2}$ could be further refined using FOCUS PEARL (see Appendix C). The resulting PEC_{pw} would be $PEC_{pw,3}$. For more information, see Section 5.2.2.3.

5.3.3. Contact exposure model

5.3.3.1. Tier 1

Table 19 presents the input and the output parameter for the contact model.

Specific input parameters for Tier 1

EF_{co} Exposure factor for contact exposure, value is set to 0.1 which equals the deposition fraction for wall treatment according to ESD PT 18 (2008).

BSF see Section 5.1.2.

For all the other parameters, see 5.3.2.1.

Calculations for Tier 1

Table 19: Spraying on walls and foundation of buildings - Tier 1 calculations for contact model

Parameters	Nomenclature	Value Res. Building	Value Non-res. Building	Unit	Origin	Source
Input						
Quantity of product applied to target surface (e.g., wall, foundation)	Q_{prod}			g/m²	S	
Fraction of a.s.	F_{ai}			[-]	S	
Treated surface (wall, foundation)	$AREA_{treated}$	wall: 125 foundation: 25	wall: 400 foundation: 50	m²	D	ESD PT 18 (2008) TAB ENV 52, 140 and 159 (2022)
Area of soil that is contaminated	$AREA_{soil}$	26	51	m²	D	ESD PT 18 (2008) TAB ENV 159 (2022)
Exposure factor for contact exposure	EF_{co}	0.1	0.1	[-]	D	Based on ESD PT 18 (2008)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm²/bee	D	Table 9
Extrapolation factor	F_{extrap}	10000	10000	m²/ha	D	
Output						
Application rate	AR			g/ha	O	
Predicted Exposure Quantity due to contact exposure	PEQ_{co}			µg/bee	O	
Calculation						
$AR = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times F_{extrap}$ **Equation 12**						
$PEQ_{co} = AR \times EF_{co} \times BSF$ **Equation 7**						

HB = honey bee, BB = bumble bee, SB = solitary bee

5.3.3.2. Tier 2

Table 20 presents the input and the output parameters for Tier 2 of the contact model. The application rate (AR) could be refined in the same way with the contamination factor (F_{cont}) as done for Tier 2 in the dietary model for above soil contamination (see Section 5.3.2.2). Applicants may also provide experimental data based on which the exposure factor for contact exposure may be refined.

Specific input parameters for Tier 2

$N_{buildings}$ see Section 5.3.2.2
F_{cont} see Section 5.3.2.2

For all the other parameters, please see 5.3.2.1.

Calculations for Tier 2

Table 20: Spraying on walls and foundation of buildings - Tier 2 calculations for contact model

Parameters	Nomenclature	Value Res. Building	Value Non-res. Building	Unit	Origin	Source
Input						
Quantity of product applied to target surface (e.g., wall, foundation)	Q_{prod}			g/m^2	S	
Fraction of a.s.	F_{ai}			[-]	S	
Treated surface (wall, foundation)	$AREA_{treated}$	wall: 125 foundation: 25	wall: 400 foundation: 50	m^2	D	ESD PT 18 (2008) TAB ENV 52, 140 and 159 (2022)
Area of soil that is contaminated	$AREA_{soil}$	26	51	m^2	D	ESD PT 18 (2008) TAB ENV 159 (2022)
Exposure factor for contact exposure	EF_{co}	0.1	0.1	[-]	D	Based on ESD PT 18 (2008)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	D	Table 9
Number of buildings on a hectare	$N_{buildings}$	16	2	1/ha	D	TAB ENV 98 and 140 (2022)
Extrapolation factor	F_{extrap}	10000	10000	m^2/ha	D	

Output						
Contamination factor	F_{cont}			[-]	O	
Application rate – Tier 2	AR_2			g/ha	O	
Predicted Exposure Quantity due to contact exposure – Tier 2	$PEQ_{co,2}$			ug/bee	O	
Intermediate Calculation						
$F_{cont} = AREA_{soil} \times \frac{N_{buildings}}{F_{extrap}}$ **Equation 13**						
End calculation						
$AR_2 = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times F_{extrap} \times F_{cont}$ **Equation 14**						
$PEQ_{co,2} = AR_2 \times EF_{co} \times BSF$ **Equation 16**						

5.4. Source of exposure – irrigation of private gardens with treated water

5.4.1. Description of source of exposure

PT 18 products can directly be applied to small-scale water habitats, e.g., water collectors, water reservoirs, tanks in private areas, and rainwater barrels for mosquito control. Larvicidal products are applied to standing water where mosquitoes could potentially lay eggs. After some time, the same treated water could be used to irrigate private gardens. This use is covered in the TAB ENV 205 (ECHA 2022b) which describes the emission scenario for the use of treated water for irrigation of private gardens where consequently the active substance is directly released to the soil compartment.

The 'Treated area' scenario is considered the only relevant exposure scenario for the irrigated garden where both the dietary model and contact model are used. This is justified to address the exposure due to contamination of plants growing in the garden as a result of shower of water sprinkled or poured over them (above soil dietary and contact exposure) as well as to address the contamination of plants which may be watered to the root instead (through soil dietary exposure). The irrigation of the garden with any systems, e.g., watering can or spraying with hose, is directed downwards. It can be assumed that there is no drift during irrigation and therefore the 'Vegetation margin' scenario is not relevant. The 'Weeds in the treated field' and the 'Plants in the treated area in the next growing season' scenarios are covered by the calculation of the 'Treated area' scenario. The plants growing in the garden are considered mixed vegetation which is in flower at the time of application (attractive to bees).

5.4.2. Dietary exposure model

5.4.2.1. Tier 1

The Tier 1 assessment is shown in Table 21.

Specific input parameters for Tier 1 – above soil contamination

Q_{irw} Amount of irrigation water to be used for irrigation of 1 m² garden, value is set to 2.86 L/m² (according to TAB ENV 205).

Cir_{1app} Concentration of active substance in irrigation water, after one b.p. application, see calculations done for the terrestrial risk assessment (according to TAB ENV 205).

Napp Number of repeated biocide applications to the water collection container, see calculations done for the terrestrial risk assessment (according to TAB ENV 205).

Cir_{Napp} Concentration of a.s. in irrigation water assuming repeated b.p. application, see calculations done for the terrestrial risk assessment (according to TAB ENV 205).

$SV_{po,du}$ Shortcut values for pollen, downward (DW) spraying taking into account that mixed vegetation is growing in the garden, see Appendix B.

$SV_{ne,du}$ Shortcut values for nectar, downward (DW) spraying taking into account that mixed vegetation is growing in the garden, see Appendix B.

EF_{di} Exposure factor for dietary exposure, value is set to 1 by default for the 'Treated area' scenario.

Calculations for Tier 1 – above soil contamination

Table 21: Irrigation of private gardens with treated water - Tier 1 calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Amount of irrigation water to be used for irrigation of 1 m² garden	Q_{irw}	2.86	L/m²	D	TAB ENV 205
Concentration of a.s. in irrigation water, after one b.p. application	cir_{1app}		mg/L	S	
Number of b.p. applications	Napp		[-]	S	
Concentration of a.s. in irrigation water assuming repeated b.p. application	cir_{Napp}		mg/L	S	
Shortcut value for pollen for above soil contamination – downward spraying	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/ developmental period	P	Appendix B
Shortcut value for nectar for above soil contamination – downward spraying	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/ developmental period	P	Appendix B
Exposure factor for dietary exposure	EF_{di}	1	[-]	D	EFSA Bee guidance Appendix B
Extrapolation factor	F_{extrap}	10000	10000	m²/ha	D

Output					
Application after 1 b.p. application	AR_{1app}		g/ha	O	
Application rate after repeated b.p. application	AR_{Napp}		g/ha	O	
Predicted Exposure Quantity due to dietary exposure for 1 b.p. or repeated b.p. applications	$PEQ_{di,1app}$ $PEQ_{di,Napp}$		µg/bee or µg/bee/day or µg/larva/ developmental period	O	
Intermediate calculation					
$AR_{1app} = Q_{irw} \times \frac{cir_{1app}}{1000} \times F_{extrap}$ **Equation 17** $AR_{Napp} = Q_{irw} \times \frac{cir_{Napp}}{1000} \times F_{extrap}$ **Equation 18**					
End calculation					
$PEQ_{di,1app} = \frac{AR_{1app}}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$ **Equation 19** $PEQ_{di,Napp} = \frac{AR_{Napp}}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$ **Equation 20**					

For the dietary model for through soil contamination, the only input parameters are the SV parameters for through soil contamination (Table 22).

Specific input parameters for Tier 1 – through soil contamination

$SV_{po,soil}$ Shortcut values for pollen, through soil dietary uptake taking into account that mixed vegetation is growing in the garden, see Appendix B. PEC_{pw} parameter for the Tier 1 SV estimation is 1 mg/kg (≙ mg/L).

$SV_{ne,soil}$ Shortcut values for nectar, through soil dietary uptake taking into account that mixed vegetation is growing in the garden, see Appendix B. PEC_{pw} parameter for the Tier 1 SV estimation is 1 mg/kg (≙ mg/L).

Calculations for Tier 1 – through soil contamination

Table 22: Irrigation of private gardens with treated water - Tier 1 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	

Calculation	
$PEQ_{di} = SV_{po,soil} + SV_{ne,soil}$	**Equation 4**

5.4.2.2. Tier 2

For the dietary model for above soil contamination in Tier 1, it is assumed that the whole treated hectare consists of attractive garden. However, this is an overly conservative assumption. Thus, a contamination factor (F_{cont}) similar to that applied for the emission scenario for spraying around the house could be used to refine the application rate.

Specific input parameters for Tier 2 – above soil contamination

$N_{buildings}$ Number of buildings within 1 ha is set to 16 according to TAB ENV 157.

$AREA_{garden}$ Size of an average European garden is set to 500 m^2 according to ESD PT 18 (2008).

F_{attr} Fraction of garden that is attractive to bees is set to 0.65. The value is derived on the basis of ESD PT 18 (2008, p. 151), according to which vegetation area in gardens could be divided into short-grass and leafy crops. Taking into account that a certain fraction of a lawn could be short-grass that is non-attractive to bees, it was decided for the sake of simplicity to divide the fraction of attractive/non-attractive lawn equally to 50:50. This leads to a Fattr of 0.65 (100%-(7.4 % any other + 6 % terrace + 5 % pathways + 33.3/2 % non-attractive lawn)).

F_{cont} Contamination factor. The F_{cont} is a fraction between 0 and 1 and describes the area within 1 ha that is covered with contaminated vegetation attractive to bees. Therefore, it accounts for the dilution of the contaminated area within the total area of 1 ha.

For all the other parameters, see Section 5.4.2.1.

Calculations for Tier 2 – above soil contamination

Table 23: Irrigation of private gardens with treated water - Tier 2 calculations for dietary model for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Amount of irrigation water to be used for irrigation of 1 m² garden	Q_{irw}	2.86	L/m^2	D	TAB ENV 205 (2022)
Concentration of a.s. in irrigation water, after one b.p. application	cir_{1app}		mg/L	S	
Number of b.p. applications	Napp		[-]	S	
Concentration of a.s. in irrigation water assuming repeated b.p. application	cir_{Napp}		mg/L	S	

Shortcut value for pollen for above soil contamination – downward spraying	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/ developmental period	P	Appendix B
Shortcut value for nectar for above soil contamination – downward spraying	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/ developmental period	P	Appendix B
Exposure factor for dietary exposure	EF_{di}	1	[-]	D	EFSA Bee guidance Appendix B
Number of buildings per ha	$N_{buildings}$	16	1/ha	D	TAB ENV 157 (2022)
Average size of private garden	$AREA_{garden}$	500	m^2	D	ESD PT 18 (2008)
Fraction of garden attractive to bees	F_{attr}	0.65	[-]	D	ESD PT 18 (2008)
Extrapolation factor	F_{extrap}	10000	m^2/ha	D	
Output					
Contamination factor	F_{cont}		[-]	O	
Application rate after 1 b.p. application – Tier 2	$AR_{1app,2}$		g/ha	O	
Application rate after repeated b.p. application – Tier 2	$AR_{Napp,2}$		g/ha	O	
Predicted Exposure Quantity due to dietary exposure for 1 b.p. or repeated b.p. applications – Tier 2	$PEQ_{di,1app,2}$ $PEQ_{di,Napp,2}$		µg/bee or µg/bee/day or µg/larva/ developmental period	O	

Intermediate calculation	
$F_{cont} = N_{buildings} \times AREA_{garden} \times \frac{F_{attr}}{F_{extrap}}$	**Equation 21**
$AR_{1app,2} = Q_{irw} \times \frac{cir_{1app}}{1000} \times F_{extrap} \times F_{cont}$	**Equation 22**
$AR_{Napp,2} = Q_{irw} \times \frac{cir_{Napp}}{1000} \times F_{extrap} \times F_{cont}$	**Equation 23**
End calculation	
$PEQ_{di,1app,2} = \frac{AR_{1app,2}}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$	**Equation 24**
$PEQ_{di,Napp,2} = \frac{AR_{Napp,2}}{1000} \times EF_{di} \times (SV_{po,du} + SV_{ne,du})$	**Equation 25**

For the dietary model through soil contamination in Tier 1, the equation for $PEQ_{j,di}$ derivation is based on a PEC_{pw} of 1 mg/kg (≙ mg/L) (Equation 4). However, this is overly conservative for biocidal uses. Therefore, the $PEQ_{j,di}$ can be refined using the actual calculated PEC_{pw} from the terrestrial risk assessment derived according to Guidance on BPR, Vol. IV, Parts B+C (2017, p. 93, equation 70). Table 24 presents the input and output parameters for Tier 2. In the SV for Tier 1, PEC_{pw} is already included in 1 mg/kg (≙ mg/L) (see Equation 5 and Equation 6), thus for Tier 2 $PEC_{pw,2}$ in Equation 10 must be considered unitless for the correct final unit (µg/bee or µg/bee/day or µg/larva/developmental period), but is given in mg/kg (≙ mg/L).

Specific input parameters for Tier 2 – through soil contamination

$PEC_{pw,2}$ Predicted concentration in porewater derived in the terrestrial risk assessment according to Guidance on BPR, Vol. IV, Parts B+C (2017, p. 93, equation 70).

For all the other parameters, see Section 5.4.2.1.

Calculations for Tier 2 – through soil contamination

Table 24: Irrigation of private gardens with treated water - Tier 2 calculations for dietary model for through soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Predicted concentration in porewater – Tier 2	$PEC_{pw,2}$		Unitless (but in fact mg/kg (≙ mg/L)	S	Biocides exposure assessment
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B

Output					
Predicted Exposure Quantity due to dietary exposure – Tier 2	$PEQ_{di,2}$		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di,2} = (SV_{po,soil} + SV_{ne,soil}) \times PEC_{pw,2}$ **Equation 10**					

5.4.2.3. Tier 3

For the dietary model for above soil contamination, if unacceptable risks remain after Tier 2, any default parameters used in the exposure calculations could be refined if applicants provide experimental data to substantiate the deviation. Proposals for such refinements need to be consulted with the evaluating competent authorities.

If needed, the dietary model for through soil contamination could be refined with $PEC_{pw,3}$ as described in the Sections 5.2.2.3 and 5.3.2.3.

5.4.3. Contact exposure model

5.4.3.1. Tier 1

Table 25 presents the input and the output parameter for the contact model.

Specific input parameters for Tier 1

EF_{co} Exposure factor for contact exposure, value is set to 1 by default for 'Treated area' scenario in accordance with EFSA Bee guidance, Appendix B, spraying application.
BSF see Section 5.1.2

For all the other parameters, see Section 5.4.2.1.

Calculations for Tier 1

Table 25: Irrigation of private gardens with treated water - Tier 1 calculations for contact exposure

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Amount of irrigation water to be used for irrigation of 1 m² garden	Q_{irw}	2.86	L/m²	D	TAB ENV 205 (2022)
Concentration of a.s. in irrigation water, after one b.p. application	cir_{1app}		mg/L	S	
Exposure factor for contact exposure	EF_{co}	1	[-]	D	EFSA Bee guidance Appendix B

Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	D	Table 9
Extrapolation factor	F_{extrap}	10000	m^2/ha	D	
Output					
Application rate after 1 b.p. application	AR		g/ha	O	
Predicted Exposure Quantity due to contact exposure, after 1 b.p. application	PEQ_{co}		µg/bee	O	
Intermediate calculation					
$AR = Q_{irw} \times \frac{cir_{1app}}{1000} \times F_{extrap}$				**Equation 26**	
End calculation					
$PEQ_{co} = AR \times EF_{co} \times BSF$				**Equation 7**	

5.4.3.2. Tier 2

For Tier 2, a refinement of the application rate could be done in line with what is proposed for the dietary model for above soil contamination (see Section 5.4.2.2) in relation to the contamination factor (Fcont) (Table 26).

Specific input parameters for Tier 2

$N_{buildings}$ see Section 5.4.2.2
$AREA_{garden}$ see Section 5.4.2.2
F_{attr} see Section 5.4.2.2
F_{cont} see Section 5.4.2.2

For all the other parameters, see Section 5.4.2.1.

Calculations for Tier 2

Table 26: Irrigation of private gardens with treated water - Tier 2 calculations for contact exposure

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Amount of irrigation water to be used for irrigation of 1 m² garden	Q_{irw}	2.86	L/m^2	D	TAB ENV 205 (2022)
Concentration of a.s. in irrigation water, after one b.p. application	cir_{1app}		mg/L	S	
Exposure factor for contact exposure	EF_{co}	1	[-]	D	EFSA Bee guidance Appendix B

Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	D	Table 9
Average size of private garden in Europe	$AREA_{garden}$	500	m^2	D	ESD PT 18 (2008)
Fraction of garden attractive to bees	F_{attr}	0.65	[-]	D	ESD PT 18 (2008)
Number of builings per ha	$N_{buildings}$	16	1/ha	D	TAB ENV 157 (2022)
Extrapolation factor	F_{extrap}	10000	m^2/ha	D	
Output					
Contamination factor	F_{cont}		[-]	O	
Application rate after 1 b.p. application – Tier 2	AR_2		g/ha	O	
Predicted Exposure Quantity due to contact exposure, after 1 b.p. application – Tier 2	$PEQ_{co,2}$		µg/bee	O	
Intermediate calculation					
$F_{cont} = N_{buildings} \times AREA_{garden} \times \frac{F_{attr}}{F_{extrap}}$					**Equation 21**
$AR_2 = Q_{irw} \times \frac{cir_{1app}}{1000} \times F_{extrap} \times F_{cont}$					**Equation 27**
End calculation					
$PEQ_{co,2} = AR_2 \times EF_{co} \times BSF$					**Equation 28**

5.5. Source of exposure – large scale spraying of specific species of trees (case A)

5.5.1. Description of source of exposure

This section concerns the assessment of exposure and risk to bees due to application by large scale spraying, in particular application of biocides on specific species of trees by aerial or ground spray against crawling and flying insects, which falls under PT 18 – Outdoor large scale spraying. For further information regarding this use, see TAB ENV 248 (ECHA 2022b).

As a result of this application bees may be exposed via overspray, spray drift or soil contamination. Considering that the exposure of bees would not be negligible for this release route, the risk has to be assessed for all relevant pathways.

Case A refers to the application on specific single species of trees (such as oaks, pines, or other woody perennials) likely to take place at forest edges, tree avenues (along the roads in the cities/countryside) or as solitary tree treatments in parks. These treatments are often performed using a cannon sprayer, motorized knapsack mistblowers (used from a lifting platform), or a helicopter. Exposure of bees is assumed due to the consumption of pollen and nectar of the

treated trees, of the plants not subject to the treatment but nevertheless affected by the spray deposition on the ground within the treated area, as well as those right next to the treated area. These might consist of flowering plants such as bushes, flowers, grass, berries, etc. At the same time bees may enter in physical contact with the spray containing biocide, or with sprayed plant matrices. Therefore, to assess the risk to bees, the following exposure scenarios need to be addressed for this source of exposure:

Treated area,
Weeds in the treated area,
Vegetation margin,
Plants in treated area during the next growing season.

In cases where non-attractive trees are treated, no risk assessment for the 'Treated area' scenario is required.

5.5.2. Dietary exposure model

5.5.2.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1.

Specific input parameters for Screening step

AR — The application rate of active substance in mass units per hectare assuming that treated trees with given crown diameter are growing densely next to each other on a hectare. The AR is based on product specific data.

N — see Section 5.1.3

B — Where the application is carried out by helicopter, downwards spraying values for constant B are recommended. Where the application is carried out by a cannon sprayer, sideward spraying values for constant B are recommended. Where details on the orientation of the spray nozzles to the treated area are not available during the assessment, values for constant B for sideward/upward spray application are applicable as a worst-case choice. See Table 10.

Calculations for Screening step

Table 27: Large scale spraying of specific species of trees (case A) - Screening step calculations for dietary exposure for above soil contamination

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Number of applications (to soil)	n		[-]	S	
Constant B	B		µg/bee or µg/bee/day or µg/larva/developmental period	P	Table 10
Output					
Predicted Exposure Quantity due to	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental	O	

dietary exposure			period		
Calculation					
$PEQdi = \frac{AR}{1000} \times n \times B$					**Equation 8**

As regards the through soil contamination, single default PEQ_{di} values are available, which are independent of application rate, as presented Table 11. The next step is to compare the PEQ_{di} values calculated by applying Equation 8 with the PEQ_{di} values reported in Table 11. For each risk case, the highest of the two PEQ_{di} values has to be considered in the risk assessment for the screening step.

5.5.2.2. Tier 1

Generally, in Tier 1 it is assumed that the biocidal application is performed on trees in spring or summer and the treated plants are attractive to bees (worst-case assumption) and while they are flowering. In case of a specific tree species is treated and where the tree species is not attractive to bees for pollen and/or nectar, 'Treated area' may not need to be assessed (if tree is not attractive for pollen, nor nectar) or some shortcut values may not need to used (if tree is not attractive for either pollen or nectar). For more information on attractiveness see EFSA Bee guidance, Appendix I and also Appendix A of this ECHA Bee Guidance.

On the basis of the above, assessment of exposure scenarios 'Treated area', 'Weeds in the treated area' and 'Vegetation margin' is based on the dietary model for above soil contamination and the respective defaults from the EFSA Bee guidance and TAB ENV 248 (2022). The dietary model for through soil contamination is relevant for the 'Plants in treated area during the next growing season' scenario.

Specific input parameters for Tier 1 – above soil contamination

EF_{di} For 'Treated area' scenario – value of 1 for spray application from the EFSA Bee guidance, Appendix B is applicable.
For 'Weeds in the treated area' scenario – value of 0.5 is to be used. The value is the worst-case value for to the most relevant surrogate crops "Olives (evergreen)" and "Pome/stone fruits" provided by the EFSA Bee guidance, Appendix B. The relevance is given by the similarity in the morphology/growth pattern of the treated trees compared to the species considered covered by these surrogate crop categories of EFSA. Generally, EF_{di} for this scenario depends on the growth stage of the treated plant (linked with interception by the treated plant).
For 'Vegetation margin' scenario – in accordance with TAB ENV 248 (2022) the highest drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides is considered relevant also as a basis for EF_{di} for this exposure scenario. TAB ENV 248 (2022) provides also drift values for different application techniques and field of uses in treatment against the oak processionary moth, which may be used when relevant.

$SV_{po,du}$; $SV_{ne,du}$ The biocides application as a worst-case assumption is considered to take place during flowering of the treated tree. Where the application is carried out by helicopter, downwards spraying SV are recommended. Where the application is carried out by a cannon sprayer, sideward spraying SV are recommended. Where details on the orientation of the spray nozzles to the treated area are not available during the assessment, a worst-case set of SVs independent of spray nozzles orientation (set up for biocides) should be used. SVs are presented in Appendix B (based on EFSA Bee guidance Appendix B). Selection of the SV is made on the basis of the number of applications during the year and the interval between multiple applications.
Since 'Weeds in treated area' and 'Plants in the Vegetation margin' are considered as habitats with mixed vegetation, sugar content of 30 % is applicable (see Table 7). Consequently, SVs for nectar for these scenarios should be calculated as follows: multiply the original $SV_{ne,du}$ by 1/3 for SB groups; by ½ for HB and BB groups.
Where treated trees are attractive for one matrix only e. g. pollen (oaks), SVs for the matrix which is not relevant (i. e. nectar in this case) are considered 0 in the Treated area scenario.

Calculations for Tier 1 – above soil contamination

Table 28: Large scale spraying of specific species of trees (case A) - Tier 1 calculations for dietary model for above soil contamination ('Treated area', 'Weeds in the treated area', 'Vegetation margin')

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Exposure factor for dietary exposure	EF_{di}		[-]		
'Treated area'		1		D	EFSA Bee guidance, Appendix B
'Weeds in the treated area'		0.5		D	EFSA Bee guidance, Appendix B
'Vegetation margin'		0.381		D/S	TAB ENV 248 (2022)
Shortcut value for pollen for during flowering situations	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for during flowering situations	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B Multiply original SV_{ne} for 'Weeds in the treated area' and 'Vegetation margin' scenarios, with 1/3 for SB; by ½ for HB and BB
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times \left(SV_{po,du} + SV_{ne,du}\right)$ **Equation 1**					

Specific input parameters for Tier 1 – through soil contamination

$SV_{po,soil}$ Shortcut values are based on a PEC_{pw} of 1 mg/kg (≙ mg/L), which can be considered as an extreme worst-case for biocides. SVs for pollen from EFSA model "through soil contamination" apply, see Appendix B.

$SV_{ne,soil}$ The shortcut values are based on a PEC_{pw} of 1 mg/kg (≙ mg/L), which can be considered as an extreme worst-case for biocides. SVs for nectar from EFSA model "through soil contamination" apply, see Appendix B.

Calculations for Tier 1 – through soil contamination

Table 29: Large scale spraying of specific species of trees (case A) - Tier 1 calculations for dietary model for through soil contamination ('Plants in treated area during the next growing season')

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = SV_{po,soil} + SV_{ne,soil}$ **Equation 4**					

5.5.2.3. Tier 2

'Treated area', 'Weeds in the treated area', 'Vegetation margin'

In Tier 2, PEQ_{di} can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2 – above soil contamination

AR — Applicants may propose typical areas relevant for the treatment of single trees, tree avenues, forest edges, where different spacing between treated trees may be assumed. In such instance, AR for Tier 2 may be derived by multiplying AR from Tier 1 with a fraction of soil defined by the treated trees crown border assumed on 1 hectare. Where Tier 2 is relevant, discussion at WG level is needed to agree on necessary default values to calculate AR. See Table 28.

$SV_{po,du}$; $SV_{ne,du}$ — Since SVs are a function of the number of applications during the year and the interval between multiple applications, lower number of applications per year and/or larger interval between applications may be considered as a form of refinement, which will result in smaller SVs. Such change needs to be confirmed by efficacy data.

EF_{di} — For 'Vegetation margin' scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 28.

For other options for refinement, see EFSA Bee guidance Sections 5.4 and 5.5. Proposals for such refinements need to be consulted with the evaluating competent authorities prior to the submission of the assessment.

'Plants in treated area during the next growing season'

Refer to Section 5.2.2.2. For the large scale spraying case A nevertheless, considerations described below apply.

Specific input parameters for Tier 2 – through soil contamination

$PEC_{pw,2}$ Predicted environmental concentration in porewater calculated from PEC_{soil} initial (in case of multiple applications after last application) after ten consecutive years of application, taking degradation into account in line with TAB ENV 248 (2022) following equation 70 of Biocides Guidance Volume IV Part B and C. Time-weighted average (TWA) concentration in soil over 180 days after the last application after 10 consecutive years of application may be considered as a second Tier (TAB ENV 237 (2022)).

5.5.2.4. Tier 3

'Plants in treated area during the next growing season'

In Tier 3, the same calculation as in Tier 1 is conducted but the shortcut values are re-calculated based on a product specific PEC_{pw}. The PEC_{pw} in Tier 2, which is calculated from PEC_{soil} in the biocides exposure assessment and is considered as a conservative value, can be refined by the modelling tool FOCUS PEARL. For more information, see Section 5.2.2.3.

5.5.3. Contact exposure model

5.5.3.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1.

Specific input parameters for Screening step

AR see Section 5.5.2.1
BSF see Section 5.1.2

Calculations for Screening step

Table 30: Large scale spraying of specific species of trees (case A) - Screening step calculations for contact exposure

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	P	Table 9
Output					
Predicted Exposure Quantity for contact exposure	PEQ_{co}		µg/bee	O	
Calculation					
$PEQ_{co} = AR \times BSF$					**Equation 9**

5.5.3.2. Tier 1

Generic considerations described under Section 5.5.2.2 Tier 1 apply.
Contact exposure is relevant for exposure scenarios 'Treated area', 'Weeds in the treated area' and 'Vegetation margin' and Tier 1 is based on the respective defaults from the EFSA Bee guidance and TAB ENV 248 (ECHA 2022b). Contact exposure is not relevant for the 'Plants in treated area during the next growing season' exposure scenario and therefore PEQ_{co} value for this scenario is equal 0 in the combined risk assessment.

Specific input parameters for Tier 1

AR see Section 5.5.2.2

EF_{co} For 'Treated area' scenario – a worst-case value of 1 associated with the flowering stage of the treated trees in case of spray application from the EFSA Bee guidance, Appendix B is applied.
For 'Weeds in the treated area' scenario – value of 0.5 is to be used. The value is the worst-case value for the most relevant surrogate crops "Olives (evergreen)" and "Pome/stone fruits" provided by the EFSA Bee guidance, Appendix B. The relevance is given by the similarity in the morphology/growth pattern of the treated trees compared to the species considered covered by these surrogate crop categories of EFSA. Generally, EF_{co} for this scenario depends on the growth stage of the treated plant (linked with interception by the treated plant).
For 'Vegetation margin' scenario – in accordance with TAB ENV 248 (2022) the highest drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides is considered relevant also as a basis for EF_{co} for this exposure scenario. TAB ENV 248 (2022) provides also drift values for different application techniques and field of uses in treatment against the oak processionary moth, which may be used when relevant.

Calculations for Tier 1

Table 31: Large scale spraying of specific species of trees (case A) – Tier 1 calculations for contact exposure ('Treated area', 'Weeds in the treated area', 'Vegetation margin')

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Exposure factor for dietary exposure	EF_{co}		[-]		
'Treated area'		1		D	EFSA Bee guidance, Appendix B
'Weeds in the treated area'		0.5		D	Based on EFSA Bee guidance, Appendix B
'Vegetation margin'		0.381		D/S	TAB ENV 248 (2022)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	P	Table 9
Output					
Predicted Exposure Quantity for contact exposure	PEQ_{co}		µg/bee	O	
Calculation					
$PEQ_{co} = AR \times EF_{co} \times BSF$ **Equation 7**					

5.5.3.3. Tier 2

In Tier 2, PEQ_{co} can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2

AR	see Section 5.5.2.3. See Table 31.
EF_{co}	For Vegetation margin scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 31.

5.6. Source of exposure – large scale spraying of mixed species of trees and bushes (case B)

5.6.1. Description of source of exposure

This section concerns the assessment of exposure and risk to bees due to application by large scale spraying, in particular application of biocides on mixed species of trees or shrubs by ground spray against crawling and flying insects (e.g., for mosquito control), which falls under PT 18 – Outdoor large scale spraying. For further information regarding this use see TAB ENV 248 (ECHA 2022b).[9]

As a result of this application, bees may be exposed via overspray, spray drift, or soil contamination. Considering that the exposure of bees would not be negligible for this release route, the risk has to be assessed for all relevant pathways.

Case B refers to the application on mixed species of trees and bushes (woody perennials). Parallels may be drawn with a surrogate crop called "ornamentals", distinguished by the EFSA Bee guidance, which refers to a diverse group of plants, grown in a variety of ways, which can vary from small herbaceous plants to large ornamentals trees. Exposure of bees is assumed due to the consumption of pollen and nectar of the treated plants, of the plants not subject to the treatment but nevertheless affected by the spray deposition on the ground within the treated area, as well as those right next to the treated area. These might consist of flowering plants such as bushes, flowers, grass, berries, etc. At the same time bees may enter in physical contact with the spray containing biocide or with sprayed plant matrices. Therefore, to assess the risk to bees, the following exposure scenarios need to be addressed for this source of exposure:

Treated area,
Weeds in the treated area,
Vegetation margin,
Plants in treated area during the next growing season.

Since mixed plants are being treated, as a worst-case assumption these are considered attractive to bees for both pollen and nectar.

For Case B there may be two ways of spraying required: 1. Treatment on large areas covered with mixed species of plants such as trees, bushes, and possibly also lawns (e. g. parks, forest edges, amenity areas); 2. Treatment on restricted areas (e. g. single trees in parks, tree avenues, single bushes, hedges, trees in gardens/amenity areas) with untreated areas in between. The assessment strategy as provided in the Section 5.6 applies to both situations, except for the first situation, where refinement of AR as given in Tier 2 in Section 5.6.2.3 is not applicable.

[9] Ultra low volume (ULV) spraying is out of scope of TAB ENV 248 (2022) and therefore also this guidance.

5.6.2. Dietary exposure model

5.6.2.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1.

Specific input parameters for Screening step

AR — The application rate of active substance in mass units per hectare assuming that treated plants are growing densely next to each other on a hectare. The AR is based on product specific data.

For further input parameters and calculations, see Section 5.5.2.1.

5.6.2.2. Tier 1

In Tier 1 it is assumed that the biocidal application is performed on plants in spring or summer and the treated plants are attractive to bees (worst-case assumption) and while they are flowering.

On the basis of the above, assessment of exposure scenarios 'Treated area', 'Weeds in the treated area', and 'Vegetation margin' is based on the dietary model for above soil contamination and the respective defaults from the EFSA Bee guidance and TAB ENV 248 (ECHA 2022b). The dietary model for through soil contamination is relevant for the 'Plants in treated area during the next growing season' scenario.

Specific input parameters for Tier 1 – above soil contamination

EF_{di} — For 'Treated area' scenario – value if 1 for spray application from the EFSA Bee guidance, Appendix B is applicable.
For 'Weeds in the treated area' scenario – the most relevant value is 1 as also suggested for the surrogate crop "Ornamentals" from the EFSA Bee guidance, Appendix B which does not have its own deposition categories (i.e., the value for the EF_{di} equals always 1). It is a worst-case surrogate crop applicable in cases when diverse group of plants are treated.
For 'Vegetation margin' scenario – assuming that the height of treated plants may vary up to the height of forest trees, in accordance with TAB ENV 248 (2022) the highest drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides, is considered relevant also as a basis for EF_{di} for this exposure scenario. TAB ENV 248 (2022) provides also drift values for different application techniques and field of uses in treatment against the oak processionary moth, which may be used when relevant.

$SV_{po,du}$; $SV_{ne,du}$ See Section 5.5.2.2 regarding the selection of SVs.
In contrast to Case A, in Case B the treatment is applied to unknown mixed species of trees and bushes. Consequently, the 'Treated area' is considered as a habitat with mixed vegetation and a sugar content of 30% (the same refers to 'Weeds in treated area' and 'Vegetation margin'). Consequently, SVs for nectar for all these exposure scenarios should be calculated as follows: multiply the original SV (nectar) by 1/3 for SB groups; by ½ for HB and BB groups.

Calculations for Tier 1 – above soil contamination

Table 32: Large scale spraying of mixed species of trees and bushes (case B) - Tier 1 calculations for dietary model for above soil contamination ('Treated area', 'Weeds in the treated area', 'Vegetation margin')

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Exposure factor for dietary exposure 'Treated area' 'Weeds in the treated area' 'Vegetation margin'	EF_{di}	 1 1 0.381	[-]	 D D D/S	 EFSA Bee guidance, Appendix B EFSA Bee guidance, Appendix B TAB ENV 248 (2022)
Shortcut value for pollen for during flowering situations	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for during flowering situations	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B Multiply original SV_{ne} by 1/3 for SB; by ½ for HB and BB
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times \left(SV_{po,du} + SV_{ne,du}\right)$					**Equation 1**

Provided that the above assumptions are maintained, 'Treated area' scenario and 'Weeds in the treated area' scenario are identical.

Specific input parameters for Tier 1 – through soil contamination

$SV_{po,soil}$; $SV_{ne,soil}$ see Section 5.5.2.2 Tier 1

In contrast to Case A, in Case B the treatment is applied to unknown mixed species of trees and bushes. Consequently, the 'Plants in treated area during the next growing season' scenario are considered as a habitat with mixed vegetation and a sugar content of 30%. Consequently, SVs for nectar for all exposure scenarios should be calculated as follows: multiply the original SV (nectar) by 1/3 for SB groups; by ½ for HB and BB groups.

Calculations for Tier 1 – through soil contamination

Table 33: Large scale spraying of mixed species of trees and bushes (case B) - Tier 1 calculations for dietary model for through soil contamination ('Plants in treated area during the next growing season')

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Shortcut value for pollen for through soil contamination	$SV_{po,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for through soil contamination	$SV_{ne,soil}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B with adjusted SV_{ne}, as explained above
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg /bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = SV_{po,\text{soil}} + SV_{ne,soil}$				**Equation 4**	

5.6.2.3. Tier 2

'Treated area', 'Weeds in the treated area', 'Vegetation margin'

In Tier 2, PEQ_{di} can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2 – above soil contamination

AR — Applicants may propose typical areas where treatment of selected spots (among those shrubs and trees) is relevant. In such instance, AR for tier 2 may be derived by multiplying AR from tier 1 with a fraction of soil defined by treated vegetation (crown) border assumed on 1 hectare. See Table 32.

$SV_{po,du}$; $SV_{ne,du}$ — Since SVs are a function of the number of applications during the year and the interval between multiple applications, lower number of applications per year and/or larger interval between applications may be considered as a form of refinement, which will result in a smaller SV. Such change needs to be confirmed by efficacy data.

EF_{di} — For Vegetation margin scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 32.

For other options for refinement, see EFSA Bee guidance Sections 5.4 and 5.5. Proposals for such refinements need to be consulted with the evaluating competent authorities prior to the submission of the assessment.

'Plants in treated area during the next growing season'

Refer to Section 5.2.2.2. For the large scale spraying case B nevertheless, considerations described below apply.

Specific input parameters for Tier 2 – through soil contamination

$PEC_{pw,2}$ Predicted environmental concentration in porewater calculated from PEC_{soil} initial (in case of multiple applications after last application) after ten consecutive years of application, taking degradation into account in line with TAB ENV 248 (2022) following equation 70 of Biocides Guidance Volume IV Part B + C. Time-weighted average (TWA) concentration in soil over 180 days after the last application after 10 consecutive years of application may be considered as a second Tier (TAB ENV 237 (2022)).

5.6.2.4. Tier 3

'Plants in treated area during the next growing season'

In Tier 3, the same calculation as in Tier 1 is conducted but the shortcut values are re-calculated based on a product specific PEC_{pw}. The PEC_{pw} in Tier 2, which is calculated from PEC_{soil} in the biocides exposure assessment and is considered as a conservative value, can be refined by the modelling tool FOCUS PEARL. For more information, see Section 5.2.2.3.

5.6.3. Contact exposure model

5.6.3.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1. For more specific information, see Section 5.5.3.1.

Specific input parameters for Screening step

AR See Section 5.6.2.1

For calculations, see Table 30.

5.6.3.2. Tier 1

Generic considerations described under Section 5.6.2.2 apply.
Contact exposure is relevant for exposure scenarios 'Treated area', 'Weeds in the treated area' and 'Vegetation margin', and Tier 1 is based on the respective defaults from the EFSA Bee guidance and TAB ENV 248 (ECHA 2022b). Contact exposure is not relevant for the 'Plants in treated area during the next growing season' exposure scenario and therefore PEQ_{co} value for this scenario is equal to 0 in the combined risk assessment.

Specific input parameters for Tier 1

AR See Section 5.6.2.2 Tier 1

EF_{co} For 'Treated area' scenario – value of 1 relevant for the flowering stage of the treated plants in case of spray application from the EFSA Bee guidance, Appendix B is applied.
For 'Weeds in the treated area' scenario – the most relevant value is 1 as also suggested for the surrogate crop "Ornamentals" from the EFSA Bee guidance, Appendix B which does not have its own deposition categories (i.e., the value for the EF_{co} equals always 1). It is a worst-case surrogate crop applicable in cases when a diverse group of plants is treated.
For 'Vegetation margin' scenario – assuming that the height of treated plants may vary up to the height of forest trees, in accordance with TAB ENV 248 (2022) the highest drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides, is considered relevant also as a basis for EF_{di} for this exposure scenario. TAB ENV 248 (2022) provides also drift values for different application techniques and field of uses in treatment against the oak processionary moth, which may be used when relevant.

Calculations for Tier 1

Table 34: Large scale spraying of mixed species of trees and bushes (case B) – Tier 1 calculations for contact exposure ('Treated area', 'Weeds in the treated area', 'Vegetation margin')

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	same as $Q_{a.i.}$ in TAB ENV 248 (2022)
Exposure factor for dietary exposure 'Treated area' 'Weeds in the treated area' 'Vegetation margin'	EF_{co}	 1 1 0.381	[-]	 D D D/S	 EFSA Bee guidance, Appendix B EFSA Bee guidance, Appendix B TAB ENV 248 (2022)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	P	Table 9
Output					
Predicted Exposure Quantity for contact exposure	PEQ_{co}		µg/bee	O	
Calculation					
$PEQ_{co} = AR \times EF_{co} \times BSF$			**Equation 7**		

5.6.3.3. Tier 2

In Tier 2, PEQ can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2

AR — see Section 5.6.2.3. See Table 34.

EF_{co} — For 'Vegetation margin scenario' – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 34.

5.7. Source of exposure – large scale spraying of natural water bodies (case C)

5.7.1. Description of source of exposure

This section concerns the assessment of exposure and risk to bees due to application by large scale spraying, in particular application of biocides on natural water bodies. In accordance with ESD PT 18 for household and professional uses (2008), spray treatment of natural water bodies to control mosquito larvae may be operated on large scale by fixed-wing aircraft or helicopters. Depending on the structure of the landscape, mosquito control may also be performed from the edge of a water body, using standard truck-mounted mosquito abatement equipment. The development of mosquito larvae can also occur in storm water treatment devices. In California,

mosquito larvicides are applied using hand-held equipment at small sites and with backpack or truck-mounted high-pressure sprayers at large sites (Metzger 2004). ESD PT 18 (2008) recommends adapting the scenarios for exposure assessment of plant protection products for crops grown in water (e.g., rice) to assess this biocidal application (European Commission, 2003b, so called MED-RICE scenario).

As a result of this application, bees may be exposed via spray drift. Considering that the exposure of bees would not be negligible for this release route, the risk has to be assessed for this pathway.

Case C refers to the application on natural water bodies which may result in deposits to a vegetation margin (banks) around the treated water bodies. Exposure of bees is assumed due to the consumption of pollen and nectar of the plants outside the treated area[10]. These might consist of flowering plants such as trees, bushes, flowers, grass or berries growing right next to the treated water body. At the same time bees may enter in physical contact with the spray containing biocide or with sprayed plant matrices. Therefore, to assess the risk to bees, the following exposure scenario needs to be addressed for this source of exposure:

Vegetation margin.

5.7.2. Dietary exposure model

5.7.2.1. Screening step

See Section 5.5.2.1 Screening step.

Specific input parameters for Screening step

AR — The application rate of active substance in mass units per hectare of treated water body surface is a necessary input into the exposure calculations. AR may need to be calculated from target concentration in the water body and based on the treated water body dimensions. In addition, for the running water, water volume flow per hour (i.e., width (m) x depth (m) x flow rate (m/hour)) needs to be considered. The AR is based on product specific data.

For further input parameters and calculations, see Section 5.5.2.1. As through soil contamination is not relevant for this source of exposure, PEQ_{di} values presented in Table 11 are not relevant.

5.7.2.2. Tier 1

In Tier 1 it is assumed that the biocidal application is performed on water bodies during spring and summer and the banks of the treated water bodies are covered by a variety of unknown plants which may flower at the time of application. Consequently, the assessment of exposure scenario 'Vegetation margin' is based on the dietary model for above soil contamination and the respective defaults from the EFSA Bee guidance and TAB ENV 248 (ECHA 2022b). Considering there is no agreed emission scenario for this type of use, the respective defaults proposed in this guidance may be replaced by different ones when such an emission scenario will become available.

Specific input parameters for Tier 1 – above soil contamination

EF_{di} — For 'Vegetation margin' scenario – as a worst-case, drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides (ENV 248), is considered relevant also as a basis for EF_{di}. Nevertheless, in case of handheld devices another drift value may be accepted based on appropriate experimental data provided by

[10] In accordance with EFSA Guidance, exposure from contaminated water is not included in the risk assessment of bees.

the applicant which should be consulted with the evaluating competent authorities prior to the submission of the studies.

$SV_{po,du}$; $SV_{ne,du}$ See Section 5.5.2.2 regarding the selection of SVs.
Since plants on the banks of treated water bodies are considered as a habitat with mixed vegetation, sugar content of 30 % is applicable for 'Vegetation margin' scenario, see Table 7. Consequently, SVs for nectar for this scenario should be calculated as follows: multiply the original SV (nectar) by 1/3 for SB groups; by ½ for HB and BB groups.

Calculations for Tier 1

Table 35: Large scale spraying of natural water bodies (case C) - Tier 1 calculations for dietary model for above soil contamination ('Vegetation margin')

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	
Exposure factor for dietary exposure 'Vegetation margin'	EF_{di}	0.381	[-]	D/S	TAB ENV 248 (2022)
Shortcut value for pollen for during flowering situations	$SV_{po,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B
Shortcut value for nectar for during flowering situations	$SV_{ne,du}$		µg/bee or µg/bee/day or µg/larva/developmental period	P	Appendix B Multiply original SV_{ne} by 1/3 for SB; by ½ for HB and BB
Output					
Predicted Exposure Quantity due to dietary exposure	PEQ_{di}		µg/bee or µg/bee/day or µg/larva/developmental period	O	
Calculation					
$PEQ_{di} = \frac{AR}{1000} \times EF_{di} \times \left(SV_{po,du} + SV_{ne,du}\right)$					**Equation 1**

5.7.2.3. Tier 2

In Tier 2, PEQ_{di} can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2

$SV_{po,du}$; $SV_{ne,du}$ Since SVs are a function of the number of applications during the year and the interval between multiple applications, lower number of applications per year and/or larger interval between applications may be considered as a form of refinement, which will result in smaller SVs. Such change needs to be confirmed by efficacy data.

EF_{di} For 'Vegetation margin' scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 35.

For other options for refinement, see EFSA Bee guidance Sections 5.4 and 5.5. Proposals for such refinements need to be consulted with the evaluating competent authorities prior to the

submission of the assessment.

5.7.3. Contact exposure model

5.7.3.1. Screening step

The screening step as described in Section 5.1.3 could be applied. If unacceptable risk is identified, the risk assessment needs to move to Tier 1. For more specific information, see Section 5.5.3.1.

Specific input parameters for Screening step

AR see Section 5.7.2.1

For calculations see Table 30.

5.7.3.2. Tier 1

Generic considerations described under Section 5.7.2.2.

Contact exposure is relevant for the exposure scenario 'Vegetation margin', and Tier I is based on the respective defaults from the EFSA Bee guidance and TAB ENV 248 (ECHA 2022b).

Specific input parameters for Tier 1

AR See Section 5.7.2.2

EF_{co} For 'Vegetation margin' scenario – as a worst-case, drift value derived for "trees (early stage, > 2m)" of 38.09 % agreed as a general default in case of assessment of biocides (TAB ENV 248 (2022)), is considered relevant also as a basis for EF_{co}. Nevertheless, in case of handheld devices another drift value may be accepted based on appropriate experimental data provided by the applicant which should be consulted with the evaluating competent authorities prior to the submission of the studies.

Calculations for Tier 1

Table 36: Large scale spraying of natural water bodies (case C) - Tier 1 calculations for contact exposure ('Vegetation margin')

Parameters	Nomenclature	Value	Unit	Origin	Source
Input					
Application rate	AR		g/ha	S	
Exposure factor for dietary exposure 'Vegetation margin'	EF_{co}	0.381	[-]	D/S	TAB ENV 248 (2022)
Body surface factor	BSF	0.0114 (HB) 0.0146 (BB) 0.00184 (SB)	dm^2/bee	P	Table 9
Output					
Predicted Exposure Quantity for contact exposure	PEQ_{co}		µg/bee	O	
Calculation					
$PEQ_{co} = AR \times EF_{co} \times BSF$			**Equation 7**		

5.7.3.3. Tier 2

In Tier 2, PEQ can be refined by changing the parameters of the calculations in Tier 1 presented above.

Specific input parameters for Tier 2

EF_{co} For 'Vegetation margin' scenario – applicants may provide experimental data on the basis of which the default drift value described above may be refined. See Table 36.

6. Effect assessments in lower tiers

The effect assessment of biocides generally relies on point estimates (EC_x, LC_x, NOEC etc.). However, the effect assessment for bees described here is based on the concept of dose-response relationships described by mathematical models.

The goal of the effect assessment is to identify the relevant toxicity endpoints for the exposure in question. To do this, toxicity endpoints from four risk cases (i.e., acute oral, acute contact, chronic, larvae; indicated by the suffix *j*) are needed (see Section 3.1.2). These are used together with the predicted exposure quantity PEQ_j, to estimate the levels of risk of the biocide use in question.

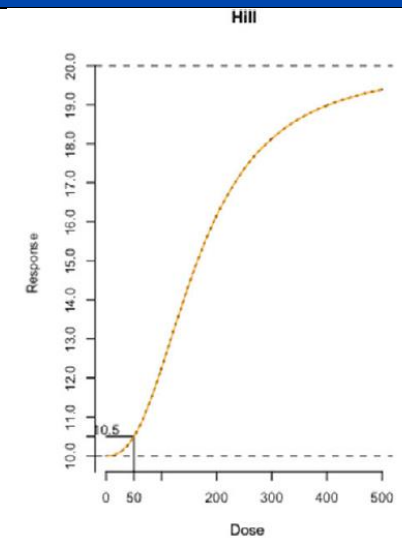
If there is evidence that the biocide under evaluation has a very specific mode of action (MoA) that affects a life stage or process that is not included in the standard data set (e.g., disruption of egg laying), specific additional data may be needed (EFSA Bee guidance).

The effect parameters (LD_{50}/*Inflection Point* and *slope*) are derived by fitting a dose-response curve (hereafter DRC_j) to the raw data of each standard laboratory test by using the four models below. The model which gives the best fit in describing the dose-response relationship for each risk case is then chosen and its parameters used in the effect assessment[11]. The effect parameters resulting from the model with the best fit are consequently chosen for further effect assessment.

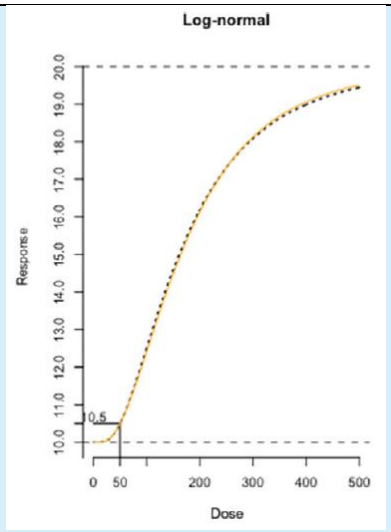
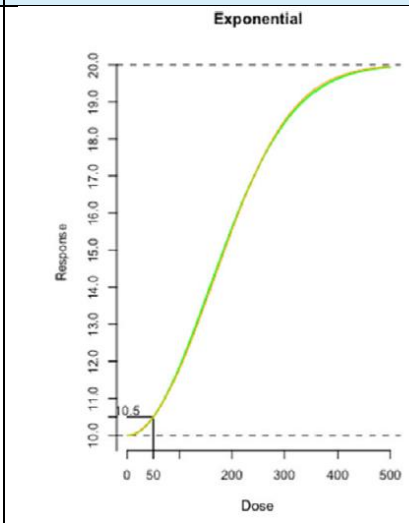
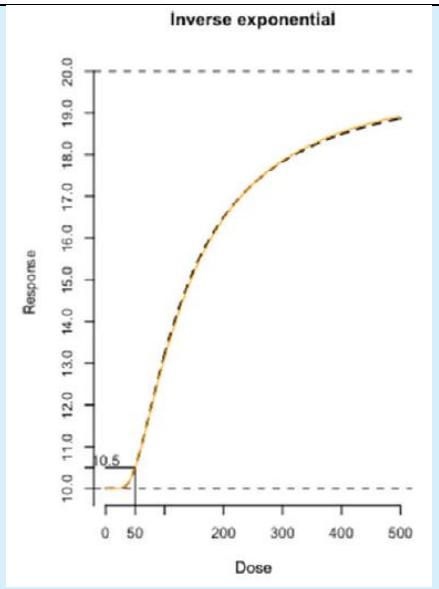
The effect endpoints are the combination of the chosen dose-response model and the values of its parameters for each specific DRC_j.

One of the four models presented in Table 37 is used to derive a DRC_j for each risk case for the bee effect assessment.

Table 37: Overview of dose-response models to be used in the bee effect assessment of biocides. In all equations it is assumed that the effect axes of the DRC ranges from 0 to 100 and is expressed in % effect. The upper limit of the DRC is always fixed to 100, i.e., 100% mortality (adapted from EFSA Supplementary Information table 37. EFSA 2023).

Dose response model	Illustration of models
Log-logistic (Hill) $$PIE_j = 100 \cdot \frac{1}{1 + \left(\frac{LD_{50,j}}{PEQ_j}\right)^{slope_j}}$$	

[11] The effect parameters resulting from the four dose-response models can be calculated with the calculator tool for the bee risk assessment (tool still under development at the time of the guidance publication).

Log-Normal $$PIE_j = 100 \cdot \Phi\left(\log\left[\left(\frac{LD_{50,j}}{PEQ_j}\right)^{slope_j}\right]\right)$$	
Exponential (Weibull-1) $$PIE_j = 100 \cdot \exp\left(-\left(\frac{IP_j}{PEQ_j}\right)^{slope_j}\right)$$	
Inverse Exponential (Weibull-2) $$PIE_j = \exp\left(-\left(\frac{IP_j}{PEQ_j}\right)^{slope_j}\right)$$	

Curve parameters: $LD_{50,j}$: Lethal concentration resulting in 50% mortality; IP_j: Inflection Point, where the convex function of the curve changes to a concave function of the curve. PEQ_j: predicted exposure concentration; PIE_j: predicted individual effect (see also chapter 7.1.1); $slope_j$: slope parameter, steepness of DRC; Φ : Probability distribution function phi, or standard normal distribution function.

A detailed overview of the suite of dose-response models to be used in the bee effect assessment can be found in Chapter 6 of the EFSA Supplementary Document (EFSA, 2023). Background information can be found in the benchmark dose Guidance Document (EFSA Scientific Committee, 2022)[12].

To ensure the use of appropriate effect parameters in risk assessment, the following aspects should carefully be considered.

- Definition of hazard parameters in experimental studies indicated by the legal requirements (Section 6.1)
- Dealing with equivalent studies performed with the same test item and the same species (Section 6.2)
- Derivation of a surrogate dose-response beyond the tested range (Section 6.3)
- Consideration of time-reinforced toxicity (Section 6.4)
- Extrapolation of the hazard parameters between species (Section 6.5).

6.1. Definition of hazard parameters in experimental studies indicated by the legal requirements

6.1.1. Legal requirements

Under Regulation (EC) No. 528/2012, the information requirements for active substances and biocidal products are set in Title 1 of Annex II and III of the BPR, respectively. For active substances with regard to bees they are described in point 9.5.1 (honey bees) and in point 9.5.2 (applicable for bumble bees, solitary bees and other non-target terrestrial arthropods) of Annex II. For biocidal products they are described in Annex III under point 9.3.

A dossier should contain toxicity tests that are necessary to identify the potential toxic effect related to a certain exposure pathway for a biocide (described in Chapter 4). The tests should be performed according to the standard guidelines, such as OECD test guidelines, or existing protocols (pending validation and adoption as new test guidelines, see overview in Table 38). In addition, relevant information from public literature and non-guideline studies can be used. Generally, the relevance and reliability of all available studies should be considered for the overall selection of endpoints.

6.1.2. Toxicity studies

Concerning the available standard test guidelines as well as the evaluation of submitted bee tests, see EFSA Bee guidance Section 6.1.2.

For the assessment of biocides, toxicity studies for bees should be provided if

1. the active substance(s), has an insecticidal mode of action

   and

2. there is a relevant exposure of the biocidal product to bees

An insecticidal mode of action is usually assumed for active substances (to be) approved in PT18.

Concerning the relevant exposure of bees, refer to ECHA Bee guidance Chapter 2 and Chapter 5.

[12] Available at https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2022.7584

Generally, data according to test guidelines presented in Table 38 should be provided for the effect assessment of bees.

For the effect assessment of bees, data should generally be submitted for all bee types for which internationally validated and standardised test guidelines are available. Data on honey bees is considered a mandatory requirement. Data on other bees may in addition be requested if it is relevant for the assessment.

Table 38: Overview of the currently available standard test guidelines for the effect assessment of honey bees, bumble bees, and solitary bees.[13]

Bee group	Test type	Test guideline
Honey bees	Acute oral toxicity	OECD 213
	Acute contact toxicity	OECD 214
	Chronic oral toxicity	OECD 245
	Toxicity to larvae	OECD 239
Bumble bees	Acute oral toxicity	OECD 247
	Acute contact toxicity	OECD 246
	Chronic oral toxicity	Standard test methods not yet available[a]
	Toxicity to larvae	Standard test methods not yet available
Solitary bees	Acute oral toxicity	Standard test methods not yet available[b]
	Acute contact toxicity	Standard test methods not yet available[c]
	Chronic oral toxicity	Standard test methods not yet available[d]
	Toxicity to larvae	Standard test methods not yet available[e]

a) Proposal for a test protocol available for *Bombus* species (Exeler et al., 2019).
b) Draft version available for *Osmia* species (Roessink et al., 2019).
c) Draft version available for *Osmia* species (Roessink et al., 2017).
d) Proposal for a test protocol available for *Osmia* species (Azpiazu et al., 2022).
e) Proposal for a test protocol available for two *Osmia* species (Claus et al., 2021).

If the applicant provides only the above-described data for honey bees, this will also be accepted for the risk assessment for bees.

In this case, toxicity extrapolation factors (T_{ef}) have to be applied (Section 6.5) to consider differences in size within and between the three bee groups (HB, BB and SB).

[13] At the time of the development of the ECHA Bee guidance, OECD TGs are only available for honey bees and bumble bees.Additional OECD TGs for bumble and solitary bees are under development and may be used once available, even if this guidance was not yet revised to include those TGs.

6.1.3. Active Substances and Biocidal Products

When the toxicity of the biocidal product cannot be reliably predicted from the active substance, studies performed with the biocidal product may be required (Annex III, BPR).

In the case of a mixture, that is a biocidal product with more than one active substance, the toxicity of the mixture cannot be predicted based on the data of the active substance alone. In this case data on the mixture are always required (see Table 39). This requirement applies only in the case of biocidal products with two or more active substances with an insecticidal MoA. Active substances with a non-insecticidal MoA that are part of the biocidal product (for example an active substance as a co-formulant) would not trigger the need of product data. For the risk assessment approach for biocidal products containing more than one active substance relevant for the risk assessment of bees, see Chapter 12.

Table 39: Summary of the data requirements for the active substance and the biocidal product (on the basis of EFSA Bee guidance)

Tier 1 study type	Study with active substance (for which there is relevant exposure) required?	Study with formulation required?	
		Formulation with one active substance	Formulation with more than one active substance with insecticidal MoA
Acute oral	Yes	Yes[a]	Yes
Acute contact	Yes	Yes[a]	Yes
Chronic oral toxicity to adults	Yes[c]	Pending on the comparison between acute studies[b]	Yes
Toxicity to larvae	Yes[c]	Pending on the comparison between acute studies[b]	Yes

a) Acute studies with the formulation can be waived when the toxicity can be predicted on the basis of the active substance (e.g. when the formulation consists of the active substance only, or of the active substance in water).

b) Generally, a study with the active substance will be sufficient; however, if there is an indication from the acute oral study that the formulation is more toxic than the active substance, then the formulation should be tested. In determining whether there is a difference then the endpoints should be expressed in terms of active substance. If the acute formulation endpoint expressed as active substance is more toxic by at least a factor of 3 than the acute endpoint for the active substance, then it can be assumed that the formulation is of greater toxicity and hence chronic and larval testing should also be carried out using the formulation. If the difference is less than a factor of 3, then testing adult chronic and larval toxicity with the active substance is sufficient.

c) In case of poorly soluble substance, a single study on the formulated product might also be appropriate as surrogate if higher solubility levels are expected with the formulated product under the test conditions.

For biocidal products containing only one active substance, at least acute (contact and oral) studies are required for both the active substance and the biocidal product, as basis for a toxicity comparison between the active substance and the biocidal product. For the biocidal product a chronic toxicity study and a honey bee brood study can be waived, if based on the comparison between acute toxicity studies, the biocidal product results in a comparable or lower toxicity than the active substance. A ratio of 3 is used to identify a potential higher toxicity of the biocidal product based on the acute toxicity endpoints (EFSA Bee guidance Section 6.7.1). Therefore, if:

- $LD_{50,acute}$ (a.s.) / $LD_{50,acute}$ (biocidal product) > 3: acute, chronic and brood data for both the active substance and the biocidal product must be provided.
- $LD_{50,acute}$ (a.s.) / $LD_{50,acute}$ (biocidal product) ≤3: no further data on the biocidal product are needed. Acute, chronic, and brood data for the active substance must be provided (see Table 42 below).

If several equivalent tests are available for the same species and the same test substance, see Section 6.2.

If for a toxicity endpoint only a right-censored, undefined LD_{50} value is available (datapoint is above a certain value, but unknown by how much: "LD_{50} > .."), see Sections 6.2 and 6.3. Further considerations about the comparison between active substance and the biocidal product are given in Section 6.7.1.

In principle, the most sensitive endpoint should always be used in the risk assessment. To select the most appropriate endpoint, a comparison should be carried out among the available studies and endpoints with the lowest e.g., LD_{50} value (i.e., most sensitive) should be used in the risk assessment, independent of being generated with the active substance or the biocidal product.

6.2. Combining equivalent studies performed with the same test item and the same species

Sometimes multiple equivalent tests on the same endpoint and test substance are available e.g., several acute contact tests with honey bees and a certain test substance. In such cases averaging of the test results is proposed, before checking whether the formulation shows higher toxicity compared to the active substance (an example is given in EFSA (2009), Section 2.4.2. See also Sections 6.1 and 6.7).

This is in line with EFSA guidance documents (e.g., EFSA, 2009), as it is expected that any set of equivalent experiments is carried out according to the same protocol. Guidance on the treatment of multiple data per species can also be found, for example, in the Guidance on BPR: Vol IV Environment Parts B+C (ECHA, 2017, Section 3.3.1.1).

If multiple equivalent studies are available, the datasets can in principle be merged before fitting any dose-response model (an example is given in EFSA (2009), Section 2.4.3). If the survival in the control differs among the experiments, it may be appropriate to transform the data using a corrected survival before merging, for instance by using the Abbott's Formula (Abbot, 1987).

In cases where the results of equivalent studies differ considerably, e.g., if the LC_{50} values are more than one order of magnitude apart (ECHA, 2017), fitting a single model to a merged dataset will lead to a large uncertainty. In such case, it is worth exploring whether the recorded difference is due to any known external factor, or whether the experiments differ in their level of reliability. It should be decided case-by-case if such experiments are excluded from the effect assessment. If no explanation can be found and the results are for the same species and endpoints, they can be aggregated into a geometric mean (ECHA, 2017).

6.3. Derivation of surrogate dose-response beyond the tested range

For some substances, e.g., substances with low toxicity or 'difficult-to-test' substances with low solubility, the highest tested dose or the 'limit dose' is expressing an effect <50%. In this case, the $LD_{50,j}$ is often referred as right-censored, undefined value (e.g., $LD_{50,j}$ > 100 µg a.s./bee). In these cases, the experimental data does not allow to derive a full dose-response curve. However, a surrogate dose-response curve can still be derived by making some conservative assumptions.

In the context of the proposed risk assessment scheme, the most important part of the dose-response is the one below the LD_{10}. This is because an effect higher than 10% would immediately trigger a concern of unacceptable risk. Thus, the derivation of a surrogate dose-response curve is mandatory in case of limit test experiments (i.e., tests with a single treatment dose) and in

case of dose-response experiments when the maximum dose did not trigger an effect >10%. In any other case, the data may be sufficient to describe at least the left part of the dose-response, and in such case the use of a surrogate is not needed.

Whenever it is not even possible to estimate a partial dose-response relationship (at least up to 10% effect), it is proposed to use the log-logistic (or Hill) dose-response model. This is mainly defined by a slope and the LD^{50} (corresponding to the inflection point, see EFSA Supplementary Document Section 6 for further details).

In general, for any specific dose *x*, a shallower slope will lead to a prediction of higher effects for any dose < *x* (see Figure 5).

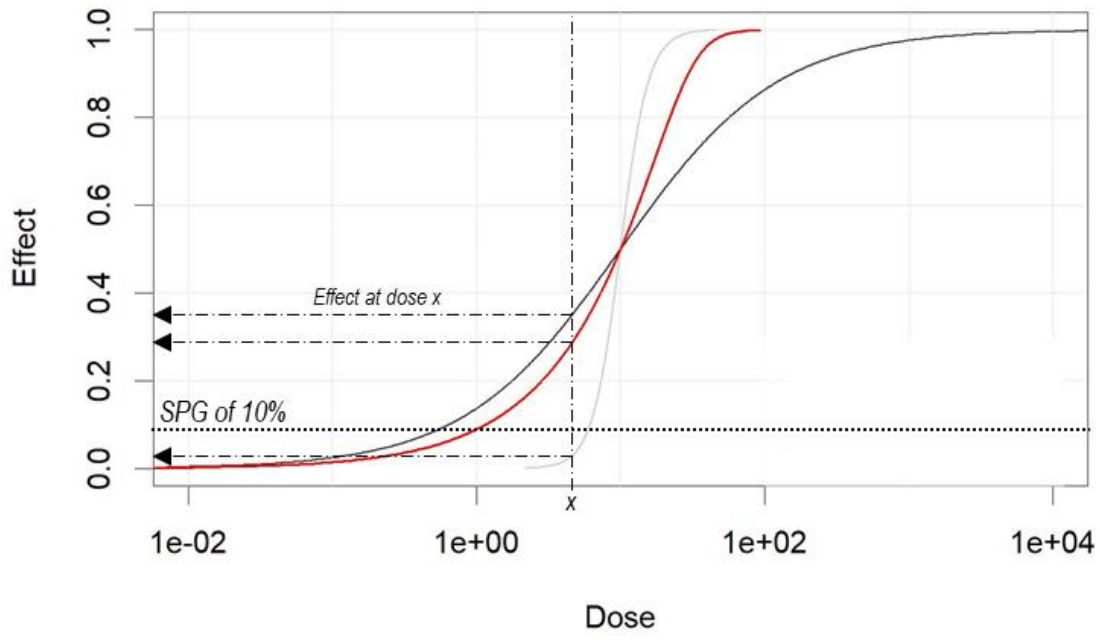


Figure 5: Illustration of the effects resulting from a dose *x* for dose-response curves with different slopes. The difference in the slope of the shown DRCs results in higher effects for shallower dose-response curves (the steeper the curve the higher the slope value). The shown DRCs are described by the log-logistic model (Hill).

As a conservative approach, a log-logistic dose response with a default $slope_j$ of 1.43 can be used whenever a specific value cannot be reliably determined from the experimental data. The generic slope value of 1.43 corresponds to the 10th percentile of the slope distribution, based on an analysis of log-logistic dose-response curves obtained from a large number of substances (Figure 6).

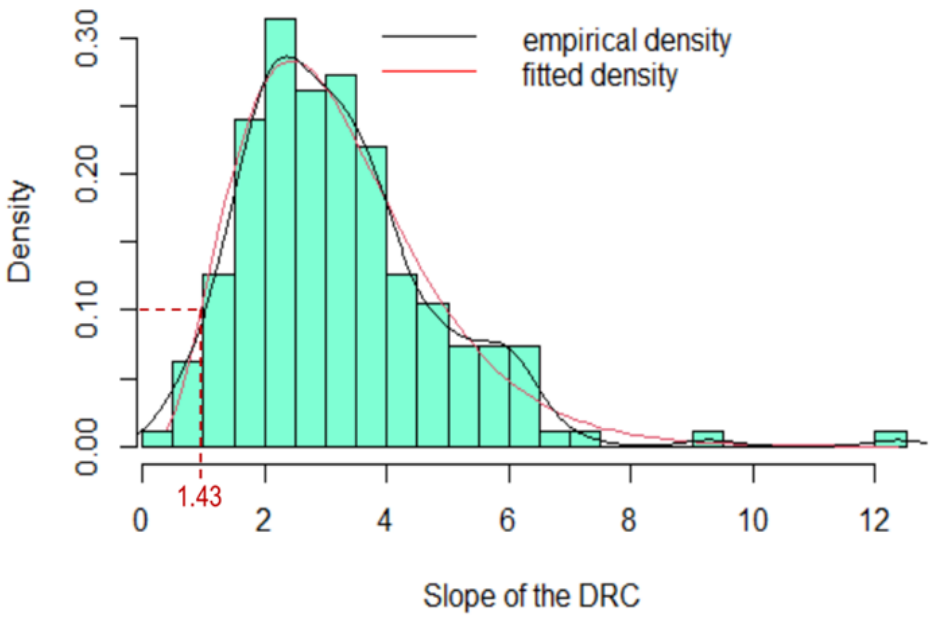


Figure 6: Histogram (green bars) and density plots of the distribution of the slopes for the determination of the default slope of 1.43 (adapted from EFSA Supplementary Document, 2023).

It is expected to predict an effect higher than the "true" effect in 90% of the times (see EFSA Supplementary Document Section 6.3). Using this generic slope value is considered conservative enough and is consistent with the SPG of 10%.

Once $slope_j$ is fixed, a surrogate $LD_{50,j}$ can be derived. This is done by multiplying the highest (or single) tested dose by an appropriate extrapolation factor (see Table 40). These extrapolation factors can be applied to all kind of tests, as no significant differences among slopes were recorded between groups of substances and test types.

Table 40: Extrapolation factors to derive a surrogate $LD_{50,j}$. (EFSA Bee guidance)

Effect observed at the highest tested dose	<10%effect	≥10 to< 20% effect	≥20 to-<30% effect	≥30 to <40% effect	≥40 to <50% effect
Extrapolation factor applied to highest tested dose	4.6	2.6	1.8	1.3	1

6.4. Time-reinforced toxicity (TRT)

The TRT of an active substance is demonstrated when the toxic effects induced after a long period of exposure to low doses are higher than the toxic effects of a short period of exposure to higher doses. In addition, depending on the properties of the active substance, the impact of low doses on bees may be underestimated by laboratory tests where the exposure period is shorter than the environmentally relevant exposure time. Therefore, the time reinforced toxicity should always be assessed (see Chapter 8).

The TRT assessment is based on the data of the honey bee chronic toxicity study. However, the study should be correctly designed to be used for the TRT assessment. If the available standard chronic study is not sufficiently reliable or if the TRT of a substance is identified, a further chronic toxicity study might be required to refine the data.

The TRT assessment allows the determination of the toxicity parameters (i.e., $LDD_{50,\,TRT}$ and the $slope_{TRT}$) that cover the whole honey bee lifespan. If the substance shows TRT properties, the lifespan dose-response obtained from the TRT assessment substitutes the 10-days dose-response obtained directly from the chronic testing with honey bees.

6.5. Extrapolation between species

The general lack of toxicity data for bumble bees and solitary bees makes it difficult to assess the risk of biocides for these bee groups. To derive suitable extrapolation factors, the question of how *LD50*s differ among bee species has been investigated from different perspectives (details about the analysis are available in EFSA Bee guidance Section 6.5 and EFSA Supplementary Document).

In some ecotoxicity experiments weight measurements for different bee species allowed establishing a generic (substance-independent) relationship between LD_{50} and bee weights, for a representative number of European bee species (~10%). This data was used to derive toxicity extrapolation factors (Tef, reported in Table 41) from standard species (*A. mellifera*, *B. terrestris*, *O. cornuta* and *O. rufa*) to smaller bumble bees and solitary bees, to protect at least 95% of European species with 95% confidence. This is considered a very conservative approach, that can be revised if more information will become available (EFSA Bee guidance).

The Tef values take into account that smaller bees are characterised by smaller $LD_{50,j}$, resulting in a higher effect. With regards to exposure estimates, however, smaller bees are characterised by lower exposure, which also depends on body weight and body surface (see EFSA Bee guidance Section 5.3.5 and 5.2.3, respectively).

For larvae, defining a Tef value was not possible as no suitable information is available to relate neither the LD_{50} nor the predicted exposure levels to the bee size (EFSA Bee guidance). Exposure estimates for larvae are based on *Bombus terrestris* for bumble bees and *Osmia* species (*O. rufa* and *O. cornuta*) for solitary bees (see Chapter 5). As the larvae of these species are not significantly smaller than honey bees, a Tef = 1 is proposed to extrapolate from honey bee larvae to bumble bees and solitary bee species (Table 41).

Table 41: Toxicity extrapolation factors (Tef). Standard $LD_{50,j}$ should be divided by these factors to obtain an estimate of an $LD_{50,j}$ protective of 95% of the species in the group (for details see Chapter 6.5 of EFSA Bee guidance and Supplementary document, respectively).

Category - extrapolation from -	Tef for extrapolation to	
	5th percentile BB weight	5th percentile SB weight
Standard honey bee adult (*A. mellifera* worker)	2.4	171
Standard bumble bee adult (*B. terrestris*[a] worker)	6.6	-
Standard solitary bee adult (*O. rufa* ♀) (*O. cornuta* ♀)	- -	144 307
Standard honey bee larva (*A. mellifera* worker)	1.0 [b]	1.0 [b]

[a] OECD test guidelines No 246 and 247 were also ring tested with *B. impatiens.* If data are available with this species, both Tef and food consumption values should be recalculated based on the appropriate body weight. For Tef, the formula is available in the supplementary document under Section 6.5.4. Camp et al. (2020) reported an average weight of 178 mg for *B. impatiens.*

[b] Tef not meant to address the 5th percentile species in terms of weight, but rather *Bombus terrestris* for bumble bees and *Osmia* species for solitary bees, i.e. species used to estimate the exposure levels to bumble bees and solitary bees.

To calculate the extrapolated $LD_{50,j}$ for bumble bees and solitary bees for each risk case, the standard or surrogate $LD_{50,j}$ from the available bee tests (Table 41, column 1) should be divided by the appropriate *Tef* (Table 41, column 2 or 3) to obtain the appropriate extrapolated $LD_{50,j}$ for bumble bees and solitary bees is calculated as follows:

$$\text{Extrapolated LD50}_j = \frac{(\text{surrogate})\ \text{Standard LD50}_j}{\text{Tef}}$$

In most of the cases, the standard LD_{50j} (which can, in some cases, be a surrogate LD_{50j}) is derived for honey bees. Nevertheless, if data are available on other standard species, those should be used in the derivation of the extrapolated LD_{50j} for their specific bee group (EFSA Bee guidance).

On a practical level, this means that the model and the parameters of the DRCj used for honey bees will remain the same, except that the parameter expressing the inflection point should be divided by the appropriate Tef (EFSA Bee guidance). For instance, DRC_{ch} obtained from chronic tests with honey bees may be used for determining the chronic dose–response of the other bee groups simply by dividing one parameter by the Tef values in the first line of Table 41.

If a dose–response is available from tests with (standard) bumble bees and/or solitary bees, this should be used as a starting point to derive the representative DRCj for their own group of bees, following the same procedure illustrated above, but using Tef values from lines 2 and 3 of Table 41.

The extrapolation between species is done at Step 1 of the lower tier risk assessment (see Section 7.1.1), after having defined a dose-response curve (DRC_j) for each relevant bee group and life stage and before calculating the predicted individual effect level (PIE_j) for each risk case.

The presented extrapolation factors are estimates based on the relationship between LD_{50} and bee weight (EFSA Bee guidance). Nevertheless, weight is not the only driver of the LD_{50}, as demonstrated from another analysis which investigated generic 'intrinsic' sensitivity of various species (see Section 6.5.3.7 of the EFSA Supplementary Document). Among those, *A. mellifera* was the most intrinsically sensitive species, which gives confidence that the extrapolation factors from this species are likely protective, despite some remaining uncertainty.

Regarding the shape (i.e., the slope) of dose-responses for bees other than honey bees almost no information is available in the literature. Nevertheless, there is no particular indication that the shape of the dose-response, which is mainly driven by toxicokinetic and toxicodynamic aspects, should vary significantly (EFSA Bee guidance). Therefore, the DRC_j obtained from tests carried out with honey bees can also be used for assessing the same endpoint for other bee groups.

6.6. Implications of time-course of the effects on exposure considerations

In some cases, the initial exposure is that causes most of the effects, which are expressed immediately, even during constant chronic exposure (e.g., for substances with fast kinetics). Expressing the exposure in terms of time-weighted average for these substances, where the initial exposure is the one causing most of the effects (even in conditions of constant exposure), may significantly underestimate the effects under the time-variable exposure expected in the field. Thus, when it is demonstrated that the effects observed are largely due to the level of initial exposure (Figure 7A) rather than to the exposure duration (Figure 7B), the only exposure that matters is the acute one Figure 7.

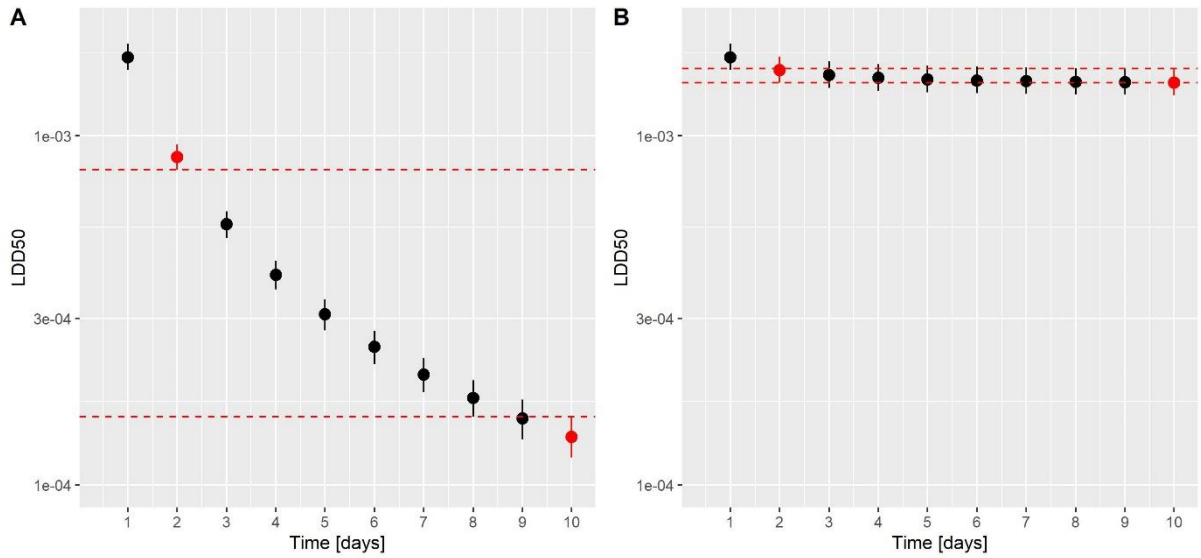


Figure 7: Examples of different situations concerning the time-course of effects. In panel A, full expression of effects depends on the exposure time. Uncertainty ranges of the LDD_{50} at 2 and 10 days are well separated (red dotted lines show lower limit of LDD_{50} at 2 days and upper limit at 10 days). In panel B, effects are almost entirely expressed after a short time. Uncertainty ranges of the LDD_{50} at day 1 and day 10 overlap. In such cases, the assumptions normally used for estimating chronic exposure are not appropriate. (EFSA Bee guidance)

In such a case, there is practically no difference between acute and chronic exposure and combining the effects of chronic and acute dietary exposure means counting twice the same process (e.g., acute exposure). As a consequence, the acute dietary case is excluded from the overall estimation of the risk (Section 7.1.3), while the chronic risk case is determined by the 10-day chronic DRC and the acute exposure level (EFSA Bee guidance).

Effects which occur only due to a short exposure window despite chronic exposure are the opposite of effects resulting from the phenomenon of TRT, where the exposure time is the main determinant of the effects. Thus, if TRT properties have not been ruled out for a substance (including those substances for which no effects were seen in the chronic test), no further check is needed (EFSA Bee guidance). On the contrary, if a lack of TRT has been demonstrated, the temporal trend of the LDD_{50} has to be checked so see whether effects are expressed immediately after the initial exposure.

This can be easily done after fitting a chronic test dataset to GUTS (General Unified Threshold model of Survival) models (something that would anyway need to be done for ruling out TRT properties – see Chapter 8). If the LDD_{50} after 2 days and after 10 days are significantly different or present a ratio > 3, it can be concluded that the exposure time plays an important role in the overall expression of effect, and thus no modification of the standard time window w is needed (EFSA Bee guidance).

If no chronic data for the product is available, assessment is based on the data for the active substance (for further details see Section 6.1.3, Table 39).

6.7. Summary of the selection of hazard parameters for the risk assessment

To select the appropriate DRC to be used for risk assessment for each group of bees and each risk case *j*, it is necessary to consider all the elements discussed above.

Standard test protocols for honey bees covering the different risk cases, are generally the starting point to derive the effect parameters for other bee species as well, considering the inter-species sensitivity. Sometimes other tests with bumble bees (*Bombus terrestris*) and solitary bees (*Osmia spp*.) are also available and should be used as a reference for the group of bees they belong to.

6.7.1. Effect parameters for the risk assessment of honey bees

To select the representative DRC_j for any test substance (either an active substance or a biocidal product containing one active substance) it is necessary to consider all the available data. The procedure is summarised in steps 1 to 4 described below:

Step 1 – if more than one equivalent test available (Figure 8)

If several equivalent honey bee tests with the same test substance are available, and their outcome is not considerably different, e.g., if the LC_{50} values are less than one order of magnitude apart (ECHA, 2017), the datasets should be merged before fitting any dose-response model (for details see Section 6.2).

If the outcome of different experiments is considerably different, and the difference is due to any known external factor, or due to differences in reliability, datasets should be selected or excluded on a case-by-case decision.

If none of the available tests allow the derivation of at least a partial dose-response curve covering at least effects ≥ 10% (e.g., in the case of limit tests), a surrogate dose-response can still be derived by applying the appropriate extrapolation factor to the maximum (or unique) tested dose to derive a surrogate LD_{50}. A log-logistic model with a worst-case default slope of 1.43 should be used in these cases as a surrogate DRC_j (Section 6.3).

STEP 1 (Section 6.2-6.3): repeat for all risk cases j and for every test item (a.s. and biocidal product).

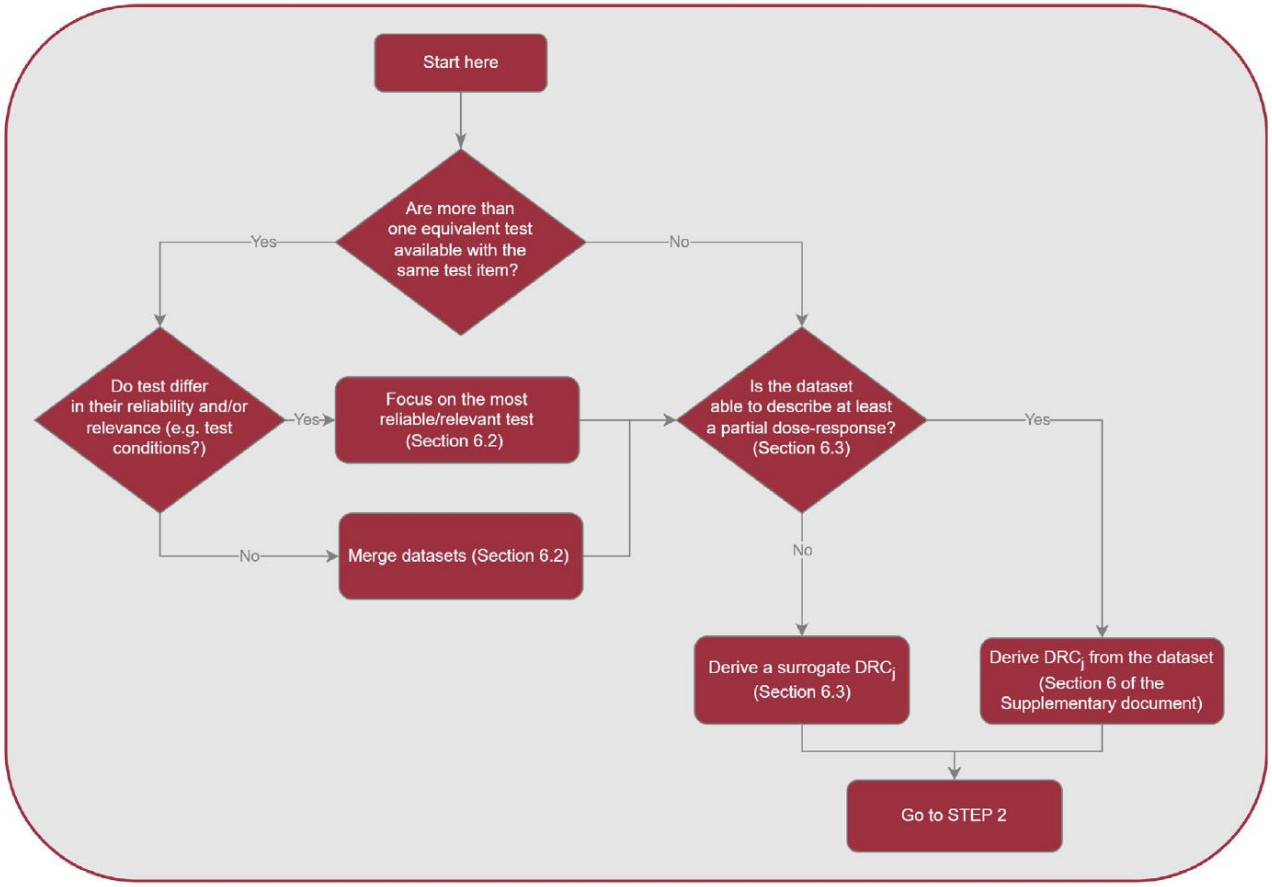


Figure 8: Flowchart illustrating the Step 1 of the process underpinning the selection of the hazard parameters for the risk assessment of honey bees (EFSA Bee guidance). In this picture, tests are considered equivalent when they relate to the same risk case. Repeat for all risk cases j and for every test substance (a.s. and biocidal product).

Step 2 – selecting most appropriate DRC_j (a.s. or biocidal product) (Figure 9)

Step 2 is not necessary if the biocidal product contains additional active substances. In such case, the mixture workflow should be followed (see Chapter 12).

In order to decide whether the DRC_j of the active substances or the biocidal product is more appropriate for honey bee risk assessment, the difference in the LD_{50} of a.s. and product (both expressed in terms of active substance) has to be explored. If the LD_{50j} for the product is more than a factor of 3 below that of the active substance, the effect parameters of the product must be selected for this risk case for the risk assessment of the active substance in the context of its inclusion/renewal (see Section 6.1).

If only one of the DRC_j of the active substance and the product is not a surrogate, this DRC_j should be used for the risk assessment, unless in one of the studies thereby higher effects at comparable doses are neglected[14]. When both DRC_j (active substance and product) are surrogates, additional case-by-case considerations must be made with regard to observed mortality and tested doses. For example, if the top/limit dose caused no mortality for either the biocidal product or the active substance, a comparison is not meaningful. In such case, it would

[14] Example: a study with the active is carried out as limit test at a dose x triggering 30% effect (only surrogate dose-response possible). The study with the formulated product is instead carried out as a proper dose-response. The effect in this second study at a dose ≈ x is considerably lower than 30%. In this case the surrogate dose-response obtained with the active should be retained for the risk assessment.

be appropriate to use for the risk assessment the surrogate dose-response obtained from the highest tested dose (expressed as active substance).

This comparison is normally performed on acute data; however, if the product is acutely more toxic, then also chronic and larvae data should be provided for the product and included in the comparison. If the product is more toxic, the risk assessment for the active substance should be based on the effect parameters derived from tests with the product.

In case of poorly soluble substances, where higher solubility levels are expected with the product under the test conditions, chronic and larval studies should be carried out uniquely with the formulated product. In this case, the DRC_j derived with the product should be considered (as surrogate) for the active substance.

STEP 2 (Section 6.1): repeat at least for the two acute risk cases. Apply to chronic dietary and larval risk cases as well in case tests with the biocidal product are available.

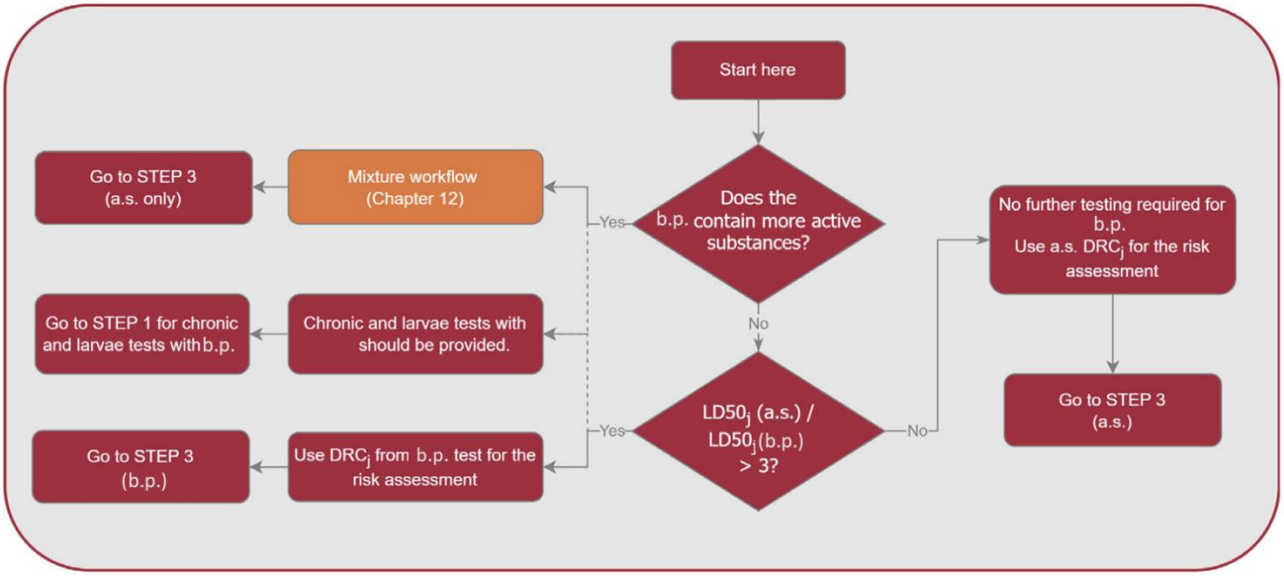


Figure 9: Flowchart illustrating the Step 2 of the process underpinning the selection of the hazard parameters for the risk assessment of honey bees (adapted from EFSA Bee guidance). Note that the comparison of the LD_{50j} between active substance and b.p. entails additional consideration in case of surrogate DRC_j (see text). (a.s. = active substance, b.p. = biocidal product)

Step 3 – Selection of effect parameter for chronic RA of honey bees (Figure 10)

When selecting the effect parameters for chronic risk assessment of honey bees, it should be considered whether the active substance (and the product, if this is triggered at the Step 2) shows time-reinforced toxicity (see Section 6.4 and Chapter 8, and for further details chapter 8 and Annex G of the Supplementary document of the EFSA Bee guidance). If this is the case, the 10-days chronic dose-response should be substituted by the life-span dose-response and an additional risk assessment for winter scenario is triggered, using a life-span dose-response for long-lived winter bees (see Section 8.2.3 for more details).

STEP 3 (Section 6.4, Chapter 8 and Annex G of EFSA Supp. document): only for chronic

- a.s.: all cases
- biocidal product only if triggered at STEP 2 and only in case it contains a single a.s.

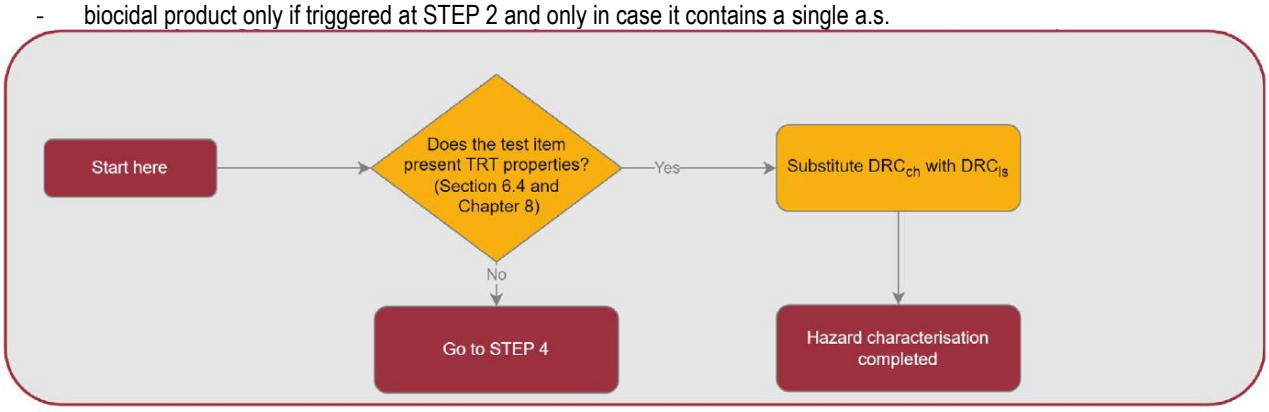


Figure 10: Flowchart illustrating the Step 3 of the process underpinning the selection of the hazard parameters for the risk assessment of honey bees (EFSA Bee guidance).

Step 4 – If effects are driven by initial exposure (Figure 11)

When effects are expressed immediately and mainly driven by the initial exposure, even in conditions of constant chronic exposure, estimating the exposure in terms of time-weighted average may significantly underestimate the effects in the field (see Section 6.6). Thus, when it is demonstrated that the effects observed are largely due to the level of initial exposure rather than to the exposure duration, the chronic dietary risk case will combine the 10-day DRC with the acute exposure estimate (EFSA Bee guidance).

This situation will not occur if a substance presents TRT properties and/or if no effects are seen in the chronic test. Section 6.6 describes how to check whether effects are expressed immediately after the initial exposure.

STEP 4 (Section 6.6): only for chronic (a.s. and biocidal product if evaluated at STEP 3)

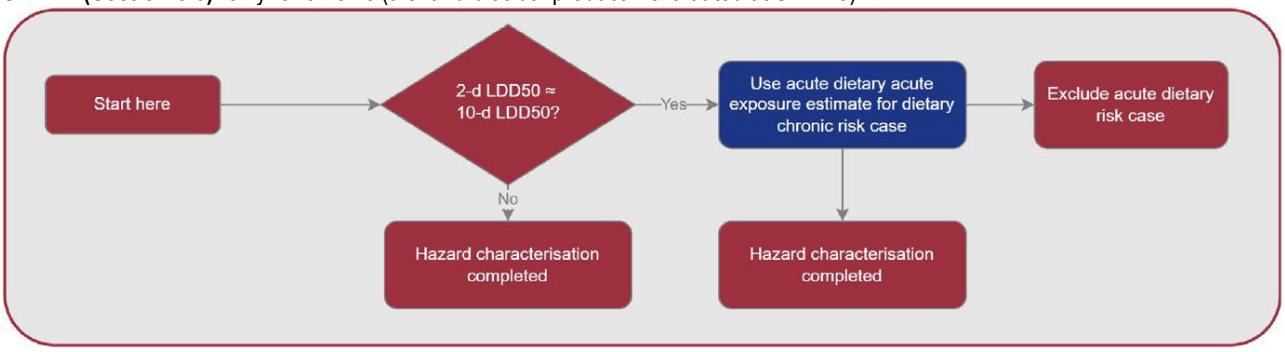


Figure 11: Flowchart illustrating the Step 4 of the process underpinning the selection of the hazard parameters for the risk assessment of honey bees (EFSA Bee guidance). The effect of the exposure length is considered minor if the LDD_{50} after 2 days and after 10 days are not significantly different and present a ratio < 3.

6.7.2. Effect parameters for the risk assessment of bumble bees

For bumble bees, OECD TG 246 and 247 acute tests may be provided in the dossier with both active substance and representative biocidal product. In addition, relevant literature data may be available. The treatment of the hazard parameters (i.e. DRC_j) from any available test with the standard species (*Bombus terrestris* and, less frequently, *Bombus impatiens*) should follow steps 1-2 summarised in Section 6.7.1 (Figure 8 and Figure 9). In contrast Steps 3-4 most likely are not applicable as they are honey bee specific, except if a standard chronic test with bumble bees is available (EFSA Bee guidance).

As the bumble bee group includes many untested species (see Section 1), toxicity extrapolation factors (Tef) should be used to cover the inter-species differences and to obtain the relevant extrapolated DRC_j (Section 6.5).

In all cases where no bumble bee data are available (e.g., chronic and larval effects) the extrapolated DRC_j should be determined by applying the appropriate Tef to the DRC_j chosen for honey bees (EFSA Bee guidance).

6.7.3. Effect parameters for the risk assessment of solitary bees

If studies based on publicly available test protocols or draft OECD TG are available (likely for acute exposure only), they can be used to derive the effect parameters for the solitary bee risk assessment (EFSA Bee guidance). When this is the case, the DRC_j from those studies could be used to obtain the extrapolated DRC_j after applying the appropriate Tef.

If no such test are available, the honey bee effect parameters should be used by applying the appropriate Tef to the honey bee DRC_j and using the respective DRC_j as explained in Section 6.5.

6.8. Options for refinement

In the rare cases, where for an active substance or biocidal product additional studies are available, there are two possibilities for a refinement of the Tier-1 effect assessment. In this case the EFSA Bee guidance considers two approaches (for further details see EFSA Bee Guidance Section 6.7):

- The geometric mean approach;
- The species sensitive distribution (SSD) approach.

Because of the lack of standardised test guidelines for many species and the general lack of knowledge on inter-species variability in the dose-response, it is currently not recommended to use the geomean or the SSD approach for bees (EFSA Bee guidance). Nevertheless, for the time being, effect information for multiple species could be considered in a weight of evidence, acknowledging that increasing the current level of knowledge would certainly improve the accuracy of the risk assessment in future.

7. Lower tier risk assessment

The aim of the lower tier risk assessment is to apply the agreed specific protection goal (SPG) of maximum 10% colony size reduction for honey bees to the proposed methodology, resulting in a conservative assessment which simultaneously identifies active substances of unacceptable risk whilst excluding the substances of low risk from further evaluation.

The suggested approach for the lower tier risk assessment for bees does not focus on single endpoints but combines the effects of different endpoints (which are extrapolated from the individual to the colony level), using the concept of response addition (Bliss, 1939). This calculation method takes into account that in real life, biocidal products can affect a honey bee colony via different endpoints and routes of exposure (see Section 1.4). Therefore, instead of determining a predicted no effect concentration (PNEC) by applying suitable assessment factors to cover for the degree of uncertainty in extrapolation from test data on a limited number of species to the real environment (BPR, Annex VI, paragraph 40. And 41), this approach combines

the predicted effects in a more mechanistic concept (for details see EFSA Bee guidance Chapter 7).

The method presented in this ECHA Bee guidance is in line with the specific protection goal (SPG) (described in EFSA Bee guidance Chapter 3), which focuses on the colony/population (see Chapter 3).

This approach allows a direct comparison between the predicted effects following the exposure to a biocidal product at colony level and the SPG defined by the trigger value of maximal 10% of colony size reduction for honey bees.

The proposed procedure for such a 'combined risk assessment' consists of three successive steps according to the EFSA Bee guidance:

1) Quantification of the effects at the individual level for each risk case (acute oral, acute contact, chronic, larvae) based on standard laboratory ecotoxicological studies, and exposure estimates;
2) Extrapolation of the individual level effects to colony/population level effects for each risk case;
3) Combination of effects for all risk cases into a single predicted effect at the colony/population level.

These steps are described in the following section. The proposed methodology may be applied in the risk assessment of all bee groups, including honey bees, bumble bees and solitary bees. However, it is noted that for bumble bees and solitary bees a threshold of acceptable effect has not yet been defined for the magnitude dimension of the SPG and therefore interpretation of the lower tier risk assessment is currently not possible for these groups of bees as also outlined in the EFSA Bee guidance.

7.1. Step-by-step explanation of the lower tier approach for honey bees

7.1.1. Step 1: Quantification of effects at individual levels

In the first step, a dose-response curve (DRC_j) is defined for each relevant bee type and life stage (for details see EFSA Bee guidance Chapter 6 and EFSA Supplementary document).

The DRC_j is then used together with the relevant predicted exposure quantity (PEQ_j) to calculate the predicted individual effect level (PIE_j). By this, the relationship between exposure to a biocide and the mortality is described and is used to indicate the proportion of bees that would be expected to die after being exposed to a specific dose.

The PIE (in unit of percentage), following the application and exposure of a biocidal product, is calculated as follows:

$$PIE_j = 100 \cdot f(DRC_j, PEQ_j) \qquad \textbf{Equation 29}$$

where j refers to a risk case as assessed in an experimental test such as acute-contact, acute-dietary, chronic-dietary or repeated-dose-larvae (EFSA Bee guidance). The PEQ_j is a realistic worst-case exposure estimate for the respective exposure assessment Tier (see Chapter 3, Table 4). The exposure estimate is defined as ecotoxicologically relevant exposure quantity, here the uptake of a biocide by an individual bee per time unit. It is represented by the dose indicating the predicted environmental exposure (PEQ_j) as well as the one in the ecotoxicological experiment. Both are given in the same unit, which is mass of a.s.·individual^{-1}(·time^{-1}, for the chronic dietary risk case).

To calculate the effect on a specific endpoint after a predicted exposure, a non-linear dose-response curve DRCj is used (Chapter 6, Table 37). The resulting mortality (%) can be interpreted as probability of one individual to die due to exposure to a certain dose, which can also be interpreted as a percentage of a cohort of individual bees to die after exposure to the identical dose (see Figure 12).

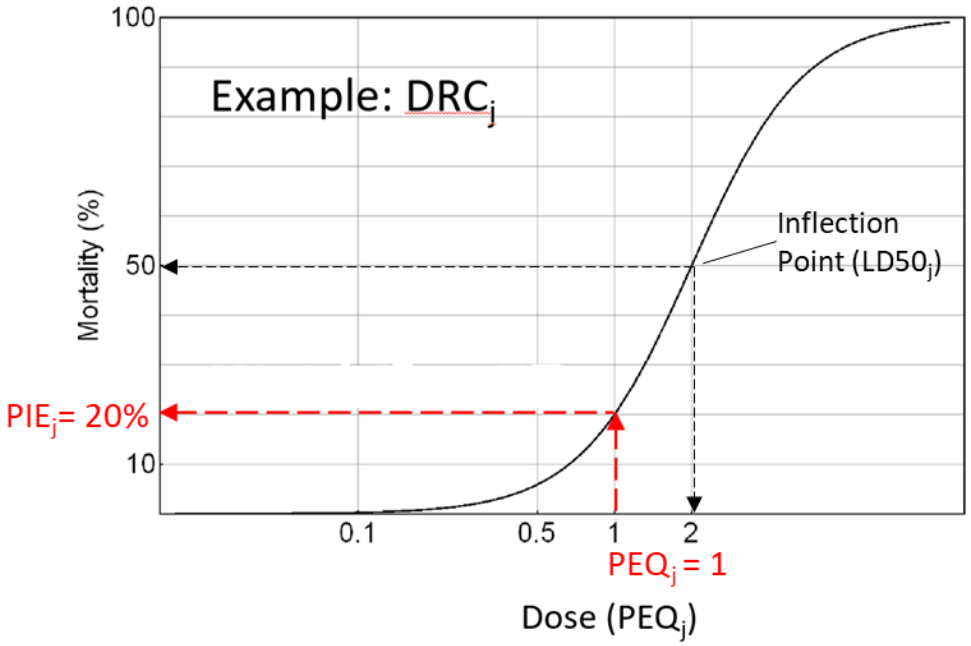


Figure 12: Graphical illustration of the proposed calculation for the effect on a specific endpoint using a non-linear dose-response curve DRC$_j$ (EFSA Bee guidance). The resulting mortality (%) can be interpreted as probability of one individual to die on exposure to a certain dose, which can also be interpreted as a percentage of a cohort of individual bees to die after exposure to the identical dose. In this illustrative case, PEQ, of 1 results in PIE$_j$ of 20%.

Table 42 gives an overview of ecotoxicological exposures as defined by a proper problem formulation and the derivation of effect parameters for honey bees (described in Chapter 4 and 6, respectively).

Table 42: Overview of exposure and the dose-response for the different life stages of honey bees, adapted for biocides (EFSA Bee guidance)

Life stage	Category	Exposure			Dose-response	
		Route	Duration	Quantification and time scale	Potency	Slope
Adult	Forager	Contact	Acute	From contact exp. model; biocide mass sticking on the forager after a single application	Determined experimentally from the acute contact test. If it cannot be derived, a worst-case surrogate is used (see Chapter 6).	
Adult	Forager	Dietary (oral)	Acute	Worst-case between the two bee roles from dietary model; biocide mass uptake per bee per day	Determined experimentally from the acute oral test. If it cannot be derived, a worst-case surrogate is used (see Chapter 6).	
Adult	In-hive (nurse)					

Adult **Adult**	Forager In-hive (nurse)	Dietary (oral)	Chronic	Worst-case between the two bee roles from dietary model; average daily biocide mass uptake per bee during: - 10 days (standard chronic assessment) - 27 days, i.e. the average lifespan of honey bee workers (for substance with TRT properties).	Standard chronic assessment Determined experimentally from the chronic oral test. If it cannot be derived, a worst-case surrogate is used (see Chapter 6). TRT assessment[a] Determined via extrapolation from the chronic oral test.
Larvae	General worker	Dietary (oral)	Chronic (prolonged)	From dietary model; average daily biocide mass uptake per larvae during 5 days.	Determined experimentally from the larvae prolonged test/repeated exposure. If it cannot be derived, a worst-case surrogate is used (see Chapter 6).

[a] When a substance has TRT properties, the risk should be evaluated for the entire honey bee lifespan for both the active (27 days) and the winter (182 days) period. Nevertheless, the winter scenario is a stand-alone assessment, which does not follow all of the steps illustrated in this Chapter. See Section 8.2.2 for more details.

The calculation of PEQ_j values is described in Chapter 5. The definition of the required hazard parameters is in detail described in Chapter 6, including the determination of values for bumble bees and solitary bees, and the procedure if no suitable dose–response relationship can be derived. Table 44 in Section 7.2.2 gives an example of the calculations done for the combined risk assessment.

7.1.2. Step 2: Extrapolation of the individual level effects to colony

In the EFSA Bee guidance, the following is explained with regards to the extrapolation of the individual level effects to colony level:

> *In lower effect-tier assessment, toxicity endpoints investigated in lab studies are expressed at levels of individuals. However, the SPG defines the relevant ecological entities as the colony for honey bees and bumble bees, and the population for solitary bees. To make the lower tier risk assessment compliant with the SPG, effects need to be extrapolated from individual levels to higher levels of biological organisation (i.e., colony or population). Since not every individual level effect might immediately propagate equally to colony levels and as there are feedback mechanisms influenced by environmental conditions, a reliable extrapolation from individual to colony levels for honey bees appears challenging. The impact of individual level effects on colony levels was therefore analysed by using the BEEHAVE model, using simulated colony level feedback mechanisms, and thereby allowing an analysis of this extrapolation between individual and colony effects under variable ecological conditions* (EFSA et al., 2021*). All analysed extrapolations are based on the general consideration of a worst-case exposure related to a certain risk case, here for larvae, foragers and in-hive bees*.

Details on the extrapolation from individual to colony/population levels can be found in Section 7.1.2 of the EFSA Bee guidance and the EFSA Supplementary document.

Overall, in the lower tier risk assessment based on lethal effects, the extrapolation step assumes a conservative 1:1 propagation of individual (PIE_j) to colony level effects (PCE_j) for all experiments, i.e. using dietary and contact exposure, formally written as

$$PCE_j = PIE_j$$ **Equation 30**

7.1.3. Step 3: Combination of effects at the colony

In the third step, effects predicted for single risk cases (PCE_j) are combined. This is justified by the consideration that under real world conditions the effects of different exposure pathways and life stages add up at the colony level, which is the ecological entity defined for the SPG for honey bees (EFSA Bee guidance). The addition of the responses of the single risk cases is based on the mathematical model of independent action (IA, or response addition), which is used for predicting the joint effect of mixtures (Bliss, 1939). It is used to calculate an overall predicted effect at colony level (PE_{SPG}), in units of % of colony size reduction and is mathematically expressed by:

$$PE_{SPG} = 100 \cdot (1 - \prod_{j=1}^{n}(1 - \frac{PCE_j}{100})) \qquad \textbf{Equation 31}$$

where PE_{SPG} is the overall predicted effect at the colony level, in units of % of colony size reduction, and the symbol Π means a multiplication of all terms from 1 to n (EFSA Bee guidance). This value is directly compared with the SPG i.e., ≤ 10% colony size reduction for honey bees. The maximum effect is mathematically limited to 100%, independent of the number of considered endpoints. Neglecting the timing of single events in the response addition calculation is common for Tier 1 methods and a conservative assumption.

7.1.4. Quantification of the contribution of a risk case to the overall predicted effect

There might be cases, where the overall predicted effect at the colony level PE_{SPG} is dominated by a single risk case (EFSA Bee guidance). This can be assessed by quantifying the contribution of one risk case on the overall predicted effect. From the definition of the PE_{SPG}, a formula can be derived for the contribution of risk case j to the overall predicted effect:

$$\Delta_j = \frac{Ln(100 - PCE_j)}{Ln(100 - PE_{SPG})} \qquad \textbf{Equation 32}$$

Depending on whether or not a single risk case dominates the PE_{SPG}, different options for refinement can be used in the higher tier risk assessment (see Chapter 10).

7.2. Implementation of the combined risk assessment in the tiered approach

As explained in Section 7.1, the quantification of an individual effect (Step 1 of the combined risk assessment) is driven by the PEQ_j from the exposure and by the DRC_j for the effect. Since for the effect assessment there are no options for refinement (see Section 6.8 and EFSA Bee guidance), the lower tier risk assessment is based on the standard ecotoxicology endpoints for the effect-tier and the different exposure-tiers (see Chapter 3). In the biocide risk assessment of bees, the exposure tiers include a screening-, Tier 1-, Tier 2- and Tier–3 exposure tier for the dietary risk cases and a screening, Tier 1- and Tier–2 exposure tier for the contact risk case, as illustrated in Figure 13 below.

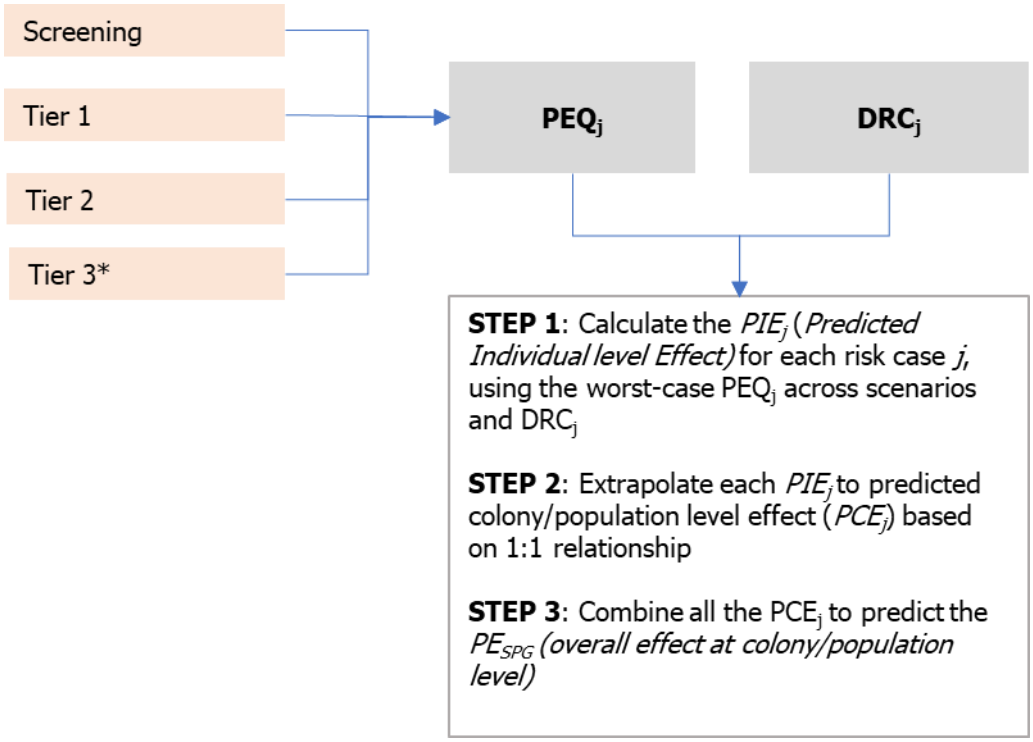


Figure 13: Combined risk assessment in relation to the exposure-tiers in the biocide risk assessment of bees (adapted from EFSA Bee guidance). (*Tier 3 applicable only for the dietary route of exposure.)

7.2.1. Screening-level risk assessment

For the screening level risk assessment, the exposure estimation for dietary and contact exposure (PEQ_j) are based on a simplified exposure model (Section 5.1.3), resulting in more conservative exposure estimations compared to the Tier 1 or higher tiers. In this context, the PEQ_{di} values derived from Equation 8 for each risk case for all sources of exposure for which above soil contamination is relevant need to be compared to the PEQ_{di} values for through soil contamination presented in Table 11. The highest of the two PEQ_{di} values has to be considered in the risk assessment for the screening step. PEQ_{co} for screening step is calculated using the Equation 9.

The predicted individual level effect (PIE) is calculated based on the screening level PEQ_i and the related DRC_j values, for each of the risk cases (acute-contact, acute-dietary, chronic-dietary and larvae-dietary) (see Section 7.1.1). Assuming 1:1 extrapolation from individual to colony level, the predicted colony level effects (PCE) for each of the risk cases are then combined to determine the overall predicted effect at the colony level (PE_{SPG}) (see Section 7.1.3), which can be compared to the SPG.

The applicant can decide whether to start the lower tier risk assessment with the screening step or directly with the Tier 1 assessment. The screening step is suggested only for large scale spraying covered in Sections 5.5, 5.6 and 5.7. If the screening level risk assessment results in acceptable risk for the evaluated use, the risk assessment can stop here, and no Tier 1 assessment is consequently required. Otherwise, the risk assessment needs to proceed with Tier 1 assessment. However, if a substance presents time-reinforced toxicity (TRT), the risk assessment must start with Tier 1 exposure estimates, because exposure estimates are calculated based on different assumptions.

7.2.2. Tier-1 risk assessment

An example is presented below to illustrate how the calculations are to be performed to estimate the risk to honey bees at colony level by combining the different risk cases. The example calculations are performed for a hypothetical biocidal product applied by spraying to the walls of residential buildings; thus, the source of exposure is the spraying of walls around buildings (for more information, see Section 5.3). The input parameters are presented in Table 43 and the calculations at Tier 1 in Table 44.

Table 43: Input and output parameters to derive the application rate for wall spraying around a residential building for Tier 1 (illustrative example).

Parameters	Nomenclature	Value	Unit
Input			
Application rate of product to target surface (e.g., wall, foundation)	Q_{prod}	12	g/m^2
Fraction of a.s. in the product	F_{ai}	0.001	
Treated surface (e.g., wall, foundation)	$AREA_{treated}$	125	m^2
Area of soil that is contaminated	$AREA_{soil}$	26	m^2
Output			
Application rate	AR		g/ha
Calculation			
$AR = Q_{prod} \times F_{ai} \times \frac{AREA_{treated}}{AREA_{soil}} \times 10000 = 576.9$			

For the Tier 1, the dietary and contact exposure estimations are performed based on the models described in Sections 5.1.1 and 5.1.2. respectively. This exposure estimation is to be performed for all relevant exposure scenarios.

For the illustrative example, the 'Vegetation margin' scenario is considered applicable. Nevertheless, since two distinct release processes are involved – spraying and run-off/wash-off, above soil as well as through soil dietary models are relevant. Therefore, Table 44 and Table 45 present the combined approach for dietary model for above soil contamination and through soil contamination for the 'Vegetation margin' scenario, respectively. The same application rate is assumed as presented in Table 43.

Table 44: Illustrative example on how to estimate the risk to honey bees with the combined approach at Tier 1 for dietary model for above soil contamination

Honey bees Tier-1 exposure (Dietary model for <u>above soil</u> contamination and contact)				
	Dietary			**Contact**
	Acute (da)	**Chronic (dc)**	**Larvae (dl)**	**Acute (ca)**
Exposure PEQ_j [µg/bee][a]	PEQ_{da} = 0.415	PEQ_{dc} = 0.170	PEQ_{dl} = 0.166	PEQ_{ca} = 0.658
Hazard parameters (DRCj): Dose-response model (Mod) LD50/IP (e)[µg/bee][a] Slope (b)	DRCda Mod: log-logistic e = 7 b = 1.84	DRCdc Mod: log-logistic e = 9 b = 1.67	DRCdl Mod: log-logistic e = 0.7 b = 2.24	DRCca Mod: log-logistic e = 15 b = 2.23
Step 1: Predicted individual level effect (PIE)	PIE_{da} = 0.5503%	PIE_{dc} = 0.1323%	PIE_{dl} = 3.808%	PIE_{ca} = 0.0936%

Step 2: Predicted colony level effect (PCE)	PCE_{da} = 0.5503%	PCE_{dc} = 0.1323%	PCE_{dl} = 3.808%	PCE_{ca} = 0.0936%
Step 3: combination of effects at colony level	$PE_{SPG} = 100 \cdot (1-(1-PCE_{da}/100) \cdot (1-PCE_{dc}/100) \cdot (1-PCE_{dl}/100) \cdot (1-PCE_{ca}/100))$ $= 100 \cdot (1-(1-0.005503) \cdot (1-0.001323) \cdot (1-0.03808) \cdot (1-0.000936))$ = **4.55%**			
PE_{SPG} i.e., ≤ 10%	Yes (acceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/dev. Period for larvae

Table 45: Illustrative example on how to estimate the risk to honey bees with the combined approach at Tier 1 for dietary model for through soil contamination

Honey bees Tier-1 exposure (Dietary model for through soil contamination and contact)				
	Dietary			**Contact**
	Acute (da)	**Chronic (dc)**	**Larvae (dl)**	**Acute (ca)**
Exposure PEQ_j [µg/bee]b	PEQ_{da} = 0.265	PEQ_{dc} = 0.250	PEQ_{dl} = 0.272	PEQ_{ca} = 0
Hazard parameters (DRCj): Dose-response model (Mod) LD50/IP (e)[µg/bee]a Slope (b)	DRCda Mod: log-logistic e = 7 b = 1.84	DRCdc Mod: log-logistic e = 9 b = 1.67	DRCdl Mod: log-logistic e = 0.7 b = 2.24	DRCca Mod: log-logistic e = 15 b = 2.23
Step 1: Predicted individual level effect (PIE)	PIE_{da} = 0.24%	PIE_{dc} = 0.25%	PIE_{dl} = 10.74%	PIE_{ca} = 0.0%
Step 2: Predicted colony level effect (PCE)	PCE_{da} = 0.24%	PCE_{dc} = 0.25%	PCE_{dl} = 10.74%	PCE_{ca} = 0.0%
Step 3: Combination of effects at colony level	$PE_{SPG} = 100 \cdot (1-(1-PCE_{da}/100) \cdot (1-PCE_{dc}/100) \cdot (1-PCE_{dl}/100) \cdot (1-PCE_{ca}/100))$ $= 100 \cdot (1-(1-0.0024) \cdot (1-0.0025) \cdot (1-0.1074) \cdot (1-0))$ = **11.18%**			
PE_{SPG} i.e., ≤ 10%	No (unacceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/dev. Period for larvae

Using the Tier 1 exposure models for both contact and dietary exposure, calculated PEQ_j values for the 'Vegetation margin' scenario result in an overall predicted effect at the colony level PE_{SPG} = 4.55% and 11.18% based on dietary model for above soil contamination and for through soil contamination, respectively. Whereas non-violation of the protection goal for honey bees is identified for the former, the protection goal for the latter is violated. Therefore, in case of this example, further refinement of the PEQ_j for the dietary model for through soil contamination can be done according to Section 5.3.

With regards to the effect parameters a DRC_j will not always be available for all substances and risk cases. If the derivation of a proper log-logistic dose-response curve is not possible because of limited data, in any case an LD_{50}/IP value needs to be derived, and a default (conservative) slope value can be used as described in Section 6.3. Leaving out one endpoint (risk case) is not acceptable, unless an appropriate default *PIE* values is used.

If a substance is found to show time-reinforced toxicity (TRT), the predicted individual level effect for the chronic dietary risk case PIE_{dc} should be calculated differently: instead of the standard 10-day LDD_{50}, a lifespan LDD_{50}-*TRT* (covering a 27-day lifespan for the active period of honey bees) should be used, together with a PEQ_i calculated for a 27-day exposure period

(see also Section 8.2.1). An additional risk assessment, covering the inactive period of honey bees during the winter period must be performed as well (see also Section 8.2.2).

If the risk assessment based on the Tier-1 exposure indicates unacceptable risk (i.e., SPG not met), and it is not possible to mitigate the risk, a risk assessment based on a Tier-2 or Tier-3 exposure assessment is necessary. It is noted that if an appropriate Tier-2 exposure assessment is not available, and the risk was not excluded at the lower tiers, the conclusion on the risk assessment will be drawn on the basis of those lower tiers.

7.2.3. Tier-2/Tier-3 risk assessment

At the Tier-2/Tier-3 exposure assessment, several of the parameters in both the contact and dietary exposure models can be refined (see Sections 5.2 to 5.7 for details on the options for refinement and the need to generate further data). Using the refined parameter values, refined shortcut values can be calculated, which in turn can be used in the models to calculate the higher exposure tier PEQ_i.

If the risk assessment based on Tier-2 or Tier-3 exposure still indicates unacceptable risk (i.e., SPG not met), a higher tier risk assessment has to be performed (see Chapter 10).

In summary, when an unacceptable risk at colony level is not excluded, any predicted individual level effect can be reiteratively refined according to the tiered approach. Higher tier effect assessment is needed when no options are available to refine the exposure estimation.

For a biocidal use to be considered safe for bees, the overall predicted effect at the colony level (PE_{SPG}) for all relevant exposure routes as well as for all relevant exposure scenarios need to be below the defined SPG of 10% for honey bees.

7.3. Implementation of the combined risk assessment approach for bumble bees and solitary bees

As mentioned in Section 1.3, for bumble bees and solitary the magnitude dimension of the SPG was set as 'undefined threshold' of acceptable effect (EFSA, 2022), and it was recommended to more frequently require higher tier data to better understand the level or protection that would be appropriate for these bee groups, in the current absence of knowledge. The EFSA Bee guidance advices the following with regard to the assessment approach for bumble bees and solitary bees:

> *Based on an 'undefined threshold', a lower tier risk assessment scheme cannot be implemented since there are no values which would allow interpretation of any quantitative lower tier outcome. However, in this guidance, exposure estimation and hazard definition for bumble bees and solitary bees is possible, although these are characterised by considerable uncertainty due to the lack of specific data. Thus, in principle the combined approach described in Section 7.1 and its implementation in the tiered approach, can be applied also for these groups of bees when a defined threshold of acceptable effects is agreed. However, it is not recommended to apply this scheme until this is defined.*
>
> *For the future implementation of such an approach, applicants and risk assessors can refer to Chapters 5 and 6 for the exposure and hazard characterisation respectively, to apply step 1 as described in Section 7.1.1. Regarding the step 2 i.e., extrapolation of effect from individual to colony/population described in Section 7.1.2, the (EFSA) WG propose to apply the same the 1:1 relationship relative to the propagation of effects from individual to colony/population since is also considered conservative for these bee groups. The combination of the effects (step 3) as described in Section 7.1.3 would also be appropriate*

for calculating the overall predicted effect based on the addition of responses at the colony/population level.

It should be considered that there are several ecological factors that could influence the vulnerability of bumble bees and solitary bees to biocidal products differently compared with honey bees. In general, the biology and ecology of bumble bees, and especially of solitary bees, suggest a lower resilience and higher vulnerability to stressors relative to honey bees. Although it is unclear to what extent each ecological factor contributes to their vulnerability, it is important to highlight that this is a remaining source of uncertainty in the risk assessment that is difficult to quantify due to the lack of data (EFSA, 2022).

In the current absence of general knowledge on bumble bees and solitary bees, it is suggested that lower tier data (standard laboratory studies) are requested for biocidal active substances (and products if necessary) to allow a better protection of these bee groups in the future.

8. Time-reinforced toxicity

According to the effect assessment approach as presented in Chapter 6, dose-response relationships (i.e., $LD(D)_{50}$ values) are estimated on the basis of standard toxicity studies for which the exposure times are assumed to reflect an "acute" and "chronic" exposure time. The standard chronic laboratory toxicity test exposes bees for up to 10 days (OECD test guideline 245). Depending on the properties of the test substance (e.g., bioaccumulative properties), the toxicity based on a 10-day study could be underestimated when the toxic effects are enhanced by the exposure time.

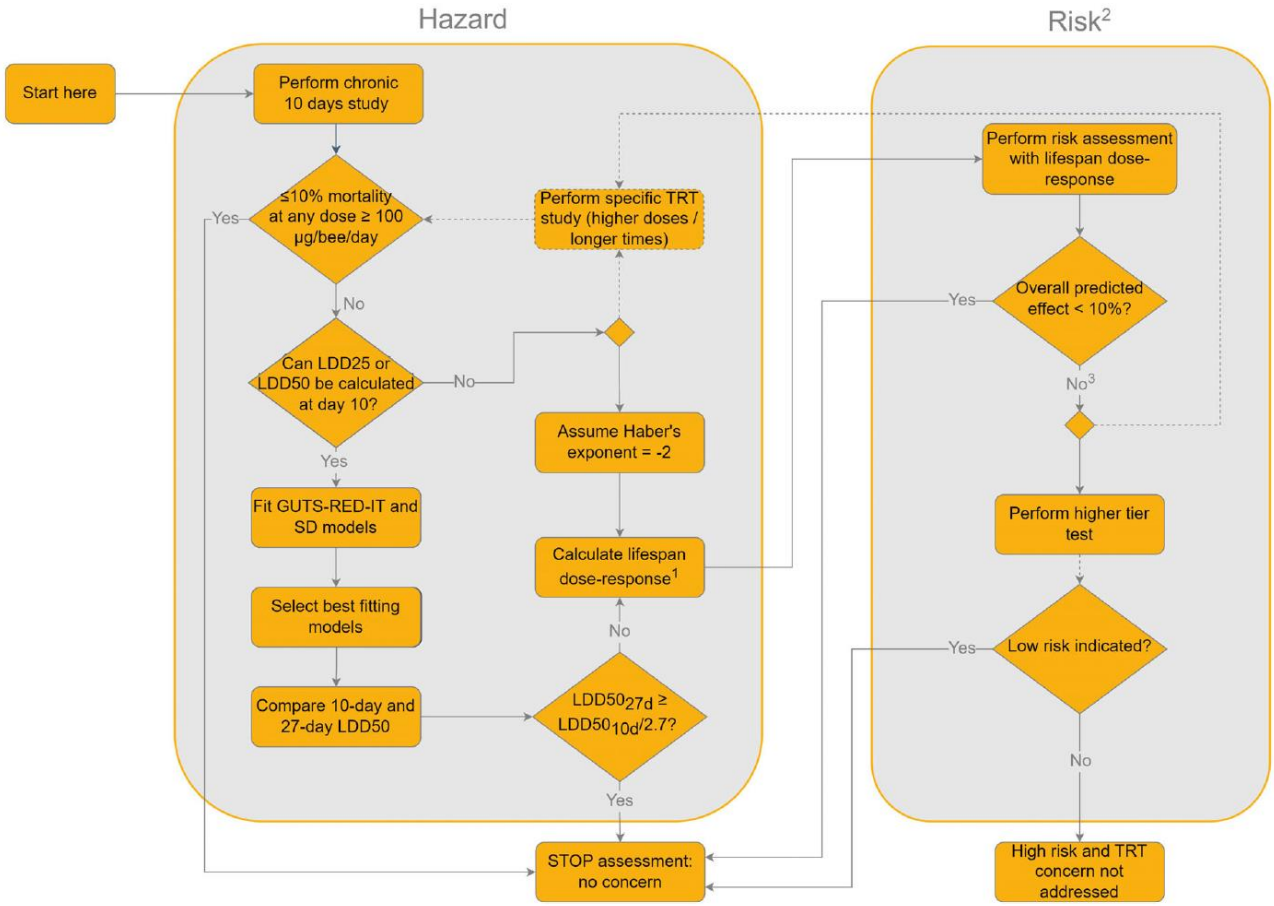
The exposure of bees to biocides is different from that of plant protection products (Chapter 4 and 5). Nevertheless, depending on the biocidal application, it is possible that bees are exposed to low doses of biocides for a period longer than 10 days, or even for the entire life span of the bees. Furthermore, the properties and mode of action of biocidal active substances may be similar or even identical to active substance of plant protection products. It was therefore decided to take over the "time reinforced toxicity" (TRT) assessment from the EFSA Bee guidance to the biocide assessment and to include it in the overall risk assessment strategy for bees (see Figure 3) in order to assess if the toxic effects of biocides at low doses over a long exposure period are higher than the effects at higher doses over a short exposure period (i.e., if toxic effects are reinforced by exposure time).

The TRT assessment is based on the extrapolation of the data coming from the standard 10-day chronic toxicity study for honey bees. The TRT assessment is divided into **two main parts** (Figure 14): the first part is the **hazard assessment** to estimate whether the biocidal active substance actually has a potential for time-reinforced toxicity, and the second part is the actual **risk assessment** for substances identified as having TRT properties in the first part. For the time being, the TRT assessment should only be performed for honey bees (for more information on this subject, see Annex G to the Supplementary Document of the EFSA Bee guidance).

The ECHA EG decided to focus the risk assessment for bees on active substances with an insecticidal mode of action and on biocidal products with relevant exposure to bees (see Chapter 2 and Section 6.1.2). Therefore, the hazard assessment, as the first part of the TRT assessment, must be carried out for those biocides that are relevant in terms of their risk to bees. The purpose of this part is to determine whether the biocide under consideration has TRT properties. When this is not the case, the standard chronic risk assessment as described in Chapter 7 is considered sufficient to address the risk from long-term exposure. However, if the biocide shows TRT properties, the risk assessment, as the second part of the TRT assessment, is performed and the TRT assessment below will take precedence over the standard chronic risk case to estimate the overall predicted effect (i.e., the toxicity endpoints from the standard chronic risk case (LDD_{50} and slope) will be replaced by the TRT endpoints (i.e., $LDD_{50,TRT}$ and $slope_{TRT}$).

The TRT property is specific to every active substance depending on its mode of action, physiochemical properties, biochemical properties and the conditions of exposure to the bees. Therefore, the TRT assessment should always be performed for the active substances relevant in terms of their risk to bees as defined above. However, a chronic toxicity study with the biocidal product is also required if, based on acute data, the product was found to be more toxic compared to the active substance, or when the biocidal product contains multiple active substances (see Table 39 in Section 6.1.3). Whenever chronic data with the biocidal product are available, the worst-case chronic toxicity endpoints (i.e., $LDD_{50,TRT}$ and $slope_{TRT}$) between the results based on the product or active substance data should be used in the TRT hazard assessment.

In case of the presence of several active substances relevant for their risks to bees in a biocidal product, the TRT assessment should be performed for all active substances. If two or more active substances show TRT, a TRT risk assessment considering all these substances should be performed, using mixture toxicity. If only one active substance shows TRT, the TRT risk assessment is only to be done for this single substance.



[1] Lifespan dose-response is calculated using the selected GUTS model (General Unified Threshold model of Survival), for both the active period and winter scenario
[2] This part has to be duplicated for the active period and the winter (inactive) period
[3] When an effect > 10% is predicted, either higher tier studies can be performed, or a specific TRT laboratory study can be executed. This second option is only applicable when this conclusion of >10% effect is reached on the basis of the worst-case assumption that Haber's exponent = -2.

Figure 14: Flowchart of the scheme for the assessment whether a substance exhibits time-reinforced toxicity. (EFSA Bee guidance)

8.1. TRT Hazard assessment

The hazard assessment part of the flowchart shown in Figure 14 intends to solve the question whether or not a biocidal active substance or product shows TRT. The steps to do so (Step 1 – 4) are described in Section 8.1.1 in line with the methodology described in the EFSA Bee guidance. If a substance is found to show TRT, a dose-response covering the whole lifespan of a honey bee has to be determined, which will be used in the risk assessment part of the TRT assessment. The calculation of the lifespan dose-response is described in Section 8.2.1.

8.1.1. Determining whether a substance shows time-reinforced toxicity

The hazard assessment is based on a methodology which relies on the use of GUTS (General Unified Threshold models of Survival),[15] TRT modelling (Jager et al. 2011). For a short introduction to the GUTS modelling framework, refer to Section 4.4.1 of Annex G to the Supplementary Document of the EFSA Bee guidance. For further details on the background for the methodology used, refer to Sections 4.4.4 and 7.1.1 of that Annex G.

Step 1: check whether an assessment is necessary
The first step of the hazard assessment is based on the data of the standard 10-day chronic honey bee toxicity study according to the OECD test guideline 245 (OECD, 2017), which is required for the standard effect assessment for honey bees (see Section 6.1.2). Therefore, the starting point is to collect data from the available study.

This first step consists in checking whether, in the 10-day toxicity study, the following conditions are met:

1. Was the mortality ≤ 10% at any dose ≥ 100 µg/bee/day?
    - If yes, then a TRT assessment is not necessary
    - If no, go to step 2.

This possibility to waive a further assessment for TRT was included to avoid unnecessary work and potential additional testing for substances of low toxicity to bees. The rationale for selecting the threshold of ≤ 10% mortality at any dose ≥ 100 µg/bee/day is described in Annex G to the Supplementary Document of the EFSA Bee guidance.

Note that if the highest dose tested in the available chronic 10-day test is below 100 µg/bee/day, Step 1 of the scheme cannot be used. In that case, proceed to Step 2.

Step 2: check whether a robust GUTS-RED model can be fitted to the data
For a robust calibration of GUTS-RED models to data from a 10-day chronic toxicity study, a level of mortality should be reached at the end of the 10-day test period that allows calculation of an LDD50 or LDD25 value for at least day 10. If an LDD_{50} cannot be determined, an LDD_{25} can be considered instead. Therefore, the following condition should be met:

2. Can a LDD_{25} or LDD_{50} be calculated at the end of the exposure period (day 10)?
    - If yes, fit both a GUTS-RED-IT and GUTS-RED-SD model to the data, and proceed to Step 3
    - If no, select one of the following options:

[15] Available for instance at: http://openguts.info/download.html

a. Perform a new 10-day chronic toxicity study (according to OECD TG 245), using higher doses, and start again at step 1 using the newly obtained data

b. Perform a new chronic toxicity study, with a longer duration. Fit both a GUTS-RED-IT and GUTS-RED-SD model to the data, and proceed to Step 3

c. Assume the substance has TRT properties, with a worst-case Haber's exponent of -2. Calculate the lifespan dose-response as described in Section 8.2.1 and proceed to the risk assessment.

When **option 2a**, which is to perform a **new chronic toxicity study using higher treatment doses,** is chosen, these doses have to be chosen so that the observed effects increase, and at least an LDD_{25} can be calculated at the end of the testing period. This should enable GUTS model fitting in Step 3a. Alternatively, when in the available study the highest tested dose was below 100 µg/bee/day, a new test including a dose of at least 100 µg/bee/day could be performed, so that this can be used in Step 1 and give the possibility to waive the TRT assessment if the mortality is ≤ 10% at dose ≥ 100 µg/bee/day. It is acknowledged that for substances of poor solubility, this might not be technically feasible using the technical active substance. In that case, performing a 10-day test (OECD TG 245) with the biocidal product instead of the technical active substance could be an option (as also proposed in Section 6.1.3). Alternatively, option 2b could be considered.

In case of **option 2b**, a **new chronic study with a longer duration** is performed. With the exception of the duration of the study, the study design should in this case also be based on OECD TG 245. To assess the validity of such a study, the validity criteria from OECD TG 245 also apply, at least to the data for the first 10 days of the test. For the time period beyond 10 days, additional validity criteria for the control are not considered necessary, as mortality in the control will inevitably increase over time. Note that the GUTS model, which is used in Step 3, is able to discriminate between background mortality and mortality from toxicant effects. Refer to Annex G to the SD of EFSA Bee guidance for some additional recommendations for the study design. Note that specific guidance on performing a study with longer duration life-long (also called 'life-long' or 'time-to-effect') test is currently not available, as this type of tests is still under development.

It should be noted that in the description of Step 2b above, it is assumed that it will be possible to use the data from the life-long test to fit a standard GUTS-RED model. It is expected that this will be possible in most cases. However, should the treatment-related mortality in the life-long test still be too low to enable a GUTS model fitting, this can be considered as an indication that TRT will not be an issue for that substance.

In **option 2c**, it is **assumed that the substance has** TRT properties, with a worst-case **Haber's exponent of -2**. An explanation on why this value of -2 is a worst-case, can be found in Section 7.1.1 of Annex G to the SD of the EFSA Bee guidance.

Step 3: Fit both GUTS-RED-IT and GUTS-RED-IT models to the data and select the best performing model

As it cannot be known *a priori* whether the GUTS-RED-SD or GUTS-RED-IT model will result in a better fit to the data, it is mandatory to use both models for TRT analysis. Both models can be fitted to the data using the currently available (semi-)automated calibration and prediction tools, thus generating the required output for the TRT assessment. The performance of both the fitted GUTS-RED-SD and GUTS-RED-IT model is compared based on the normalised root mean square error (NRMSE) for model calibration, and the model with the lower NRMSE value is the better performing one and should be selected.

Note that in all standard GUTS implementations background mortality is described by a single

parameter. While this is generally sufficient for 10-days test when the control mortality must remain below 15%, it may not be the case for life-long test, where background mortality is not expected to be constant over time. In such case, a modified version of GUTS, which uses a 2-parameter model for control mortality has to be used.

Please refer to Section 5.1 of Annex G to the SD of the EFSA guidance for a detailed explanation of this modified version to be considered.

Step 4: Compare the 10-day and 27-day LDD_{50} and decide on TRT
As a final step to determine whether a biocidal substance shows TRT, the 10-day and 27-day LDD_{50} values should be derived from both the GUTS-RED-SD and GUTS-RED-IT model fitted to the data. If

$$LDD_{50,27d} \geq \frac{LDD_{50,10d}}{2.7}$$

then there is no concern about TRT. However, if this condition is not fulfilled i.e., $LDD_{50,27d} < LDD_{50,10d} / 2.7$, TRT cannot be excluded.

Since it is possible that the outcome is different between GUTS-RED-SD and GUTS-RED-IT, the following decision scheme should be followed to know on which model to base the conclusion:

- If neither of the GUTS-RED-SD and -IT model does indicate TRT: it can be concluded that the substance does not show TRT. No further TRT risk assessment is required
- If both the GUTS-RED-SD and -IT model indicate TRT: it can be concluded that the substance shows TRT. Calculate the lifespan dose-response as described in Section 8.2.1, and proceed to the risk assessment.
- If one model indicates TRT and the other does not: use suggested metrics (NRMSE based on calibration data) to decide which of the two models to use.
    - If one model clearly fits better (shows lower NRMSE values), base the conclusion for TRT on the outcome from that model (i.e., compare the $LDD_{50,10d}$ and $LDD_{50,27d}$ derived from that model). Depending on the outcome, no further TRT risk assessment is required, or a lifespan dose-response as described in Section 8.2.1 needs to be calculated before proceeding to the risk assessment.
    - If there is no clear difference between both models, use the worst case (which will most likely be the SD model). In that case, it can be concluded that the biocidal active substance shows TRT. Calculate the lifespan dose-response as described in Section 8.2.1 and proceed to the risk assessment.

8.2. Risk assessment based on TRT

For biocidal active substance or product for which there is concern for TRT following the steps described above of the hazard assessment, the standard chronic risk assessment (see Chapter 7) might underestimate the risk from long-term exposure. Therefore, for such substances, a specific risk assessment, which covers the whole lifespan of a bee, should be performed. This specific risk assessment supersedes the standard chronic risk assessment. The different steps of the TRT risk assessment following the methodology outlined in the EFSA Bee guidance are presented in Sections 8.2.1 to 8.2.4.

8.2.1. Calculating the lifespan dose-response (LDD_{50} and slope)

If a biocidal active substance or product is identified as showing TRT, or a worst-case approach

assuming a Haber's exponent of -2 is followed, a risk assessment which covers the whole lifespan of a bee should be performed. In order to be able to perform such a risk assessment, the toxicity endpoint (LDD_{50} and slope) for a period of exposure that covers the whole lifespan should be known.

To estimate the toxicity endpoint for an exposure period corresponding to the lifespan of a honey bee, two scenarios are considered in the EFSA Bee guidance: a scenario that covers the active period of the bees (i.e., summer scenario), and a second that covers the inactive period of the bees (i.e., winter scenario). A dose-response relationship for the whole lifespan should therefore be calculated for both scenarios, using a lifespan of 27 and 182 days for summer and winter bees (i.e., $LDD_{50,27d}$ and $slope_{27d}$, and $LDD_{50,182d}$ and $slope_{182d}$, respectively), as described respectively in Section 8.2.2 and 8.2.3.

GUTS-RED models fitted to the chronic toxicity data can be used to determine the dose-response at any timepoint. If in Step 3 of the workflow described in Section 8.1.1, one of the models (either GUTS-RED-IT or GUTS-RED-SD) was identified as better matching the data, the parameterization from the best model should be used to determine the 27- and 182-day dose-response. If there is no clear difference between both models, the one resulting in worst-case estimated 27- and 182-day dose-response should be used.

In case a worst-case approach assuming a Haber's exponent of -2 is followed, the linear C vs. t relationship (on a log-log scale) is used as a basis to calculate the lifespan dose-response. Since this option will likely be used in cases where the maximum effect is small (i.e., no reliable LDD_{25} can be obtained from the data), a surrogate 10-days dose-response can still be derived.

The input parameters for the TRT risk assessment to calculate the exposure for the two lifespan scenarios are discussed in Sections 8.2.2. and 8.2.3.

8.2.2. Risk assessment for the active period

For the lifespan risk assessment during the active period, it is assumed that a honey bee will live for 27 days (see EFSA Bee guidance for more information on this value). Given that the standard chronic risk assessment also focuses on bees during the active period, the same method for estimating the dietary exposure can be used in the lifespan risk assessment (Section 5.1). The values for the different parameters (e.g., Residue per unit dose of pollen/nectar (RUD) and DT_{50} in pollen and nectar, used to calculate the Predicted Concentration per Unit Dose in pollen/nectar (PCUD)), as used in the standard risk assessment, can also be applied here. However, there are two specific parameters for assessing the TRT of the active period:

- **The time window for calculating time-weighted average concentrations (called "w")**, needed for the calculation of the PCUD. A time window of 27 days (corresponding to the median lifespan of an active honey bee) is used instead of 10 days (corresponding to the duration of a standard chronic oral toxicity study).

- **Pollen and nectar consumption.** During their entire lifespan, honey bee workers undergo changes in their diet in relation to the tasks they execute. Thus, for this specific case, a combination of subsequent diets was considered. Specifically, it was assumed that bees perform nursing activities for 10 days (pollen and nectar consumption), then 8 days of additional in-hive tasks (nectar consumption similar to the nursing phase, no pollen consumption), and 9 days of foraging activity (higher nectar consumption due to flying activities, no pollen consumption). See Section 5.3.4.5 of the Supplementary Document of EFSA guidance for more details.

Taking the above parameters into account results in specific shortcut values, which are given in ECHA Bee guidance Appendix B. The shortcut values are then used to estimate the dietary exposure PEQ for the active lifespan (i.e., 27 days) with both the "above-soil contamination" and

the "through soil contamination" exposure models. As in the standard chronic risk assessment, the predicted individual level effect (PIE) is then calculated using the active period lifespan PEQ and the $LDD_{50,27d}$ and $slope_{27d}$. This is combined with the other three risk cases (i.e., acute oral, acute contact and larvae; see Section 7.1.3) to estimate the overall predicted effect at the colony level.

For the illustrative example (introduced in Section 7.2.1), referring to a hypothetical biocidal product applied to walls by spraying and assuming the substance shows TRT, example calculations for the active period are presented in Table 46 and 47. Since the 'Vegetation margin' scenario is applicable for this kind of use and both above soil contamination and through soil contamination are relevant, the results are presented in two tables, Table 46 and Table 47, respectively. In this case, the dietary chronic predicted individual level effect is calculated based on the hazard parameters for the whole lifespan of a summer bee (27 days), and an exposure period of 27 days, instead of the 10 days as in the standard risk assessment for bees. For the other three risk cases and for the calculation of the PE_{SPG}, there are no differences from the standard risk assessment.

Table 46: Illustrative example on Tier 1 exposure assessment – Active period for a substance showing TRT properties (Dietary model for above soil contamination and contact).

Honey bees Tier-1 exposure (Dietary model for above soil contamination and contact)				
	Dietary			**Contact**
	Acute (da)	**Chronic (dc) (27 days)**	**Larvae (dl)**	**Acute (ca)**
Exposure PEQ_j [µg/bee] [a]	PEQ_{da} = 0.415	PEQ_{dc} = 0.092	PEQ_{dl} = 0.166	PEQ_{ca} = 0.658
Hazard parameters (DRCj): Dose-response model (Mod) Inflection point (e)[µg/bee][a] Slope parameter (b)	DRCda Mod: log-logistic e = 7 b = 1.84	DRCdc Mod: log-logistic e = **1.25** b = 1.67	DRCdl Mod: log-logistic e = 0.7 b = 2.24	DRCca Mod: log-logistic e = 15 b = 2.23
Step 1: Predicted individual level effect (PIE)	PIE_{da} = 0.5503%	PIE_{dc} = 1.27%	PIE_{dl} = 3.808%	PIE_{ca} = 0.0936%
Step 2: Predicted colony level effect (PCE)	PCE_{da} = 0.5503%	PCE_{dc} = 1.27%	PCE_{dl} = 3.808%	PCE_{ca} = 0.0936%
Step 3: Combination of effects at colony level	$PE_{SPG} = 100 \cdot (1-(1-PCE_{da}/100) \cdot (1-PCE_{dc}/100) \cdot (1-PCE_{dl}/100) \cdot (1-PCE_{Ca}/100))$ $= 100 \cdot (1-(1-0.005503) \cdot (1-0.0127) \cdot (1-0.03808) \cdot (1-0.000936))$ = **5.64%**			
PE_{SPG} i.e., ≤ 10%	Yes (acceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/developmental period for larvae

Table 47: Illustrative example on Tier 1 exposure assessment - Active period for a substance showing TRT properties (Dietary model for through soil contamination and contact).

Honey bees Tier-1 exposure (Dietary model for <u>through soil</u> contamination and contact)					
		Dietary			**Contact**
		Acute (da)	**Chronic (dc) (27 days)**	**Larvae (dl)**	**Acute (ca)**
Exposure	PEQ_j [µg/bee][a]	PEQ_{da} = 0.265	PEQ_{dc} = 0.164	PEQ_{dl} = 0.272	PEQ_{ca} = 0
Hazard parameters (DRCj): Dose-response model (Mod) Inflection point (e)[µg/bee][a] Slope parameter (b)		DRCda Mod: log-logistic e = 7 b = 1.84	DRCdc Mod: log-logistic e = **1.25** b = 1.67	DRCdl Mod: log-logistic e = 0.7 b = 2.24	DRCca Mod: log-logistic e = 15 b = 2.23
Step 1: Predicted individual level effect (PIE)		PIE_{da} = 0.24%	PIE_{dc} = 3.26%	PIE_{dl} = 10.74%	PIE_{ca} = 0.0%
Step 2: Predicted colony level effect (PCE)		PCE_{da} = 0.24%	PCE_{dc} = 3.26%	PCE_{dl} = 10.74%	PCE_{ca} = 0.0%
Step 3: Combination of effects at colony level		$PE_{SPG} = 100 \cdot (1-(1-PCE_{da}/100) \cdot (1-PCE_{dc}/100) \cdot (1-PCE_{dl}/100) \cdot (1-PCE_{ca}/100))$ $= 100 \cdot (1-(1-0.0024) \cdot (1-0.0326) \cdot (1-0.1074) \cdot (1-0))$ = **13.86%**			
PE_{SPG} i.e., ≤ 10%		No (unacceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/developmental period for larvae

In the above example, the overall predicted effect at the colony level PE_{SPG} of 5.13% for above soil contamination is higher than what was predicted without consideration of TRT for the active period. Nevertheless, in this example, the SPG is not violated. However, the PE_{SPG} for the through soil contamination is 13.80%, which is above the acceptable threshold of 10% of effect on the colony. In fact, the TRT characteristics lead to more sensitive hazard parameters, thus to a lower LDD_{50} after 27 days, but also to lower exposure, since the relevant period is no longer 10 days but instead 27 days as a typical lifespan of a summer bee.

8.2.3. Risk assessment for winter bees

During the winter, honey bees will not forage for fresh pollen and nectar, but will feed on the food stored in the hive (i.e., honey). Therefore, the exposure of the bees to the biocides depends on the presence of residues in the honey. Given the differences in climatic conditions and agricultural and beekeeping practices in Europe, it is complicated to realistically estimate the extent of oral exposure of bees to contaminated honey. As a worst-case and lower tier assessment, it is therefore assumed that the winter bees are fed 100% contaminated honey throughout the winter period (i.e., 182 days). For more information on the residues in honey and the lifespan of six months (i.e., 182 days), refer to Section 7.2.2 of Annex G to the Supplementary Document of the EFSA Bee guidance.

Assuming that winter bees feed on 100% contaminated honey, the dietary exposure is estimated consistently with the exposure models presented in Chapter 5. The specific parameters for the winter scenario needed for the TRT risk assessment of the inactive period are the following:

- **Sugar consumption from honey**: bees consume **8.8 mg of sugar/day** in temperate regions, during winter to maintain the nest temperature at 5-8°C in the periphery and 15-20°C in the centre.

- **Sugar content in honey**: as the water content of honey is assumed to be 18%, the

sugar content of honey would then be **82%**.

- **Dissipation rate in honey**: Given that there is currently no data available on the DT_{50} of active substances in honey, it was agreed to use a worst-case value for the lower tier risk assessment (i.e., **1000 days**), which corresponds to no substantial dissipation.

As for the risk assessment of the active period, the dietary exposure PEQ of the winter bees can be calculated either with the "above-soil contamination" and the "through soil contamination" model as presented in Sections 8.2.3.1 and 8.2.3.2, respectively. As in the standard chronic risk assessment, the predicted individual level effect (PIE) is then calculated using the winter period lifespan PEQ and the winter lifespan dose-response (i.e., $LDD_{50,182d}$ and $slope_{182d}$).

For the winter scenario, only chronic dietary exposure through honey consumption is considered. It is to be noted that the contamination in honey originates from nectar foraging only. Therefore, the winter bee scenario is relevant only if the exposed plants are attractive for nectar. In addition, as there are no larvae during winter, the dietary chronic PIE is calculated as a stand-alone assessment and the other risk cases (i.e., adult acute contact and dietary, and larvae) are not relevant for the winter scenario. The predicted individual level effect will correspond to the overall predicted effects at the colony level (PCE).

8.2.3.1. Above-soil contamination

The following equation is used to calculate the PEQ due to dietary exposure for the winter bee scenario in case of relevant biocidal application (i.e., spraying on walls and foundation of houses, irrigation of gardens, large scale spray):

$$PEQ_{di} = \frac{AR}{1000}\ SV \qquad \textbf{Equation 33}$$

Where: AR = Application rate (g/ha)
SV = shortcut value for dietary exposure through honey

The shortcut values are calculated using the following equation:

$$SV_{wi,above} = \frac{1}{1000}\ PCUD_{ho}\ \frac{CMP_{su,wi}}{S_{ho}} \qquad \textbf{Equation 34}$$

Where:
$CMP_{su,wi}$ = 8.8 mg/day is the consumption of sugar in winter
S_{ho} = 0.82 is the sugar content of honey
$PCUD_{ho}$ = Predicted concentration per unit dose in honey (mg/kg)
= $RUD_{ho}\ \frac{1-e^{-kw}}{kw}$, with k= ln(2)/DT_{50}, DT_{50} in honey = 1000 days (if no data available on dissipation in honey) and w = 182 days.

For more information on the database for RUD values in honey, refer to the Appendix B in Annex G to the Supplementary Document of the EFSA Bee guidance. Based on this database, a 90th percentile RUD for residues in honey of 3.0 mg/kg was derived. Shortcut values are presented in Table 48. Following from the illustrative example (introduced in Section 7.2.1), an example calculation for winter bees is given for above soil contamination in Table 49 for a substance that shows TRT.

Table 48: Shortcut values for dietary exposure through honey (winter bees) for above soil contamination.

Application	Shortcut value (µg/bee/day) For crop/grass/treated plant on the treated area	Shortcut value (µg/bee/day) For mixed vegetation
Spray applications	0.03	0.015

Table 49: Illustrative example on Tier 1 exposure assessment – Winter bees for a substance showing TRT properties (Dietary model for above soil contamination and contact).

Honey bees Tier-1 exposure (Dietary model for above soil contamination and contact)				
	Dietary			Contact
	Acute (da)	Chronic (dc) (182 days)	Larvae (dl)	Acute (ca)
Exposure PEQ_i [µg/bee] [a]		PEQ_{dc} = **0.009**		
Hazard parameters (DRCj): Dose-response model (Mod) Inflection point (e)[µg/bee][a] Slope parameter (b)		DRCdc Mod: log-logistic e = **1.25** b = 1.67		
Step 1: Predicted individual level effect (PIE)		PIE_{dc} = 0.025%		
Step 2: Predicted colony level effect (PCE)		PCE_{dc} = 0.025%		
Step 3: Combination of effects at colony level	$PE_{SPG} = 100 \cdot (1- (1-PCE_{dc}/100))$ $= 100 \cdot (1-(1-0.00025))$ $=$ **0.025%**			
PE_{SPG} i.e., ≤ 10%	Yes (acceptable risk identified)			

[a] Units are mentioned for brevity as µg/bee, but they are in fact µg/bee/day for chronic and µg/bee/developmental period for larvae

8.2.3.2. Through soil contamination

Consistent with what was agreed for the above-soil contamination model, the 90th percentile value from the database for RUD values in honey (3.0 mg/kg) is considered for through soil contamination as well. As presented in Section 5.1.1., residues in nectar for contamination via soil can be estimated by using PEC_{pw}. The same is considered to be true for residues in honey. Thus, the following equation is used to calculate the PEQ due to dietary exposure for the winter bee scenario in case of relevant biocidal applications (i.e., manure/sewage sludge application on soil, spraying on walls and foundation of houses and irrigation of gardens).

$$PEQ_{di} = SV_{wi,so} = \frac{1}{1000} \times PEC_{pw} \times \frac{CMP_{su,wi}}{S_{ho}} \times \frac{1 - e^{-kw}}{kw}$$ **Equation 35**

Where: PEC_{pw} = Predicted Environmental Concentration in pore water (mg/L = mg/kg)
$CMP_{su,wi}$ = 8.8 mg/day is the consumption of sugar in winter
S_{ho} = 0.82 is the sugar content of honey
k = $\ln(2)/DT_{50}$ and w = 182 days.

In the Tier 1 of the exposure assessment, the PEC_{pw} is assumed to be 1 mg/kg, for any application regime where the cumulative application rate is not higher than 4.5 kg/ha. In cases

where the cumulative application rate is higher than 4.5 kg/ha, Tier 2 exposure estimation have to be conducted by refining PEC_{pw}, as described in Chapter 5.

Note that all parameters used for Tier 1 exposure estimations are in this case fixed. The results of the exposure estimations for winter scenario and through soil contamination model are shown in Table 50.

Table 50: Shortcut values (SV) for dietary exposure through honey (winter bees) for through soil contamination.

	Shortcut value (µg/bee/day) For crop/grass/treated plant on the treated area	Shortcut value (µg/bee/day) For mixed vegetation
Through soil contamination	0.011	0.0055

Table 51: Illustrative example on Tier 1 exposure assessment – Winter bees for a substance showing TRT properties (Dietary model for through soil contamination and contact).

Honey bees Tier-1 exposure (Dietary model for <u>through soil</u> contamination and contact)				
	Dietary			**Contact**
	Acute (da)	**Chronic (dc) (182 days)**	**Larvae (dl)**	**Acute (ca)**
Exposure PEQ_j [µg/bee]		PEQ_{dc} = **0.0055**		
Hazard parameters (DRCj): Dose-response model (Mod) Inflection point (e)[µg/bee]a Slope parameter (b)		DRCdc Mod: log-logistic e = **1.25** b = 1.67		
Step 1: Predicted individual level effect (PIE)		PIE_{dc} = 0.012%		
Step 2: Predicted colony level effect (PCE)		PCE_{dc} = 0.012%		
Step 3: Combination of effects at colony level	$PE_{SPG} = 100 \cdot (1- (1-PCE_{dc}/100))$ $= 100 \cdot (1-(1-0.00012))$ = **0.012%**			
PE_{SPG} i.e., ≤ 10%	Yes (acceptable risk identified)			

In case of the illustrative example of the hypothetical biocidal product applied to walls by spraying, where release processes contribute to both, above soil contamination and through soil contamination, the overall predicted effect at the colony level on winter bees PE_{SPG} is 0.025% and 0.012% for the above soil and the through soil contamination, respectively (Table 49 and Table 51). The risk is therefore acceptable. The PEQ_{dc} value of 0.0055 mg/bee/day corresponds to the shortcut value for the mixed vegetation, which is relevant for biocide exposure.

8.2.4. Refinement options

If the Tier 1 risk assessment for the active period and/or the winter bee scenario shows an unacceptable risk (i.e. the SPG is not met), it is possible to refine either some parameters of the exposure equations, as can be done for the standard risk assessment (see Chapter 5), or to perform a specific TRT study, as also described in Section 8.1.1. (step 2 of the hazard assessment). The latter would especially be useful for those substances for which an LDD_{50} or LDD_{25} cannot be determined, and for which it could therefore be assumed a worst-case Haber's exponent of -2 for calculating the lifespan dose-response.

For the lifespan risk assessment for the **active period**, the dietary exposure estimations can be refined using all options presented for the standard risk assessment (see Sections 5.2 to 5.7).

For the lifespan risk assessment for the **winter bees**, the only two parameters in the dietary exposure model that could be refined using substance-specific data, are the residues in honey and the DT_{50} in honey. However, data on the residues in honey is usually not available in the biocide dossier. For further information on the refinement of residues in honey, refer to Section 7.2.3 of Annex G to the Supplementary Document of the EFSA Bee guidance. Eventually, a refinement of the PEC_{pw} could be possible, as presented in Sections 5.2 to 5.7.

The lifespan of a winter bee of 182 days is a very rough and conservative estimate. In theory, this value could be refined if more detailed data would be available. However, it should be noted that any refinement of the winter bee lifespan would have only a rather minor effect on the estimated lifespan-LDD_{50} (see Annex G to the Supplementary Document of the EFSA Bee guidance for details). Therefore, this kind of (general) refinement is not considered very useful, unless the outcome of the risk assessment is borderline or it can be demonstrated that the length of the winter period is substantially less than three months, which is hardly the case for Europe.

Chronic toxicity data can further be refined by conducting higher tier effect field studies (see Chapter 10). Generally, these requirements are the same for substances that show time-reinforced toxicity and those substances that do not. However, for substances with TRT properties, a field study must be sufficiently long to ensure that potential effects following long-term exposure are taken into account. In practice, this means that the study should not be started later than September and last until next spring, thus including overwintering and observing the honey bees for at least half a year. This type of study designed for plant protection products is challenging and may not be technically feasible for biocides. Therefore, refinement of chronic toxicity data might be a difficult option for biocides. Nevertheless, any available higher tier data could be evaluated on a case-by-case basis.

9. Sublethal effects on honey bees in risk assessment

9.1. Overall strategy

Sublethal effects result from an exposure dose that does not directly cause death. Therefore, it is difficult to establish a direct link between the sublethal effects observed in a standard study and the strength of the honey bee colony (see definition of SPGs, Section 1.3). Nevertheless, a wide range of adverse sublethal effects on behaviour, physiology, longevity, or reproduction have been reported for many bee species in the open literature (see Annex K to the Supplementary Document of the EFSA Bee guidance). Thus, due to growing concerns and specific requests for greater consideration of sublethal effects in the risk assessment of pesticides for bees, it was decided to include the assessment of sublethal effects in the EFSA Bee guidance. For the same reasons, the ECHA EG also decided to consider, in parallel to the TRT assessment, the sublethal effects in the risk assessment of biocides for bees (see Figure 3). Therefore, even if the standard lower tier risk assessment, based on mortality endpoints, indicates an acceptable risk, the sublethal effect assessment must be carried out. Note that if the lower tier assessment were to lead to a higher tier assessment with higher tier studies, and the higher tier studies show compliance with the SPG, it would be assumed that the sublethal effects are covered by these studies and that there is no concern for sublethal effects on foraging behaviour.

As the spectrum of observed sublethal effects is wide and there is a lack of standardisation of studies to assess these effects, it was decided for the EFSA Bee guidance to focus on sublethal effects that may alter bee behaviour, in particular feeding and foraging behaviour. Indeed, it is assumed that a significant change in the diet of a colony, caused by a significant alteration in foraging, can indirectly have a negative impact on the colony strength. In addition, it is to be noted that observations of feeding behaviour are already included in the OECD test guidelines of the standard laboratory studies that are required for standard risk assessment. This could allow potential risks related to sublethal effects to be identified quite easily without the need for

further lower or higher tier studies. Nevertheless, if a concern of sublethal effects were to be raised, it could still be further investigated in specific higher tier studies (see Section 9.3). Furthermore, as most of the standard tests are carried out and most of the data is available for honey bees, it was also decided to limit the assessment of sublethal effects to honey bees.

Sublethal effects are strongly linked to the mode of action of a chemical. As the ECHA Bee guidance will focus primarily on insecticidal substances/products (see Section 2.1) and various sublethal effects related to the insecticidal mode of action have already been reported in the open literature (see also Annex K to the Supplementary Document of the EFSA Bee guidance), the assessment of sublethal effects, in parallel to the lower tier assessment, is justified and is also required for the risk assessment of biocides for bees. However, as the link between sublethal effects on foraging behaviour and colony strength is still to be confirmed, the outcome of the sublethal effect assessment, as described below, can therefore only be "concern for sublethal effects indicated" or "no concern for sublethal effects indicated". In addition, it is expected that further recommendations and improvements of the approach can be provided as experience is gained.

9.2. Strategy for identifying concern for sublethal effects from lower tier information on honey bees

Based on the EFSA Bee guidance, a strategy for assessing sublethal effects for biocides is presented in Figure 15 and explained in the following sections (9.2.1 to 9.5). Further information to the overall strategy is also available in Chapter 9 of the Supplementary Document to the EFSA Bee guidance.

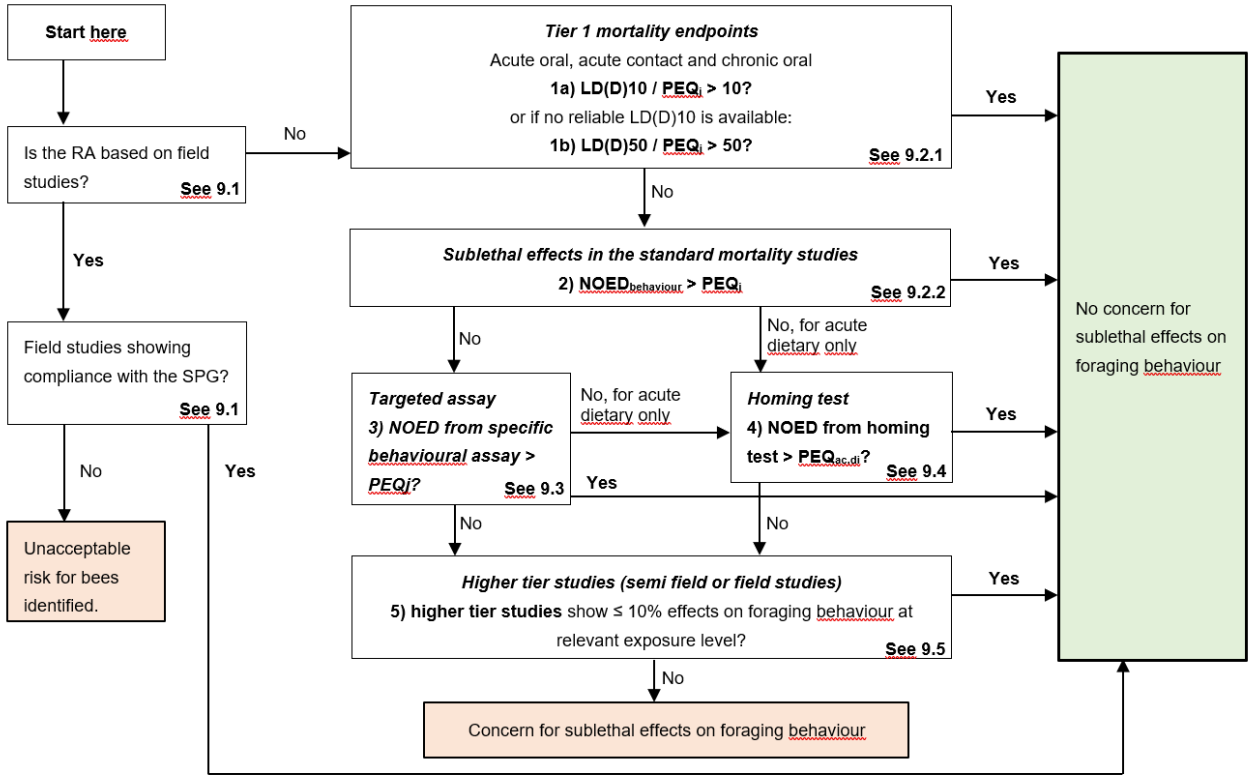


Figure 15: Assessment strategy for sublethal effects (adapted from EFSA Bee guidance). SPG = Specific Protection Goal; LDD = Median Lethal Dietary Dose; PEQ = Predicted Exposure Quantity, where *j* denotes the risk case and *ac,di* refers to acute, dietary; NOED = No Observed Effect Dose

9.2.1. Toxicity/exposure ratio using mortality endpoints

The first step in the assessment of sublethal effects is to estimate the "level of no concern", i.e., a sufficiently low exposure (i.e., PEQ_j) at which no effect on foraging behaviour is expected. The "level of no concern" can be calculated as the $LD(D)_{10}$ divided by 10, evaluated separately from the standard laboratory tests on honey bees (i.e., acute oral, acute contact and chronic oral) (for more details on the toxicity/exposure ratio calculation, see Section 9.2.1 to the Supplementary Document of the EFSA Bee guidance). If a reliable $LD(D)_{10}$ cannot be calculated, the $LD(D)_{50}$ divided by 50 can be used, which would be a worst-case "no concern level". The "level of no concern" is then compared to the corresponding PEQ. A concern for sublethal effects is triggered if:

- 1a) **$PEQ_j > LD(D)_{10} / 10$, i.e., $L(D)D_{10} / PEQ_j > 10$,**
  or if no reliable $L(D)D_{10}$,

- 1b) **$PEQ_j > LD(D)_{50} / 50$, i.e., $L(D)D_{50} / PEQ_j > 50$**

Where:

- the PEQ_j (j indicates different PEQ values for the relevant risk cases) values are calculated according to Chapter 5. The most refined PEQ_j available can be used.
- For limit tests in which no significant mortality is observed, the following stands: $LD(D)_{50} = LD(D)_{10} = NOED$, meaning that the case 1a) is applicable.
- If TRT properties are determined, the lifespan LDD_{50} or data from the specific TRT study should be used. Since foraging behaviour is only relevant for summer bees, the 27-d $LD(D)_{50}$ and corresponding PEQj should be used (see Chapter 8 for more information).

If the PEQ_j is lower than the 'no concern level' (1a, 1b) for each of the three standard toxicity studies on honey bees, i.e., acute contact, acute dietary and chronic dietary, then no concern for adverse effect for foraging behaviour can be concluded and no more consideration is needed. If the PEQ_j is higher than the 'no concern level' a potential concern is identified, and the risk assessor should consider the next step (step 2 in 9.2.2).

9.2.2. Using pattern of sublethal effects seen in the laboratory tests

The second step consists in measuring, again on the basis of standard toxicity test data, the abnormal feeding behaviour of bees and the amount of food consumed. For this purpose, it is proposed in the EFSA Bee guidance to use the regular observations required in the OECD test guidelines 213, 214 and 245. From these behavioural observations in standard laboratory tests, it should be possible to determine if exposure to biocides influences the behaviour of bees in laboratory experiments (i.e., determine the `no concern level´ $NOED_{behaviour}$). Further standardisation can be achieved by following the recommendations in the supplementary materials of Tosi and Nieh (2019).

Given that in older studies, the behavioural observations may be insufficient, it is necessary to evaluate the reliability of the behavioural data. The reliability can be assessed by considering the age of the study and/or the level of detail of the description of the behavioural observations in the methods section. For example, studies that generated data prior to the publication of OECD test guidelines 231, 214, and 245, which provided an initial description of the abnormal behaviours to be recorded (forming the basis of the study). Abnormal behaviours that should be recorded (forming the basis of the assessment in this guidance document), may be considered less reliable. However, such studies may be considered reliable if they provide a description of the methods of observation and a description of the categories of behavioural endpoints that should be recorded, and that these categories are consistent with those recommended by the OECD 245. Consideration may also be given to whether the observations are consistent with other studies and information on the tested active substance.

Sections 9.2.2.1 to 9.2.2.3 of the EFSA Bee guidance explain how to derive a $NOED_{behaviour}$ value for abnormal bee behaviour from the standard laboratory tests.

There is no concern indicated from the sublethal effects on foraging behaviour if:

- 2) **the $NOED_{behaviour}$ > PEQ_j**

If this is not the case, additional studies are needed, see step 3 in Section 9.3 and step 4 in Section 9.4.

As no direct link can be established between the behaviour of bees in a laboratory context and mortality, any statistically significant difference in behaviour between the treatment and control groups should be treated as indicative of a potentially important sublethal effect that requires further investigation.

9.2.2.1. Statistical analysis of behavioural effects to derive 'no concern level' ($NOED_{behaviour}$)

Ideally, the appropriate statistical model should be able to describe not only the dose-response trend, which is the focus of this analysis, but also the temporal pattern of abnormal observations. This may be problematic, however, as individuals showing behavioural abnormalities may revert to normal behaviour over time, which may result in the absence of a clear and consistent trend over time, in contrast for mortality studies where the trend is, by definition, increasing. Accounting for the temporal pattern may thus hinder the analysis and produce results which are difficult to interpret. Therefore, it is recommended in the EFSA Bee guidance to analyse the data aggregated across the experimental period. A single aggregate proportion should be calculated for each cage (the sum of daily observations of abnormal behaviour divided by the sum of the daily count of live bees). The analysis should then focus on investigating whether the aggregate proportions show an increasing trend with dose levels.

OECD recommendations (OECD, 2006) have been followed to use a statistical test for trend combined with a 'step-down' procedure. This test is focussed on the detection of a monotonic (increasing) trend and should be appropriately selected among the range of options presented in OECD (2006). A good choice, to provide an example, could be the Rao-Scott adjusted Cochran-Armitage test (RSCA) (Rao and Scott, 1992), which has several desirable advantages: it is generally the most robust choice for quantal data (proportions), it allows for overdispersion, and it takes experimental replication into account (Green et al., 2018).

An appropriate statistical test for trend is chosen and the data is analysed using a step-down procedure following the method described in OECD (2006), meaning:

- The test for trend should be performed for data from all the treatment groups including the control. The cages should be incorporated as subgroups (or clusters) in the test.
- If the test is significant ($\alpha = 0.05$) then there is an increasing response across all dose levels. The high dose group is omitted and the test for trend is repeated with the remaining dose groups.
- The procedure is continued until the test is non-significant – there is no increasing response across the remaining dose groups. The highest concentration remaining at this stage is the NOEC.

9.2.2.2. Statistical analysis of behavioural effects to derive the 'no concern level' for food consumption ($NOED_{behaviour,food}$)

In acute and chronic dietary studies, the applicant should also test to see if the biocides induce changes in food consumption. The analysis should be based on an analysis of variance and include a dose, day, and day by dose interaction in order to be able to compare the mean volume of food consumed between each treatment group and the control. The comparison should be

done for each day; a Bonferroni-Holm correction for multiple comparisons can be used.

9.2.2.3. Worked example of how to analyse the behavioural observations

The EFSA Bee guidance provides a worked example to demonstrate the calculation of a NOEC from data taken from an anonymised dossier study.

The data consists of a 10-day chronic exposure study where the number of abnormal behaviours, as described in OECD test guideline 245, were recorded every 24 hours. The data is presented in Figure 16 below and was analysed using a RSCA test with a step-down procedure, as described in Section 9.2.2.1. The model returned significant p-values for the first four steps (all $p < 0.001$) indicating that each of the four highest concentrations of the product had a higher proportion of abnormal behavioural observations than the control.

When the model included only the control and the lowest tested concentration of the product, 156.25 mg a.s./kg, the trend was not significant ($p = 0.483$), indicating that this can be considered as the NOEC value from this experiment. In the 156.25 mg a.s./kg treatment group, the accumulated mean uptake of the test item was 43.99 µg a.s./bee/day. Thus, the interpretation of this test would be that a $PEQ_{di,ch}$ of < 4.399 µg a.s./bee/day would not trigger a concern for sublethal behaviour, a $PEQ_{di,ch}$ ≥ 4.399 µg a.s./bee/day would trigger a concern for sublethal effects and the applicant should proceed to further targeted behavioural tests (see steps 3 and 4 below).

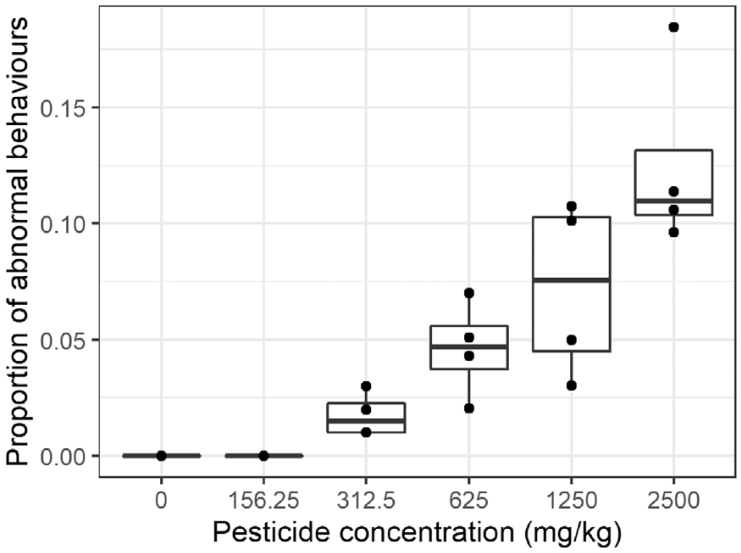


Figure 16: Boxplot showing the proportion of abnormal behaviour across a range of plant protection product concentrations aggregated across the entire experiment, note that the y axis is truncated at 0.2. (EFSA Bee guidance)

9.3. Specific behavioural assays on honey bees

As a third step, if a potential concern is raised on the basis of abnormal behavioural observations in the standard laboratory studies, targeted behavioural tests can be carried out. These studies can be similar to the standard toxicity tests, but performed at lower doses, while ensuring that the predicted exposure of the exposure assessment tiers is covered in the test. In addition, these studies should implement the following modifications to improve the data quality on sublethal effects:

- Even with training, interpreting the behaviour of an animal can be subjective. In order to minimise any unconscious bias, it is necessary that all behavioural results are generated blind (i.e. the observer does not know which treatment was given to which group).

- The minimum number of replicates should be increased. The applicant has to demonstrate that the experiment is large enough to detect an effect size of at least 10% more observations of abnormal behaviour in the treatment group relative to the control group with an alpha of 0.05 and a power of 0.8.

- Further standardisation of behavioural assessments can be achieved with the recommendations in the supplementary materials of Tosi and Nieh (2019).

In addition, it has to be noted that using the OECD design, the behavioural observations can only be monitored as the proportion of individuals behaving abnormally at any point, making the unit of replication the cage. If individual bees can be marked, either by using paints or identification tags, then the behaviour of each individual can be recorded at each timepoint, potentially making the unit of replication the individual and increasing the statistical power of the test to detect an effect. This is however considered a potential future improvement, not implementable at present.

The modifications mentioned above (blind observer and increased number of replicates) can also be implemented directly in the mortality tests. It is recommended to consider these in future modifications of the OECD guidelines for acute and chronic adult bee toxicity tests.

The $NOED_{behaviour}$ from the targeted assay should be compared to the relevant exposure level.

There is no concern indicated from sublethal effects on foraging behaviour if:

- 3) **the $NOED_{behaviour}$ from targeted assays > PEQ_j**

9.4. Homing flight study

The Annex K of the Supplementary Document of EFSA Bee guidance provides an overview of possible tests to investigate sublethal effect, some of which have a behavioural trait as an endpoint. Most of them are not standardized tests, except the homing flight study (OECD Guidance Document 332, 2021). This study aims at assessing effects of acute oral exposure to sublethal doses of a substance on the homing flight of worker honey bees. As the study only assess the effect of acute exposure, it can only be used as a refinement of a concern identified from the acute exposure. The dose level in the test needs to cover the acute daily intake of a forager bee.

From the data in this study, a $NOED_{homing\ test}$ can be determined. As mentioned above for other non-standardised targeted behavioural tests, there is no concern indicated from acute sublethal effects on foraging behaviour if:

- 4) **$NOED_{homing\ test}$ > $PEQ_{ac,di}$**

9.5. Higher tier endpoints

The last step gives the possibility to use data from higher tier studies in cases where they are available, but not reliable enough to perform a higher tier risk assessment. As mentioned in Section 9.1, should a higher tier assessment with higher tier studies be available or be carried out for a biocide, it will cover the risks of sublethal effects.

However, it is possible to assess effects on foraging behaviour in these higher level studies to obtain additional information on the mode of action of biocides. In this case, a negative effect

on foraging behaviour can be determined if there is a 10% reduction compared to the untreated control in one or more of the following parameters:

- The amount of pollen collected per flight (in mass)
- The number of bees returning with pollen
- The duration of a foraging flight (in minutes/flight)

More details on the assessment of these parameters can be found in the EFSA Bee guidance Section 9.5.

- 5) **If no effect >10% was seen at this exposure level, there is no concern from sublethal effects on foraging behaviour.**

10. Higher tier risk assessment

Higher tier risk assessment may be triggered when an unacceptable risk is identified in the lower tier risk assessment. The goal of the refinement is to reduce uncertainty through increased amount of information from studies conducted under more representative environmental conditions than standard laboratory tests. Regarding honey bees, the objective of the higher tier assessment is to check whether the agreed SPG is met (similar to lower tier risk assessment). For bumble bees and solitary bees, no SPG is currently defined due to current absence of knowledge and thus comparison of higher tier risk assessment output to SPG is not possible. Until the SPGs for bumble bees and solitary bees are defined, the EFSA Bee guidance advices to require more frequently higher tier studies to allow better protection of these bee species.

In the EFSA Bee guidance, three types of higher tier effect studies are described: field studies, semi-field studies and colony feeder studies (Table 52). The EFSA Bee guidance provides recommendations for the circumstances when each study type would be useful in relation to the outcome of the lower tier risk assessment (EFSA Bee guidance Section 3.3, Figure 4 and Section 10). In higher tier studies, it is necessary to measure the concentration of residues in pollen and nectar in order to ensure appropriate exposure levels.

Table 52: Overview of the higher tier study types for honey bees (HB) presented in the EFSA Bee guidance. See EFSA Bee guidance Chapter 10 and Annex C for further information.

Study (HB)	Description
Field study	Colonies with free flying bees are studied in open field conditions in (agricultural) landscapes. Colony size is the endpoint used for statistical comparison.
Semi-field	Bee colonies enclosed in large cages in field conditions. Forager mortality and foraging behaviour are the endpoints used for statistical comparison.
Colony feeder	Free flying colonies in open field conditions but with limited food (spiked sugar solution). The number of covered brood cells is the endpoint used for statistical comparison.

In the case of biocides, the performance of a higher tier risk assessment would be dependent on the case since these studies designed for plant protection products are challenging and may not be technically feasible for biocides. It has therefore been considered that higher tier field studies

for plant protection products may not be directly applicable for biocidal products[16]. However, if the design is adapted, the tests may be applicable to biocide uses as well. For instance, semi-field studies could be relevant for biocidal products if an unacceptable risk is identified in the lower tier risk assessment.

It is advised for the applicant to consult the corresponding section of the EFSA Bee guidance (Chapter 10), and to discuss with the evaluating competent authority, before performing any type of higher tier studies to ensure that an adequate testing protocol will be applied. In addition to the description of the aim, methodology, main considerations, and the endpoints for each higher tier study type, the EFSA Bee guidance provides instructions for a weight of evidence and uncertainty analysis for the use of the studies in the higher tier risk assessment. Under the biocide risk assessment, any available higher tier data needs to be evaluated on a case-by-case basis.

11. Metabolite assessment

11.1. Method

For biocides, an environmental risk assessment should be performed for active substances, as well as their metabolites (as stated in BPR Annex VI Art 8, 32 and 73, in Introduction to guidance on the Biocidal Products Regulation, Part A: Information requirements, Volumes I – IV, Chapter 4 (ECHA, 2022c), and in Guidance on the Biocidal Products Regulation, Volume IV, B+C, Section 2.1 and further (ECHA, 2017)). Metabolites trigger concern for bees when they are identified in plant materials like pollen, nectar, or other attractive plant matrices. For biocide applications, assessment of metabolites in these plant materials is not a standard practice. BPR Annex II includes information requirements for biodegradation and transformation in water, manure, soil, and air. However, the information requirement in BPR Annex II 10.4 to support the approval of an active substance states 'Additional studies on fate and behaviour in the environment'. This includes any relevant environmental compartment or matrix. Hence, when bees are expected to be exposed (see Chapters 2, 4 and 5) and a risk assessment is triggered, data measuring the residues and metabolization processes of the active substance and its degradation products in the relevant matrices are required. As experience with the generation of relevant metabolite data in plant matrices is very limited for biocides and the risk assessment for the active substance covers the metabolites in many cases (refer to EFSA Bee guidance, Appendix C), a stepwise approach depending on the source of exposure/scale is used for the metabolite risk assessment.

As a first step, the metabolite risk assessment will only be required for larger scale applications, including large scale spraying, irrigation, and manure/sewage sludge applications (see Chapter 5). Residue data and metabolism studies in nectar, pollen, and plants with the active substance and its possible metabolites are required only if it is anticipated that the substance is used in products that are sprayed outdoors (e.g., large scale spraying and irrigation). For through-soil exposure (manure/sewage sludge application) a different approach is taken, using data which are more generally available for active substances under the BPR and more easily obtained for biocidal uses. In this approach, metabolites formed in soil are assessed and this information can be obtained from the assessment for the terrestrial compartment. Refer to Figure 17 for a decision tree for the metabolite assessment for biocidal uses, and to the paragraphs below for further information on the assessment for large scale spraying, irrigation, and manure/sewage

[16]Minutes of the 92nd meeting of representatives of Members States Competent Authorities for the implementation of Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products, available at https://health.ec.europa.eu/events/92nd-meeting-expert-group-implementation-biocidal-products-regulation_en

sludge applications.

11.1.1. Assessment for large scale spraying and irrigation

Since typically data on plant residues and metabolism are not available in the biocide dossiers, a non-test screening step is introduced in the assessment of large scale spraying and irrigation applications. For this screening effect tier, no further metabolite assessment is needed if the risk for the (parent) active substance is unacceptable. In addition, in the screening step no further data are initially required for metabolites if the risk for the active substance is acceptable. Instead, if an acceptable risk was identified in the risk assessment of the (parent) active substance, then a screening for any potential metabolites should be performed, assuming a 10-times higher toxicity compared to the parent. In practise, this means that the standard risk assessment is re-run with the 10-times more toxic effect values. For this screening effect tier, no further data are required for metabolites if the risk for the metabolite is acceptable (i.e., PE_{SPG} ≤ 10%) with the 10-times higher toxicity. If using this approach, the risk is unacceptable, further data are required on metabolites.

When the screening indicates an unacceptable risk for potential metabolites, an assessment should be performed in line with the EFSA Bee guidance, for large scale outdoor spraying and irrigation. Applicants should contact the evaluating competent authority prior to the start of any study and prior to the submission of a dossier containing studies on metabolites. To select the most appropriate testing strategy, the problem formulation for metabolite assessment should be considered in light of the outcome of the lower tier risk assessment, relevant exposure pathway(s) and plant species. For information on residue data, refer to the EFSA Bee guidance Appendix C. However, for biocides the exposed plants in most cases are of an unknown and mixed composition. If one plant species is treated with the biocide, then this species should be included in the residue/plant metabolism studies. If a mixture of plant species may be exposed to the biocide, a surrogate species should be included in the studies. In line with the EFSA Bee guidance (Appendix C), the submission of a residue trial performed in a surrogate flowering species (for example Phacelia, oilseed rape, or sunflowers) may be considered for the purpose of identification of relevant metabolites in pollen and nectar.

A risk assessment for the metabolites identified in the available studies (e.g., plant metabolism studies) is triggered when:

- residues of metabolites are found at or above 10% TRR (Total Radioactive Residue) **and** 0.01 mg eq/kg (OECD, 2007) in residue studies in pollen and nectar or metabolism studies in treated plants,

  OR

- residues of metabolites are found at or above 10% TRR (Total Radioactive Residue) **or** 0.01 mg eq/kg in residue studies in pollen and nectar or metabolism studies in treated plants, **and** their parent substance is of acute toxicity to bees (i.e., LD_{50} < 0.01 µg/bee).

When a metabolite requires further assessment, based on the criteria above, relevant information on the hazard and exposure of the metabolite to bees must be provided. The assessment should be in line with the EFSA Bee guidance Chapter 11, following the exposure assessment approaches for biocide sources of exposure detailed in ECHA Bee guidance Chapter 5).

11.1.2. Assessment for manure/sewage sludge application

In the assessment of manure/sludge application, a risk assessment for metabolites is triggered when:

- metabolites in soil formed ≥ 10% on a molar basis, of the active substance in soil or appearing

at two consecutive sampling points at amounts ≥ 5% on a molar basis, or if at the end of the soil degradation study the maximum of formation is not yet reached but accounts for ≥ 5% on a molar basis, of the active substance at the final time point (in line with Guidance on BPR: Vol IV ENV Parts B+C), **and**

- their parent substance is of acute toxicity to bees (i.e., LD_{50} < 0.01 µg/bee).

As a first step, screening can be performed for the relevant soil metabolite(s), assuming a 10-times higher toxicity compared to the parent. For this screening effect tier, no further data are required for metabolites if the risk is acceptable (i.e., PE_{SPG} ≤ 10%). If the risk is unacceptable on the basis of first tier porewater calculations, the porewater concentration can be refined (PEARL groundwater calculations, in line with agreements under FOCUS PEARL standard assessment). If on the basis of these calculations an unacceptable risk is still identified, further data are required investigating the uptake characteristics of these metabolites in plants, pollen and nectar and/or the toxicity of the relevant metabolite(s) to bees. Applicants should contact the evaluating competent authority prior to the start of any study and prior to the submission of a dossier containing such studies on metabolites. In order to select the most appropriate testing strategy, the problem formulation for metabolite assessment should be considered in light of the outcome of the lower tier risk assessment, relevant exposure pathway(s) and plant species.

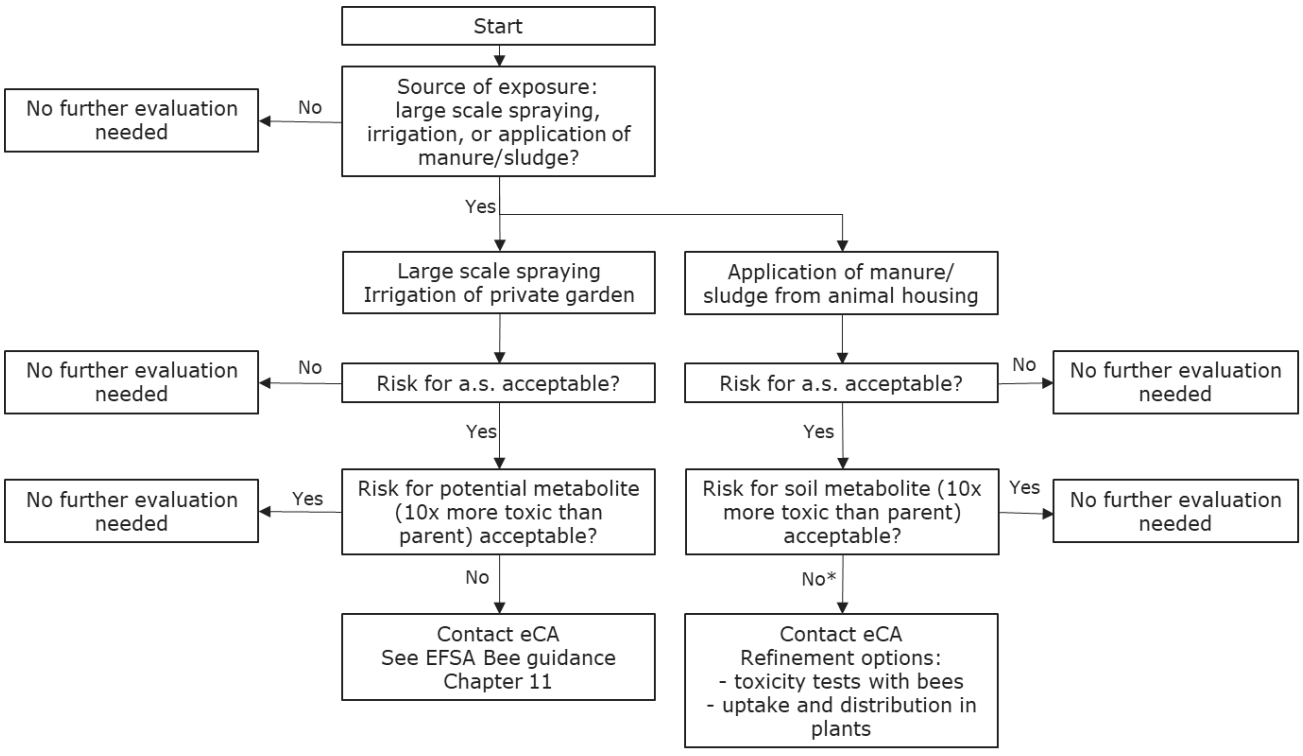


Figure 17: Decision tree for the metabolite risk assessment for biocides. *If in the risk for metabolites for manure/sewage sludge application is unacceptable, the porewater concentration can be refined. eCA = evaluating competent authority.

11.2. Risk assessment scheme for metabolites

When a need for the metabolite risk assessment is triggered, as described in Section 11.1, an assessment in line with the EFSA Bee guidance Chapter 11 will be followed to cover the assessment of metabolites.

With regards to toxicity studies, the information requirements on metabolites are in principle the same as for the parent active substance. However, studies on acute contact risk to bees are not considered relevant as exposure to metabolites in nectar and pollen via contact is negligible. Furthermore, the EFSA Bee guidance provides different scenarios based on the availability of metabolite data in a dossier. Three different options for the hazard assessment are described in situations where A) the dossier is complete, B) dossier is partially complete and C) data in dossier is missing (screening tier). If no bee toxicity data are available on the metabolite, the EFSA Bee guidance provides a possibility to estimate the toxicity based on the results of toxicity studies conducted with the metabolite for other invertebrate species, or by estimating the toxicity of a metabolite with non-testing methods like (Q)SAR, or presence of the toxophore.

For the exposure estimation, the acute and chronic dietary exposure is considered for the adult bees and chronic exposure for the larvae in the metabolite assessment. The exposure assessment may be started with a screening step and proceeded to Tier 1 if necessary, allowing a stepwise approach for the risk assessment. Higher tier studies (field effect studies) may eventually be conducted to address the suspected risk from the metabolites if the screening level and lower tier assessment results in unacceptable risks to bees (Chapter 10).

12. Mixtures

In this chapter, an approach is presented on how to address the risk of biocidal products containing more than one active substance (mixtures) for honey bees, bumble bees and solitary bees. Mixture toxicity only has to be assessed if two or more active substances with an insecticidal MoA are present in the product. Risk assessment of the mixture is not triggered due to active substances with non-insecticidal MoA that are part of the biocidal product, for example an active substance as a co-formulant (see also Section 6.1.3). The basic concept of the risk assessment for bees is that they are exposed to residues of active substances in the environment, e.g., via their diet. This approach differs in two ways from the standard risk assessment for mixtures for aquatic and terrestrial organisms as presented in the BPR Guidance Vol. IV, Parts B+C (2017):

- The risk assessment for biocidal products for bees in this guidance is a simplified version of the approach for PPPs presented in Chapter 12 of the EFSA Bee guidance. It is the logical consequence of following the approach of the risk assessment for single active substances as presented in this guidance (see Chapters 5 to 7), which is also based on the concept presented in the EFSA Bee guidance.

- Whereas for aquatic and terrestrial organisms according to the BPR Guidance Vol. IV, Parts B+C (2017), where the assessment for mixture toxicity is performed for the most sensitive trophic level, in this guidance the assessment for mixture toxicity for bees is required only for honey bees (and only qualitatively for bumble bees and solitary bees as long as no defined specific protection goal is available for these two bee groups).

12.1. Legal requirements

Regarding the conditions for granting an authorisation for a biocidal product, the BPR states in Article 19(2) that "the evaluation of whether a biocidal product fulfils the criteria set out in point (b) of paragraph 1 shall take into account the following factors: […] (d) cumulative effects, (e) synergistic effects." Cumulative and synergistic effects need to be addressed because biocidal products are usually multi-component mixtures of one or more active substances and a range of co-formulants that serve different purposes, e.g., as preservatives, anti-foaming agents, stabilizers, pigments, emulsifiers, solvents, or diluents.

The approach presented in the EFSA Bee guidance builds on existing methods and scientific experience in assessing chemical mixtures. For the sake for harmonisation, the same approach,

but simplified, is presented here and applied for biocidal products. Usually, mixture effects are the sum of the individual effects of the active substances at a certain dose (also known as concentration/dose addition). However, sometimes, interactions of mixture components can cause either significantly increased (synergistic) or decreased (antagonistic) effects compared with the effects predicted by concentration/dose addition. Especially interactions that increase the toxicity of a mixture need to be checked carefully. Although synergism occurs rarely, there are already known combination of active substances that result in synergistic effects, especially if known synergists, like piperonyl butoxide (PBO), are included in biocidal products. In a recent study, synergistic effects on honey bees were observed for mixtures containing thiamethoxam in combination with cyfluthrin and permethrin, respectively (Li et al., 2023).

Both regulations (BPR and PPPR) base the mixture toxicity risk assessment on two options that are considered most adequate for the assessment of hazards and risks of mixtures: measured ("whole mixture" approach) and calculated mixture toxicity ("component based" approach). Generally, calculated mixture toxicity is the preferred option since no additional testing is required. This approach is usually done for the standard aquatic and terrestrial risk assessment for biocidal products. However, for the present bee risk assessment, effect studies with biocidal products that contain two or more active substances are always required (see Table 39 in Section 6.1.3). Therefore, in most cases, the risk will be estimated using measured mixture toxicity unless experimental testing of the product is technically not feasible. In the latter case, the calculated mixture toxicity approach would be applied. Based on the mixture toxicity (measured or calculated) selected for each risk case (i.e., acute-contact, acute-dietary, chronic-dietary and larvae-dietary), a combined risk assessment can be conducted for each bee group, in line with the approach presented in Chapter 7.

12.2. Risk assessment for mixtures

12.2.1. Defining the effects

For the **measured mixture toxicity**, the selection of the relevant effect parameters ($DRC_{j,mix\text{-}meas}$) will follow the same rules as explained in Chapter 6. It is important that the selection of dose-response model and the corresponding effect parameters ($DRC_{j,mix\text{-}meas}$) would ensure that the mortality is not underestimated at the lower doses by choosing a model with a too steep slope (see also Section 6.3).

If no effect data of the biocidal product are available, the effect parameters for the **calculated mixture toxicity** ($DRC_{j,mix\text{-}calc}$) need to be estimated. With a dose addition approach, for a mixture of *n* components, a specific $LD_{x,mix\text{-}calc}$ resulting in an effect level *x* is calculated as follows:

$$LDx_{mix-calc} = \left(\sum_{i=1}^{n} \frac{p_i}{LDx_i} \right)^{-1}$$

Equation 36

Where:
n: number of mixture components
i: index from 1…n mixture components
p_i: the i^{th} component as a relative fraction of the mixture composition (note: Σp_i must be 1)
$LD_{x,i}$: dose of component i provoking x% effect

This means that, when the dose-response relationships and thereby different effect levels ($LD_{x,i}$ from 1% to 99%) and the relative fractions (p_i) of the *n* components of the mixtures are known, it is possible to calculate the $LD_{x,mix\text{-}calc}$ for a range of effect levels. This allows describing the dose-response curve of the mixture in a rather precise way, by using the most suitable model to extract the effect parameters ($DRC_{j,mix\text{-}calc}$).

Even if the calculated $LD_{x,mix\text{-}calc}$ for a range of effect levels cannot be approximated by a log-logistic model or any other model (see Chapter 6), and none of the models results in a $DRC_{j,mix\text{-}calc}$ with a good fit, an estimation of the $LD_{x,mix\text{-}calc}$ of the mixture due to a specific level of exposure can still be made, by predicting with reasonable accuracy, the effects caused by a certain exposure level by using Equation 36 only.

12.2.2. Defining the exposure

The calculated or measured $LD_{50,mix}$ of a mixture with active substances can be conceived as an LD50 of a single virtual compound. Therefore, in analogy, it can also be assumed for the exposure side that that mixture components together constitute a virtual compound and thus the individual PEQ_j of each active substance can be added up. This concept is a standard procedure for the standard risk assessment for aquatic and terrestrial risk assessment for biocidal products (ECHA 2017).

The dietary and contact exposure level of the mixture ($PEQ_{j,mix}$) can be calculated with Equation 37. With this equation, it is assumed that the PEQ_i of all active substances present in the biocidal product will occur at the same moment and are not separated in time (i.e., worst-case $PEQ_{j,mix}$).

$$\boldsymbol{PEQ_{j,mix} = \sum_i^n PEQ_{j.i}} \qquad \textbf{Equation 37}$$

With:

PEQ_i = Predicted Exposure Quantity of active substance i for risk case j. This is the output of the exposure estimation (see Chapter 5).

PEQ_{mix} = Sum of the individual PEQ_i

It should be carefully checked whether **metabolites** of ecotoxicological relevance have to be included into the PEQ_{mix} or not (see Chapter 11). Usually, metabolites of ecotoxicological relevance need to be included in the risk assessment of biocides, and therefore also in the mixture toxicity assessment. However, as described in Chapter 11, exposure and effect data are not straightforward to obtain for biocidal uses. In case the applicant pursues to perform studies on metabolites, it should be carefully checked that the gathered information is also useful for the risk assessment of mixtures. For this first version of the ECHA Bee guidance, however, in absence of specific study designs for metabolites, the focus of the mixture toxicity remains on the active substances and thus metabolites shall not be considered for the time being. However, if reliable data on both exposure and effect can be collected, the approach how to conduct the mixture toxicity risk assessment including metabolite is described in Chapter 12 of the EFSA Bee guidance.

If on this basis unacceptable risk is not excluded, no further refinement is possible because in contrast to PPPs, more detailed consideration of time-dependent exposure patterns (i.e., shift in the composition in the environment) is not foreseen for the exposure side (see Chapter 5).

So far uncertainty remains on the real fate of other **co-formulants** present in the mixture that is applied (EFSA Bee guidance). Co-formulants may in some cases dissipate slower than the active substances and are not covered at the screening level and Tier 1 risk assessment. In absence of specific data, uncertainty remains on the actual exposures of bees to these compounds.

Thus, only if exposure and effect data of an ecotoxicologically relevant co-formulant is available, the concerned co-formulant could be included in the risk assessment of the mixture as an additional compound in the calculated mixture effect (see step 2 in Figure 18).

12.2.3. Risk assessment scheme

A detailed stepwise decision scheme is presented in Figure 18. The scheme needs to be iterated for each risk case.

The steps are identical to the scheme presented in Chapter 12 of the EFSA Bee guidance, apart from the simplification based on omission of step 3 presented in Figure 22 in the EFSA Bee guidance. This step 3 is relevant for PPPs when the exposure estimations were refined based on substance-specific parameters that result in re-calculating the SV parameters with a Monte Carlo method (see Section 5.5.7 of the EFSA Bee guidance). Since for the exposure estimation of biocidal products no such re-calculation of SV parameters is foreseen (only a change in a single value parameter depending on the source of exposure, see Chapter 5), this step can therefore be omitted.

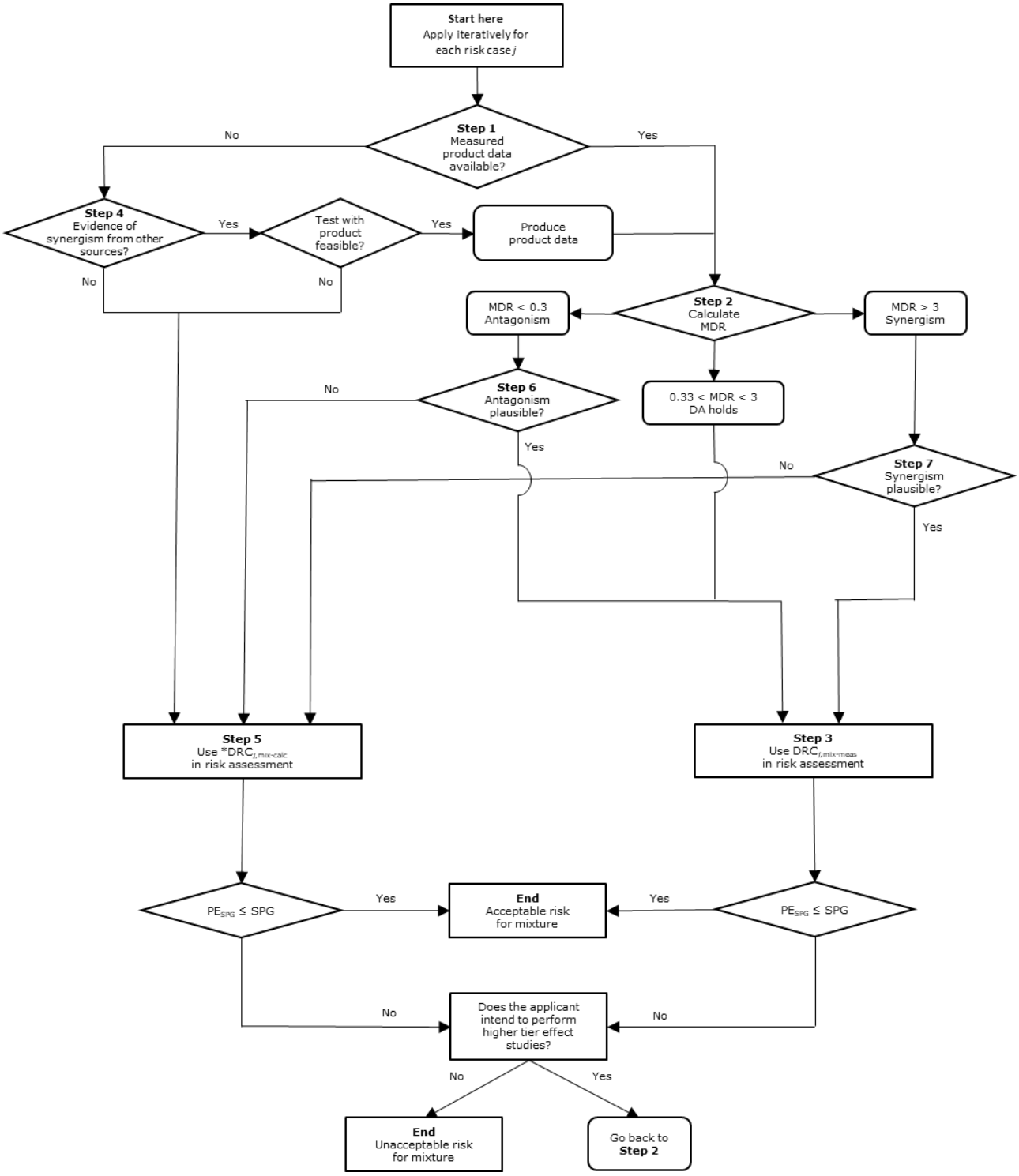


Figure 18: Workflow illustrating the risk assessment scheme for mixtures (modified from EFSA Bee guidance). $DRC_{j,mix\text{-}meas}$ = measured dose-response curve for risk case j, $DRC_{j,mix\text{-}calc}$ = calculated dose-response curve for risk case j, MDR = model deviation ratio; PE = overall predicted effect at colony level; SPG = specific protection goal. *$DRC_{j,mix\text{-}calc}$ may need to be corrected by an appropriate MDR from other risk cases/species when synergism is plausible (see step 4).

Step 1. Are measured mixture toxicity data ($LD_{50j,mix\text{-}meas}$) with the product available for the given risk case?

No, (only data for the a.s. ($LD_{50j,a.s.}$) are available): Go to step 4

Yes, (both data for mixture ($LD_{50j,mix\text{-}meas}$) and active substances ($LD50_{j,a.s.}$) are available: Go to step 2.

Step 2. Check the plausibility of the calculated mixture toxicity $LD50_{j,mix\text{-}calc}$ (derived with equation 36) against the measured mixture toxicity ($LD50_{j,mix\text{-}meas}$) on the basis of the mixture composition of the active substances in the product by means of the Model Deviation Ratio (MDR, see equation 38).

Notes:
In order to determine if the active substance may act more (i.e., synergistically) or less (i.e., antagonistically) than expected by dose addition, a comparison of the calculated $LD50_{j,mix\text{-}calc}$ versus the measured $LD50_{j,mix\text{-}meas}$ endpoints is informative.

This comparison may also indicate that other co-formulants not included in the calculation of $LD50_{j,mix\text{-}calc}$ could contribute to the overall mixture toxicity in an appreciable way. When this is the case, they can be included in a refined calculation, however only if the respective single-compound toxicity data of the co-formulant is available which is very rarely the case. Possible outcome of the MDR calculation is the following:

$$MDR = \frac{LD50_{j,mix-calc}}{LD50_{j,mix-meas}}$$ **Equation 38**

- **0.33≤MDR≤3.** The measured and calculated $LD50_j$ are considered in agreement if the MDR is between 0.33 and 3. That is the dose addition hypothesis holds. This convention is in line with the recent EFSA recommendations related to pesticide RA (Pesticide Peer Review Meeting 185, 9–12 October 2018). In relation to 'when a formulation should be considered more toxic than the active substance', the proposal was to account for a difference of a factor of three, as recommended in the guidance from the Directorate-General for Health and Food Safety (SANCO/10597/2003 rev. 10.1) (European Commission, 2012) on the equivalence of batches. Thus, if the MDR lies between these two values, it is considered that the dose addition hypothesis holds
- **MDR is < 0.33.** Less than additive (i.e., antagonistic) mixture toxicity is indicated if the MDR is below 0.33.
- **MDR is > 3.** More than additive (i.e., synergistic) mixture toxicity is indicated if the MDR is > 3.

A careful interpretation of the MDR is mandatory, especially if not all components that potentially contribute to the measured mixture toxicity (e.g., co-formulants) have been considered in the calculated mixture toxicity. Care should also be taken that the counter-checking of measured and calculated $LD50_j$ refers to the same basis, that is, the relative proportion of mixture components must be consistent (e.g., to the sum of active substances of a given biocidal product if co-formulants are not included in the dose addition calculation).

If MDR = 0.33–3 (dose addition approximately holds for the mixture): Go to step 3 (use measured mixture toxicity)
If MDR < 0.33 (mixture less toxic than dose addition): Go to step 6
If MDR > 3 (mixture more toxic than dose addition): Go to step 7

Step 3 (Step 4 in EFSA Bee guidance). Use the measured mixture dose-response ($DRC_{j,mix\text{-}meas}$) and proceed to the risk assessment as described in Chapter 7.

PE_{SPG} ≤ SPG: Acceptable risk
PE_{SPG} > SPG: Acceptable risk not demonstrated

Step 4 (Step 5 in EFSA Bee guidance). Is there evidence that synergistic interactions between mixture components might occur[17] which cannot be ruled out for the given endpoint with sufficient certainty?

Note: If synergistic effects cannot be excluded, the risk assessment should preferably be based on measurements, as synergistic interactions are not predictable by dose addition nor by other concepts such as independent action/response addition alone. If experimental testing of the mixture is no option (e.g., for technical reasons) for certain species and endpoints, but synergism is known form other studies, the risk assessment may be performed by **shifting the calculated $DRC_{j,mix\text{-}calc}$ by the MDR** obtained from other risk cases/species if available.

Yes (mixture toxicity calculation not feasible): Measured mixture toxicity data required for risk assessment

- If measured mixture toxicity becomes available: Go to step 2
- If measuring the mixture toxicity is not technically feasible, but a reliable MDR is available from other risk cases/species, shift the calculated $DRC_{j,mix\text{-}calc}$ by the MDR and go to step 5

No (mixture toxicity calculation feasible): Go to step 5 (use calculated mixture toxicity)

Step 5 (Step 6 in EFSA Bee guidance). Use the calculated $DRC_{j,mix\text{-}calc}$ to estimate the effect for the risk case of concern and proceed to the risk assessment.

PE_{SPG} ≤ SPG: Acceptable risk
PE_{SPG} > SPG: Acceptable risk not demonstrated, check single-substance refinement options

Note that PE_{SPG} represents the combined effects of all the risk cases taken in consideration in the RA. To comply with the SPG, any risk case can be refined independently of the others and thus a refinement on another risk case may suffice.

Step 6 (Step 7 in EFSA Bee guidance). Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data ($DRC_{j,mix\text{-}meas}$) regarding potential impacts of the default assumption of dose addition (e.g., check for heterogenous input data, i.e. different study designs/endpoints). Does the apparent antagonism hold?

Note: If plausible toxicological explanation for this apparent antagonism can be provided (e.g., special feature of the formulation type), the risk assessment should be based on the measured mixture toxicity. Otherwise, the calculated mixture toxicity is a better option. No correction for MDR is needed, as the calculated mixture toxicity represents a worst-case.

Yes (antagonism holds): Go to step 3 (use measured mixture toxicity)
No (antagonism does not hold): Go to step 5 (use calculated mixture toxicity)

Step 7 (Step 8 in EFSA Bee guidance). Carefully recheck the apparent synergism as observed in the measured mixture toxicity data ($DRC_{j,mix\text{-}meas}$) regarding potential impacts of heterogeneous input data (testing conditions/endpoints should be homogenous) and of co-

[17] e.g., based on toxicological knowledge from literature, some indications are given in Appendix 11 in the BPR Vol. IV Parts B+C, ECHA 2017

formulants ignored in the dose addition calculation. Does the apparent synergism hold?

Note: If plausible toxicological explanation for this apparent synergism is available or if this check reveals the presence of a toxic co-formulant, the risk assessment should be based on the measured mixture toxicity. Otherwise, the calculated mixture toxicity is a better option.

Yes (synergism holds): Go to step 3 (use measured mixture toxicity)
No (synergism does not hold): Go to step 5 (use calculated mixture toxicity)

13. Risk mitigation measures, instructions for use, and warning sentence

13.1. Risk mitigation measures

If an unacceptable risk to bees is indicated, consideration of risk mitigation measures (RMMs) is one possible option to reduce the exposure of bees and to refine the risk assessment. RMMs can be integrated to an exposure assessment re-estimation at any tier, except the screening level and/or they can be proposed to reformulate the problem formulation. Any suggested mitigation must reduce the risk sufficiently so that the risk assessment indicates an acceptable risk. Furthermore, risk mitigation measures should be practicable (e.g., for the proclaimed user category), suitable for the intended use, and enforceable. It must be assured that risk mitigation phrases are seen by a relevant person (product user).

RMMs can be classified into two broad categories with the aim to reduce the exposure to bees:

1. Specific mitigation measures are targeted actions which are needed to mitigate an identified risk due to biocide exposure. The mitigation must be demonstrated quantitatively. Any suggested mitigation must be accompanied by an appropriate risk assessment for which additional data may be needed. Specific mitigation measures can be proposed by the applicant within the risk assessment process.

2. Generic mitigation measures are those actions which are undertaken to manage the risk to bees. Generic mitigation measures can be considered as risk management options within the decision-making process for the approval of active substances or the authorization of biocidal products.

It is important that the effect of implementing risk mitigation measures on other sections is taken into account, especially the effect on efficacy.

Specific risk mitigation measures can include for example the reduction of the application rate or the number of applications and/or increase of interval between applications. Note that there is an overlap of these measures with the refining options during risk assessment.

Currently the only (generic) harmonized risk mitigation phrase aimed at reducing the exposure and hence the risk to bees is the following SPe8[18]:

> *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).*

[18] Regulation (EU) No 547/2011 under revision at the time of this guidance development

This phrase, or parts of it, is considered to cover risk mitigation for PPPs for bees, specifically honey bees. The sentences may be considered for applications of biocides that are comparable to those of PPPs, specifically for large scale spray applications (see Sections 5.6 to 5.7) and potentially for small-scale spraying on walls and foundation of houses (see Section 5.3). It must be emphasized that the sentences would need to be adapted for biocide use. For example, for biocides there is usually no distinct differentiation between "crop" and "field margin". Furthermore, as most biocides are applied on or in the proximity of mixed vegetation, it might not be possible to avoid flowering plants/weeds during application.

Furthermore, parts of this phrase are associated with uncertainty regarding the practicability, and potentially have unclear or even undesired effects.

For the biocides source of exposure due to manure/sewage sludge application (see Section 5.2), there are no appropriate RMMs to reduce the consumption of contaminated pollen and nectar by bees, other than the measures to reduce the amount of biocide reaching manure/sludge. Manure as an agricultural asset is exported/traded and therefore restrictions, such as limiting the amount of manure that is applied or the type of land that it is applied to, cannot be expected to be complied with via supply chain. Exposure of bees can only be limited indirectly by measures limiting the amount of biocide reaching manure. RMMs or instructions for use that are generally aimed at reducing environmental input (i.e., by preventing the product from reaching the manure), and therefore only having an indirect effect on bee exposure, are not considered in this guidance. These can be applied in accordance with other available guidance and current practice.

13.2. Instructions for use for baits

RMMs are applied when an unacceptable risk is identified based on the conclusions of the risk assessment. For bait application, no quantitative risk assessment is proposed in this guidance. Baits are generally not expected to be attractive to bees for several reasons (see Section 2.1.2). Indirect exposure of bees through soil is not considered relevant for baits because the area of soil contaminated from leaching or run-off is very local and small-scale (see Section 2.1.2).

One key factor, that determines whether bees can orally take up bait formulations, is the viscosity. It is known that viscous sugar-based substrates need to be liquefied before bees would be able to take it up. To prevent baits that are placed outdoors from being liquified, it is recommended to include the following instruction for use on the label of the bait product:

Apply only in areas that are not liable to submersion or becoming wet, i.e., protected from rain, floods, and cleaning water.

This instruction for use is already commonly used for bait products with outdoor application because it prevents the formulation from entering the environment. It is applicable to most bait formulations, i.e., bait boxes, granules, and gels.

Several RMMs and instructions for use for baits have been proposed and discussed by the Environment Working Group of the Biocidal Products Committee, and by the representatives of Member State Competent Authorities and the European Commission. The discussed measures/sentences were aimed at preventing direct consumption of baits by bees. In the discussion by the biocide competent authorities, it was noted that the proposed sentences might be disproportionate considering there is no risk identified due to the lack of appropriate risk assessment tools[19]. Furthermore, it was proposed that the sentences are considered in the

[19] CA-Dec20-Doc.4.1 WARNING SENTENCE AND RMM FOR BEES

context of the ECHA Bee guidance development.

During guidance development, the ECHA EG conducted a survey among stakeholders and experts to assess whether these and similar sentences are considered efficient in preventing bees and other pollinators from accessing the baits. The responses to the survey generally lacked data to support the statements (e.g., literature, laboratory, or field studies) and therefore only limited recommendations are made in the current ECHA Bee guidance.

The provided answers generally suggested that *bait boxes* with small enough openings are considered to prevent bees from accessing the bait inside the bait box. Bait boxes can therefore be considered as safe for bees. However, applying a product in a bait box without further efficacy testing is not an option in most cases.

Misuse in general and specifically of bait products is not covered in the ECHA Bee guidance. Users are generally expected to follow instructions for use. However, several incidents with biocides with fatal bee hive intoxication were reported within the last years in Switzerland for example. In few cases, baits were directly used in bee hives to combat ants. To raise awareness and prevent such incidents, the following sentence may be added to the instructions for use:

Do not apply in or near bee hives.

13.3. Requirement for Warning Sentence

Prior to the development of the ECHA Bee guidance, the procedure to include a hazard-based warning sentence in the authorisation of biocidal products was discussed by the Member States Competent Authorities[19]. The following wording was agreed for biocidal products, based on a toxicity threshold of 11 µg/bee (acute toxicity):

"*This biocidal product contains (active substance name) which is dangerous to bees*".

At the time of the development of the ECHA Bee guidance, the attribution criteria of the warning sentence to biocidal products and the harmonisation with PPPs was discussed. Instructions on the requirements for the warning sentence is not addressed in this guidance and instead is provided as a stand-alone document[20] .

14. Conclusions

The ECHA Bee guidance document provides applicants and competent authorities with the methodology to assess the risk to honey bees, bumble bees and solitary bees from the use of biocidal products. The guidance takes into account the available guidance for plant protection products (EFSA 2023), having made the necessary adaptations to biocides when needed.

With regards to arthropod pollinators other than bees, future development of guidance is needed since at the time of the preparation of this guidance sufficient information was not available for developing a risk assessment methodology for non-bee pollinators.

[20] CA-Dec20-Doc.4.1 Warning sentence and RMM for bees_final.docx. Available at https://circabc.europa.eu/ui/group/e947a950-8032-4df9-a3f0-f61eefd3d81b/library/5e6cf719-8286-4cbf-9b1e-f01eade08bb7/details

15. Recommendations for future development and research

This section describes the knowledge gaps and research needs identified for the bee risk assessment of biocides in particular. For further information and for other general recommendations, please refer to the Chapter 15 of the EFSA Bee guidance.

A description of the knowledge gaps and recommendations for future research with regards to non-bee pollinators are available in Section 1.6 and in the related ECHA publication (ECHA 2022a).

15.1. Inclusion of potentially important matrices/exposure routes/life stages

Some bees may be continuously exposed to biocides due to their way of life, for instance larvae of some Megachilidae: 1) leaf cutting bees that use leaves of plants, including ornamentals, to build their nests, or 2) bees that use mud as a nesting material, such as mason bees (Osmia spp.), which may be exposed for instance in an irrigation scenario. In these cases, larvae could be continuously exposed to contaminated material in the nests, leading to chronic larvae-contact risk. Further research would be needed on these routes of exposure in order to potentially include them in the risk assessment.

15.2. Exposure Assessment

With regards to exposure assessment of bees, further research and generation of data is needed in respect to

- substance retention rate, environmental fate with an emphasis on the microbial metabolism of the substance, its bioavailability in the next season, effect and dynamics of metabolites, their dissociation rate from the soil particle, and, finally, to be able to assess the concentration of the parent or metabolite substances in pollen;
- potential exposure of larvae through contact with contaminated material brought into the nest/hive by adults;
- the derivation of biocide-specific values in relation to interception in particular concerning the large scale spraying (see Section 5.5 to Section 5.7);
- irrigation scenario: use of mud as a nest building material for many pollinators could be a route of exposure to insecticides for larvae. This route could potentially be important for such pollinators as mason bees (Osmia spp.), which use mud to build nest partitions (see 15.1);
- small scale applications: Lack of knowledge with regards to exposure from bait applications, attractiveness of baits, and direct consumption of bait, which prevents to develop a realistic scenario for bee risk assessment via this route of exposure.

In addition, it is noted that the following exposure routes have not been covered in the first version of the ECHA Bee guidance (see Chapter 5):

- the consumption of contaminated water (e.g., puddles formed during spraying, contaminated surface water, or guttation water),
- the consumption of other contaminated plant matrices (e.g., honey dew, extrafloral nectaries, resin, wax etc.),

- contact exposure of bee species which are breeding in contaminated soil or wood, or which use other contaminated materials (e.g., leaves, mud) to build their brood cells.

15.3. Effect assessment in lower tiers

With regards to solitary bees and bumble bees the lack of standardised test guidelines was highlighted. Furthermore, due to lack of toxicity data for bumble bees and solitary bees, extrapolation between bee species is difficult and includes many uncertainty factors (see Section 6.5).

15.4. Lower tier risk assessment

With regards to bumble bees and solitary bees, it was highlighted that uncertainty in the risk assessment results from the lack of data on the ecological factors that may influence the vulnerability of these bee species in relation to honey bees (see Section 7.3).

15.5. Sublethal effects on honey bees in risk assessment

For sublethal effects, in general there is a need to gain more experience since the method provided in the EFSA Bee guidance and applied consequently in the biocide assessment constitutes the preliminary instruction to perform such an assessment. The current main deficiencies are related to the missing link between the observed sublethal behaviours and the SPG, as well as lack of standardised sublethal effects assessments for bumble bees and solitary bees.

15.6. Higher tier risk assessment

In general, experience is needed in relation to higher tier risk assessment for bees and higher tier studies in the assessment of biocides. Especially it is unlikely that field studies according to the currently available test guidelines would be conducted for biocides. This lack of knowledge at the time of the writing of this guidance prevents providing detailed instructions or recommendations for higher tier risk assessment and testing.

Regarding the limitations in higher tier risk assessment, it was suggested that for honey bees the higher tier effect refinement is only potentially limited to contact risk (semi-field studies) and larval risk (colony feeding studies), as full-scale field studies are likely to be unachievable for biocides, and thus not resulting in higher tier refinement options for dietary risks.

15.7. Metabolites

In general, there is a need to gain more experience on assessment of metabolites for the risk to bees due to biocidal use. It is currently uncertain if the calculations sufficiently represent the actual situation in field, as metabolite residue data in pollen and nectar are lacking for biocidal uses. In addition, the lack of instructions for testing also apply to the assessment of metabolites.

15.8. Mixtures

The first version of the ECHA Bee guidance is focussing on active substances with insecticidal mode of action (usually approved under PT18) and sources of exposure arising from PT 18

emission scenarios. However, under environmental conditions, bees may be exposed to mixtures of biocides in the context of their foraging activity or in the collection of materials for hive/nest construction. Mixtures on insecticides with other non-insecticidal biocides such as fungicides are currently not covered by the guidance. It is recommended that this aspect will be taken into consideration in future updates of the guidance in order to address concern pointed out in literature (e.g., Belden 2022, Almasri et al. 2020, Sgolastra et al. 2016).

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Glossary and abbreviations

Abbreviation/Acronym	Explanation
a.s.	Active substance
AR	Application rate
B	Constant B (used in PEQ calculation)
BB	Bumble Bee
BBCH	Growth stage; uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie)
BEEHAVE	Computer model to simulate the development of a honey bee colony and its nectar and pollen foraging behaviour in different landscapes
b.p.	Biocidal product
BSF	Body surface factor (dm^2/bee)
ca	contact - acute
CA	Competent Authority
GCEG	Guidance Consultation Expert Group
Cir	Concentration of active substance in irrigation water
CMP	Food consumption, e.g., CMPsu = sugar consumption
co	Contact
Colony	A colony consists of a number of individuals of the same species living in close association with each other.

Colony strength	Colony size, defined here as the number of adults that forms the colony
da	dietary - acute
dc	dietary – chronic
di	dietary
dl	dietary - larvae
DT_{50}	Disappearance Time 50, time required for 50% degradation of a substance, (defined method of estimation)
du	During flowering
DRC	Dose-Response Curve, the parameter describing the steepness of the dose-response relationship obtained from standard laboratory tests
DW	Downward spray, it includes all application methods where the spray is directed to the ground
EA	Exposure Assessment
EC_{50}	Effective Concentration 50, concentration required to obtain a 50% effect on a test population after a specified test duration
eCA	Evaluating competent authority
EC_x	Concentration with x % level of effect compared to the control
ECHA	European Chemicals Agency
EDx	Effective Dose, e.g., ED50 = Effective dose for 50% of the organisms tested
EED	Estimated Exposure Dose (in higher tier effect studies), measured or calculated dose of a substance to which an organism is likely to be exposed

EF	Exposure Factor
EfAGs	Effect Assessment Goals. The EfAG operationalises the Specific Protection Goals with respect to the effect assessment, e.g., definition of relevant model species, type of toxicity, measured endpoints for the relevant species, extrapolation between species
EFSA	European Food Safety Authority
EG	Expert group
EREQ	Ecotoxicologically Relevant Exposure Quantity. Conceptual interface between the effect and exposure tiers. It is based on ecotoxicological considerations and defines the type of exposure quantity that in a mechanistic sense best explains observed effects in an ecotoxicological experiment
EU	European Union
EU COM	European Commission
ExAGs	Exposure Assessment Goals. The ExAG operationalises the Specific Protection Goals with respect to the exposure assessment in the environment, e.g., definition of the environmental exposure, type, duration, matrix, and level of conservativeness of the exposure estimate.
Fai	Fraction of an active substance in the product
Fattr	Fraction of a garden that is attractive to bees
Fcont	Contamination factor
FOCUS	FOrum for Co-ordination of pesticide fate models and their USe, an initiative of the EU COM to harmonise the calculation of predicted environmental concentrations of active substances of plant protection products
FVI	Flower-Visiting Insect
GD	Guidance Document

GUTS-RED	General Unified Threshold model for Survival – Reduced. GUTS is a framework for deriving toxicokinetic–toxicodynamic models, which account for effects of toxicant exposure on survival in time.
GUTS-RED-IT	General Unified Threshold model for Survival – Reduced - Individual Tolerance GUTS is a framework for deriving toxicokinetic–toxicodynamic models, which account for effects of toxicant exposure on survival in time.
GUTS-RED-SD	General Unified Threshold model for Survival – Reduced - Stochastic Death GUTS is a framework for deriving toxicokinetic–toxicodynamic models, which account for effects of toxicant exposure on survival in time.
Guttation	Appearance of drops of xylem sap on the tips or edges of leaves of some vascular plants
HB	Honey Bee
Hive	Enclosed, man-made structure in which some honey bee or bumble bee colonies with their nets are kept.
ho	Honey
Honey dew	A sugary secretion produced by aphids and other insects
IP	Inflection point. Points of the curve where the curvature changes its sign
LC50	Median Lethal Concentration, the concentration of a chemical that kills half of a test population after a specified test duration
LD50	Lethal Dose, the dose required to kill half of a test population after a specified test duration
LDD_{50}	Median Lethal Dietary Dose (Chronic dietary experiments), a statistically calculated dietary dose of a substance that can cause death in half of the test organisms at the end of the test period

LF	Landscape factor, describes the proportion of the food intake of a bee colony or population that originates from the treated field: e.g., LFpo = landscape factor for pollen
MDR	Model Deviation Ratio
MoA	Mode of action
n	Number of applications (to soil)
Napp	Number of repeated biocide applications to the water collection container
NBP	Non-Bee Pollinator
Nbuildings	Number of buildings on a hectare
ne	Nectar
Nest	A nest is a structure built by the bees to hold eggs, offspring, and the adult form(s) itself. E.g., honey bees, bumble bees and solitary bees can have nests
NOEC	No Observed Effect Concentration, the highest tested concentration which causes no effect
NOED	No Observed Effect Dose, the highest tested dose which causes no toxicity
OECD	Organisation for Economic Co-operation and Development
PCE	Predicted colony level effect
PCUD	Predicted Concentration per Unit Dose
PEARL	Pesticide Emission At Regional and Local Scales
PEC	Predicted Exposure Concentration
PEQ	Predicted exposure quantity

PE_{SPG}	Overall predicted effect at the colony level
PIE	Predicted individual level effect
po	pollen
PPP	Plant Protection Product
PPR Panel	EFSA Scientific Panel on Plant Protection Products and their Residues
Protection goal	The objective of environmental policies, typically defined in laws or regulations.
pw	Porewater
Qirw	Amount of irrigation water
Qprod	Quantity of product applied
(Q)SAR	(Quantitative) structure-activity relationship; mathematical models that can be used to predict the physicochemical, biological and environmental fate properties of compounds from the knowledge of their chemical structure.
RMM	Risk Mitigation Measures. Actions which are needed to mitigate/manage a risk to bees due to chemical exposure
RUD	Residue per Unit Dose, a parameter expressing the residue concentration of a pesticide molecule in pollen and in nectar, standardised on an application rate of 1 kg/ha
SANCO	European Commission Health and Consumer Protection Directorate General
SB	Solitary Bee
S_{ho}	Sugar content of honey
SN	Sugar content of nectar

SPG	Specific Protection Goal
SSD	Species Sensitivity Distribution
STP	Sewage treatment plant
su	Sugar
SUW	Sideward and Upward spray, it includes all applications where the spray is directed sidewards or upwards (this can be air assisted or without air assistance)
SV	Shortcut value, the 90th percentile of a distribution of residue intake per bee (or larvae) over a colony (or population, for solitary bees)
TEF	Toxicity Extrapolation Factor, numerical values derived from standard species to smaller bumble bees and solitary bees for a generic (substance-independent) relationship between LD50 and bee weights
TRR	Total Radioactive Residue
TRT	Time-Reinforced Toxicity, the potential of a compound under evaluation for showing increased toxic effects due to long-term exposure to low doses, compared to what would be expected based on short-term exposure to higher doses
TWA	Time-Weighted Average
w	Time window for deriving time-weighted average concentrations for chronic exposure
wi	Winter

Appendices

Appendix A - Attractiveness of different shrubs and wild trees

Flowering shrubs can be an excellent food source for bees because they tend to grow larger than herbaceous perennials, and therefore produce a larger number of flowers. Some species bloom all summer (Mach and Potter, 2018).
Flowering trees are critical to providing an ample food source for bees because of their large size and thousands of flowers. A blooming linden or black locust produces so much pollen and nectar that it dwarfs the amount provided by most garden flowers in comparison. Among other trees attractive to bees are red maple, hawthorn, chestnut, willow, etc (Mach and Potter, 2018; Donkersley, 2018).
Wind pollinated trees are abundant in temperate forests. **Wind-pollinated trees do not produce nectar, but bees may take advantage of them as an abundant source of pollen.** Male flowers cast pollen into the wind in random search of a mate. In early spring, it is not uncommon to see bees and other insects visiting the male flowers in search of pollen, but they are foragers, not pollinators. Among the most frequently visited wind-pollinated trees are ash, birch, elm, hickory, oak, poplar, maple and willow. Pollen from the wind-pollinated trees may be collected by bees because of a favourable nutritional value, the large amount of pollen produced, or because it is available at times when other food sources are scarce (Donkersley, 2018; Splitt et al, 2021). Oaks are self-incompatible, incapable of pollinating themselves to produce viable acorns. The bees are not pollinators unless they carry their collected pollen to female flowers on another tree. **Oak trees should be therefore considered as attractive to bees for pollen only.** Also, several genera of wind-pollinated angiosperms are routinely visited by bees to collect pollen (Smitley et al, 2019; Bogdziewicz, et al, 2017).
Pines, spruces and nearly all gymnosperms are not usually visited by bees unless it is to gather sap used for propolis, a sticky substance used to fill crevices and seal hives. Such trees **should be considered as non-attractive to bees for pollen nor nectar**.[21,22]

Appendix B – Shortcut values for contact and dietary exposure

See document *ECHA_Bee_guidance_Appendix_B*.

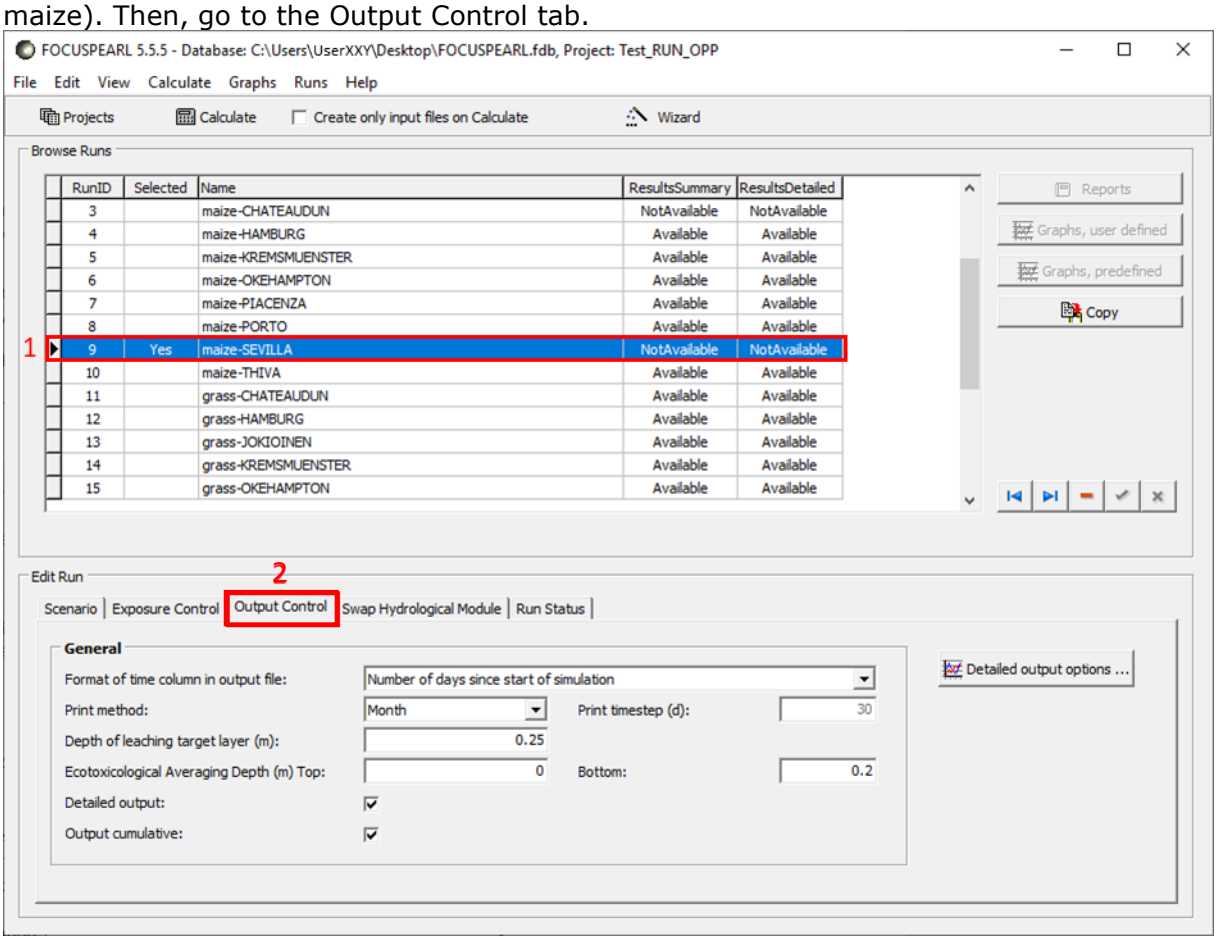
Appendix C – Manual for Refinement of PECpw with FOCUS PEARL

So far, FOCUS PEARL has only been used for groundwater modeling in the Biocides assessment. A refinement of PEC_{pw} (in alignment with Tier3-A of EFSA Guidance) is possible with FOCUS PEARL 5.5.5.
A detailed approach to obtain output on the pore water concentration in FOCUS PEARL 5.5.5 is given below. The starting point for creating an output on pore water concentration are the "runs" produced when modelling groundwater concentrations. For this, the basic settings on the substance, application and scenario selection (which crops) are required (please also consider TAB ENV 23, 165 and 166 (2022)):

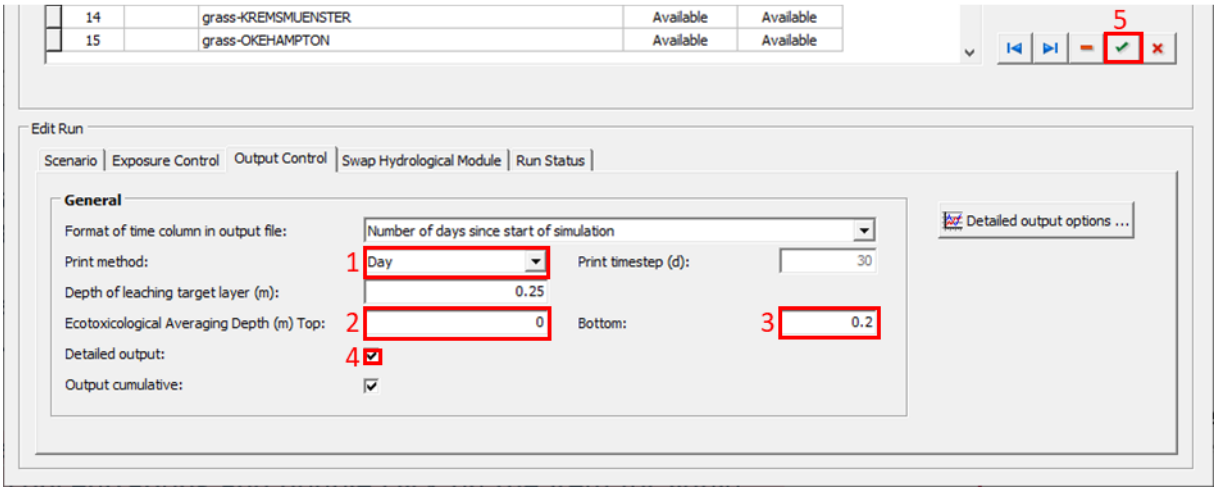
- 1 Select the run of interest, go to the Output Control tab.
  This approach should be followed for all locations and application schemes (arable land/grassland). Here in the example, it was made for RunID 9 (Sevilla arable land with

[21] According to the EFSA imidacloprid conclusion, conifers (grouped with non-orchard trees) are noted as not foraged by bees for nectar and pollen/ considered as non-attractive to bees for pollen and nectar. (https://statics.teams.cdn.office.net/evergreen-assets/safelinks/1/atp-safelinks.html)
[22] According to https://ecologyisnotadirtyword.com/2016/10/30/unlikely-plant-pollinator-relationships/ conifers are an important source of resins for some bee species, who use it to build nests and as chemical defence against predators.

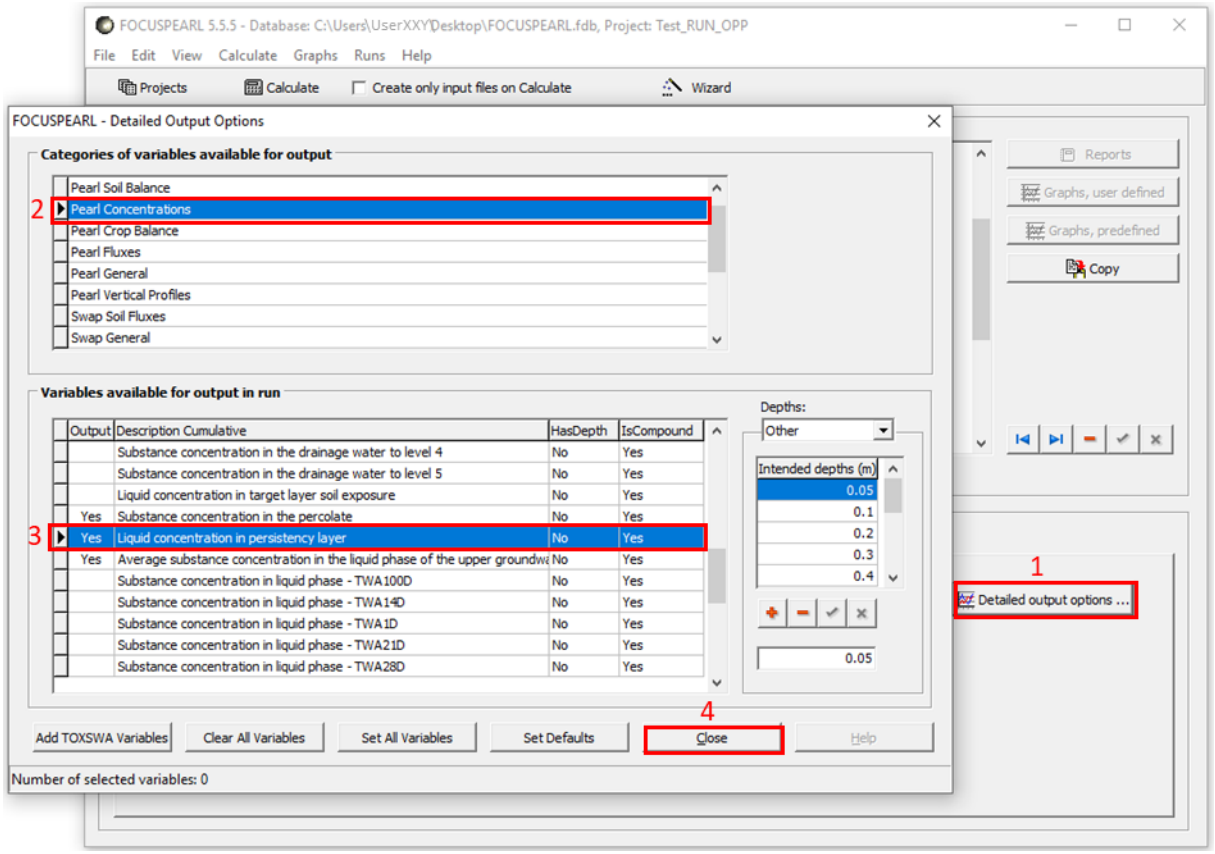
maize). Then, go to the Output Control tab.



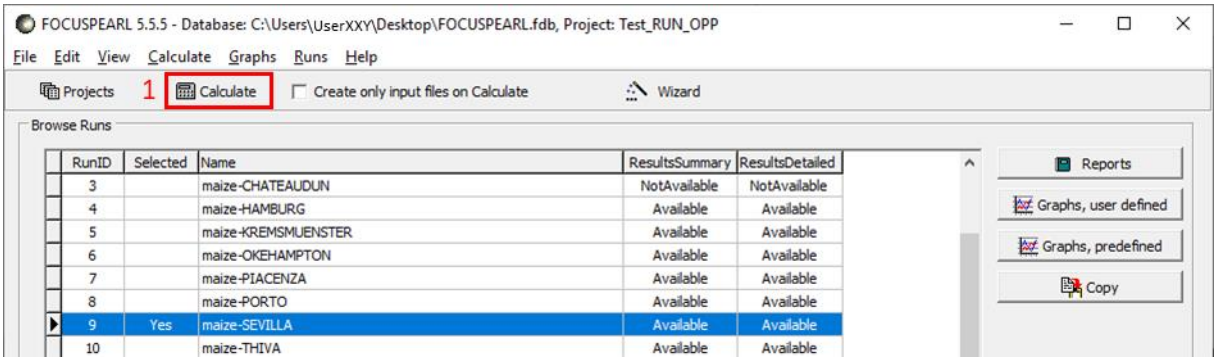
- 2 Set the print method on "Day", Set the top of the "Ecotoxicological averaging depth" to 0 m and the bottom boundary to the soil depth 0.2 m (see EFSA Guidance, Chapter 5.5.15).
  Mark the checkbox for detailed output. Click on the post edit button (the '√' button on the bar on the Browse part of the main screen)



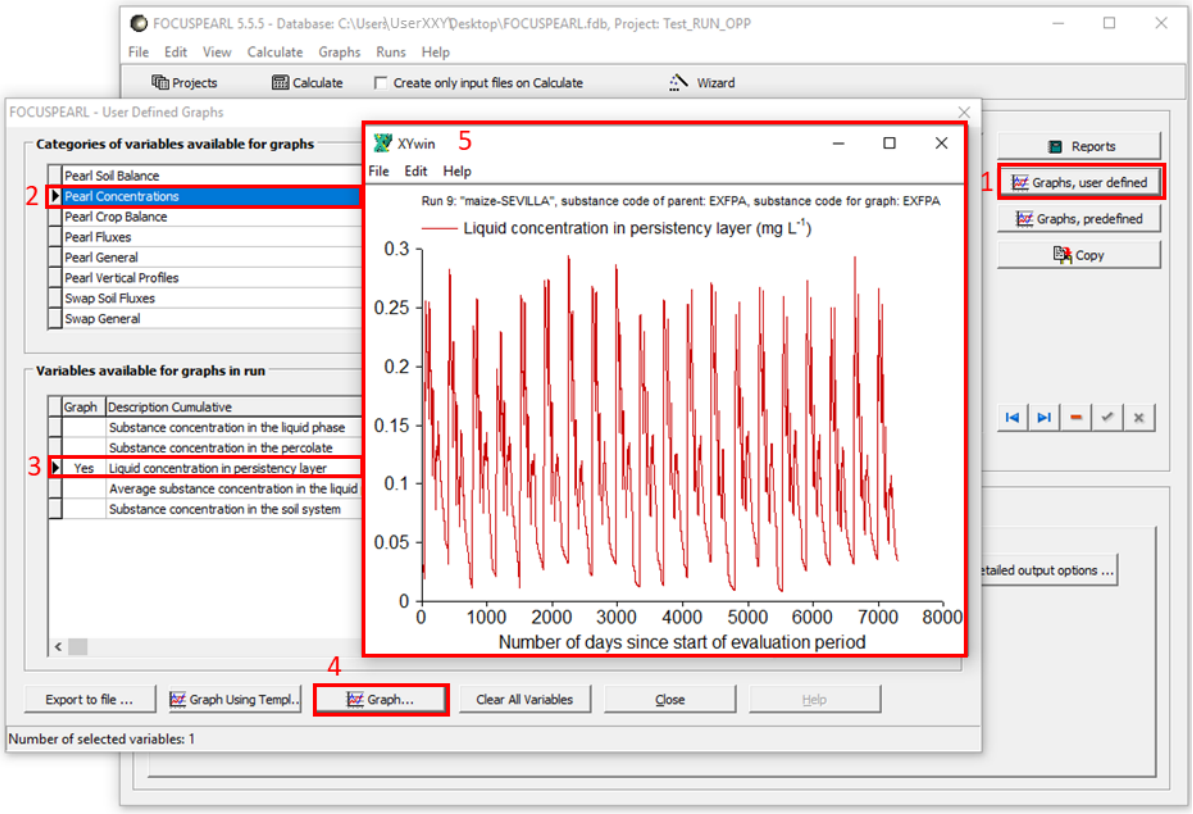
- 3 Click on the button 'Detailed output options' and select 'PEARL concentrations'. Next double click on the output item for 'Liquid concentration in persistency layer', i.e. the top 0.2 m layer.



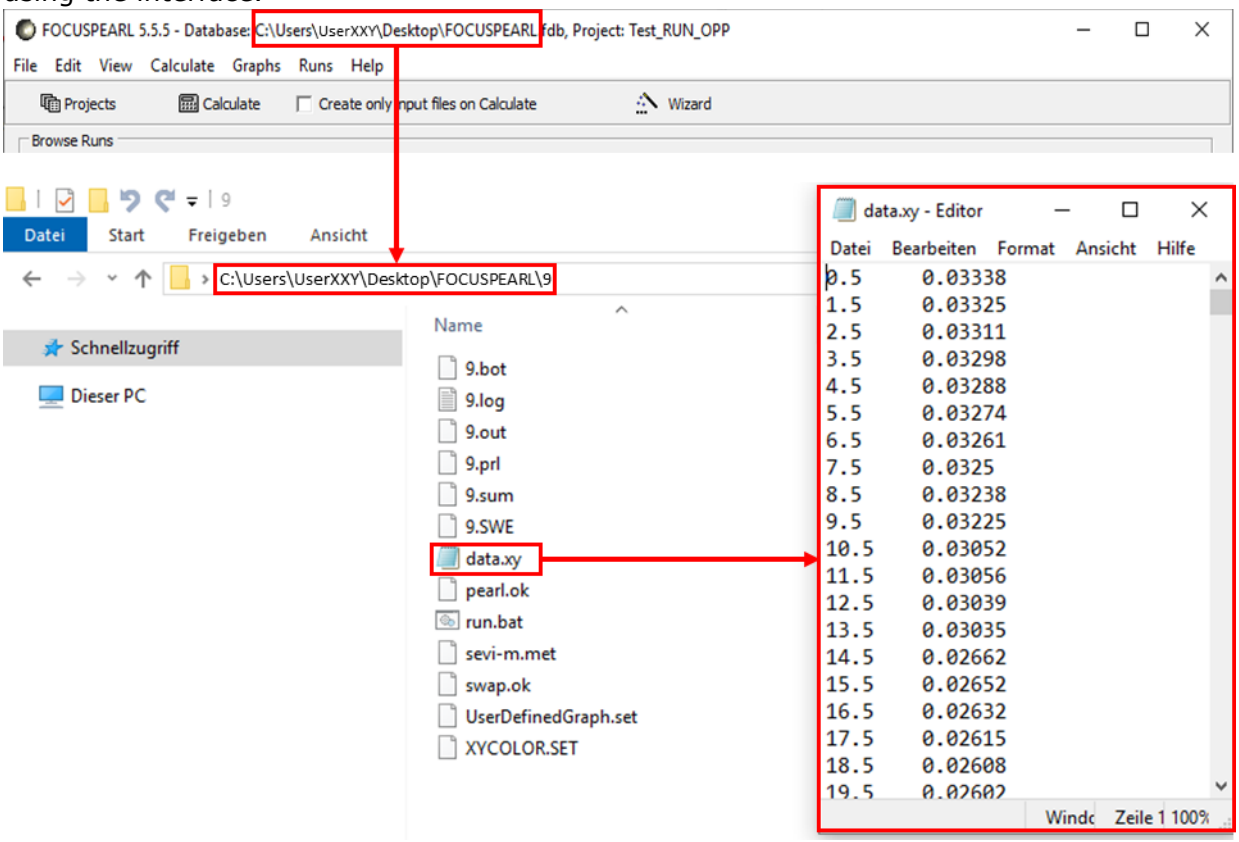
- 4 Execute the assessment



- 5 After completion of the assessment click on the button ‘Graphs User Defined’, select the category PEARL concentrations and double click on the item for liquid concentration in the persistency layer. Next click on the ‘Graph’ button. A graph will be shown with the pore water concentration in the top 20 cm layer plotted against time.


FOCUSPEARL 5.5.5 - Database: C:\Users\UserXXY\Desktop\FOCUSPEARL.fdb, Project: Test_RUN_OPP
File Edit View Calculate Graphs Runs Help
Projects
Calculate
Create only input files on Calculate
Wizard
FOCUSPEARL - User Defined Graphs
Categories of variables available for graphs
Pearl Soil Balance
Pearl Concentrations
Pearl Crop Balance
Pearl Fluxes
Pearl General
Pearl Vertical Profiles
Swap Soil Fluxes
Swap General
Variables available for graphs in run
Graph
Description Cumulative
Substance concentration in the liquid phase
Substance concentration in the percolate
Yes
Liquid concentration in persistency layer
Average substance concentration in the liquid
Substance concentration in the soil system
Export to file ...
Graph Using Templ...
Graph...
Clear All Variables
Close
Help
Number of selected variables: 1
Reports
Graphs, user defined
Graphs, predefined
Copy
1
2
3
4
5
XYwin
File Edit Help
Run 9: "maize-SEVILLA", substance code of parent: EXFPA, substance code for graph: EXFPA
Liquid concentration in persistency layer (mg L-1)
0.3
0.25
0.2
0.15
0.1
0.05
0
0 1000 2000 3000 4000 5000 6000 7000 8000
Number of days since start of evaluation period

- The data for this graph are output to the data file data.xy; this file is available in the folder [RunID] (in the example RunID 9) containing the PEARLdb.fdb file (path shown on top of the main screen). Please note that this file is overwritten if you plot another graph using the interface.



The relevant time points for bee assessment are 120 days for grassland and 150 days for arable land (see EFSA Guidance, chapter 5.5.15).

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